Synergistic tumor-suppressive effect of apatinib (rivoceranib), a selective VEGFR-2 inhibitor, in combination with immunotherapy in a syngeneic murine lung cancer model

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Background

- While angiogenesis inhibition is best known for its ability to limit the sprouting of new vessels, it is also known to normalize immature tumor vessels, a mechanism which is hypothesized to decrease the immunosuppressive tumor microenvironment and increase the number of tumor-infiltrating lymphocytes in the tumor environment. These mechanisms should enhance the antitumor immune response and suggest potential synergy between antiangiogenic and immunotherapy agents.
- A number of preclinical studies have supported the benefit of combining antiangiogenesis therapy with immunotherapy: monoclonal antibodies targeting VEGF and VEGFR-2, as well as multi-kinase, small-molecule inhibitors targeting VEGFR have shown synergistic efficacy in various tumor models.[1]
- Those studies determined that the combination may limit tumor growth due to increased antitumoral T-cell populations (e.g. $CD4^+$ and $CD8^+$ T-cells) and decreased immunosuppressive immune cell populations (e.g. T_{reg} and MDSCs).[2]
- Clinically, combination treatments have reached Phase III development for renal cell, non-small cell lung carcinoma, and ovarian cancer patients.
- Apatinib (official global nonproprietary name "rivoceranib" outside of China) is an orally-available, small-molecule tyrosine kinase inhibitor of VEGFR-2 with high selectivity and potency (IC_{50} = 1.0 nM) as well as a low toxicity profile proven in multiple clinical trials. Apatinib (rivoceranib) is approved in China for third-line gastric cancer and many clinical trials have been conducted worldwide for multiple tumor types including an ongoing global Phase III gastric cancer study (NCT03042611).[3]
- Herein, we present preclinical outcomes that apatinib (rivoceranib) synergistically suppressed tumor growth when combined with anti-PD-1 immunotherapy in a mouse model of lung carcinoma.

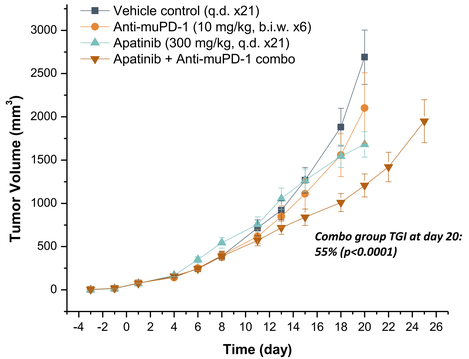
Methods

Disease model	• LL/2 murine lung carcinoma model • Tumor cells were inoculated into subcutaneous hind flank of 6–8 weeks-old C57BL/J6 mice
Dosing period	• After tumor size reached 60–80 mm^3 • 25-day dosing followed by 1-week observation
Dosing groups (n=10 for each group)	• Vehicle control (p.o., q.d.) • Anti-muPD-1 antibody monotherapy (10 mg/kg, i.p., b.i.w.) • Apatinib monotherapy (300 mg/kg, p.o., q.d.) • Combination of apatinib + anti-muPD-1 antibody
Sample analysis	*Every 2–3 days:* • Tumor volume and body weight measurement *Days 6 & 13*: • Flow cytometry: tumor-infiltrating immune cells ($CD3^+$, $CD8^+$, T_{reg}, MDSC) *Day 25:* • Histology & IHC: tumor-infiltrated immune cells ($CD3^+$, $CD8^+$, T_{reg}, MDSC) • Tumor PD-L1 expression

Results

Tumor growth inhibition

Figure 1. Tumor growth curve.



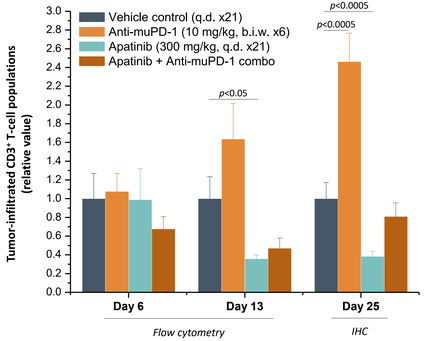
%TGI (tumor growth inhibition) = {{[mean day 20 control] - [mean day 0 control]} - {[mean day 20 combo group] - [mean day 0 combo group]}} / {[mean day 20 control] - [mean day 0 control]} * 100%

- At day 20, tumor growth inhibition (TGI) of anti-muPD-1 monotherapy was 22% (p<0.05), and TGI of apatinib monotherapy was 37% (p<0.0001).
- The degree of TGI in the combination treatment group was greater than either agent in monotherapy at 55% (p<0.0001).

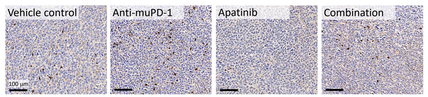
Immunophenotyping of tumor-infiltrating immune cells

Figure 2. (A) Tumor-infiltrating $CD3^+$ T-cells quantified by flow cytometry at day 6 & 13 and by IHC imaging analysis at day 25, and normalized by vehicle control group in each day. (B) Representative IHC snapshots (20× magnification) of tumor-infiltrated $CD3^+$ T-cells.

(A) Changes of $CD3^+$ T-cells from vehicle control group



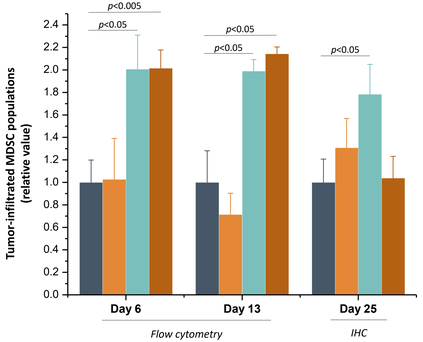
(B) IHC of $CD3^+$ T-cells located in the tumor tissues



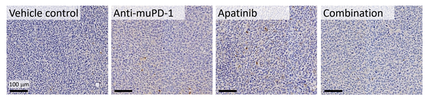
- Tumor-infiltrating $CD3^+$ T-cells in the anti-muPD-1 monotherapy group were increased 150%, whereas those in the apatinib monotherapy group decreased 62% from vehicle control at day 25.
- Combination treatment recovered tumor-infiltrated $CD3^+$ T-cells to a similar level seen in the vehicle control group at day 25.
- The level of tumor-infiltrating $CD8^+$ T-cells showed a similar trend to that seen with $CD3^+$ T-cells (data not shown).

Figure 3. (A) Tumor-infiltrating MDSCs quantified by flow cytometry (days 6 & 13) and IHC (day 25), and normalized against vehicle control on each day. (B) Representative IHC snapshots (20× magnification) of tumor-infiltrating MDSCs.

(A) Changes in MDSCs from vehicle control group



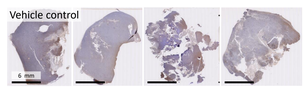
(B) IHC of MDSCs located in the tumor tissues



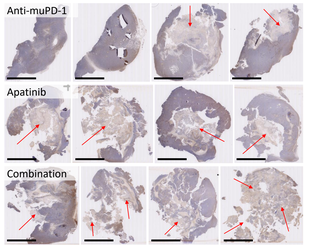
- Tumor-infiltrating MDSCs in apatinib monotherapy and combination treatment were increased 100% from vehicle control on days 6 & 13, whereas those of anti-muPD-1 monotherapy were not changed.
- By day 25, tumor-infiltrating MDSCs in the combination treatment group returned to levels seen in the vehicle control group.

Histopathology assessments of tumor tissue

Figure 4. Representative entire tumor sections and notable features. Red arrows indicate tumor necrosis area.



- Tumor mass is well maintained.
- Necrosis is rarely observed.



- Tumor mass is mostly maintained.
- Necrosis is partially observed.
- Outer rim of tumor mass is mostly maintained.
- Central necrosis is prominent.
- Necrosis is widely distributed over tumor.

- Central tumor necrosis, which is a typical feature of hypoxia, was prominently observed in the apatinib monotherapy group and partially observed in the anti-PD-1 monotherapy group.
- Tumor necrosis was prominent and more widely-distributed in the combination treatment group compared to other treatments.

Conclusions

- Both apatinib (rivoceranib) monotherapy and the combination of apatinib with anti-PD-1 induced a higher degree of tumor necrosis than anti-PD-1 monotherapy in this preclinical setting; however, since apatinib monotherapy did not appear to significantly mediate changes in the anti-tumoral immune environment, further follow-up is required to determine the underlying mechanisms of synergy between the agents.
- The combination of apatinib (rivoceranib) with an anti-PD-1 antibody significantly improved tumor growth suppression over each agent in monotherapy, which is encouraging for ongoing and upcoming clinical trials of apatinib in combination with anti-PD-1 therapies. (NCT03396211, NCT03407976)

References

1. R. Jain, et al. *Cancer Cell* (2014) 26:605-622.
2. S. Zhao, et al. *Journal of Thoracic Oncology* (2017) 12:S147, IASLC 2016 Abstract #OA11.07.
3. Y-K. Kang, et al. ASCO 2017 Abstract #TPS4138 (NCT03042611).

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