TU2218, a novel ALK5/VEGFR2 dual inhibitor, overcomes tumor endothelial cell anergy and enhances anti-PD1 immunotherapy efficacy 5080

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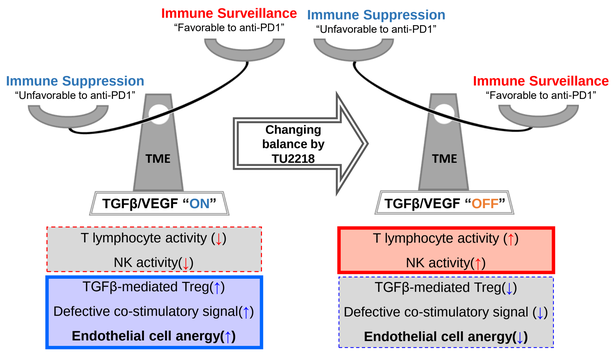
Abstract

Immune tolerance by TGF-β and VEGF is inextricably related with poor outcomes of approved anti-PD-(L)1 therapy. Accordingly, a dual target for ALK5 and VEGFR2 via single or combination treatments can be an unequivocal tactic to tune tumor-microenvironment (TME) favorable to ICI, and to essentially overcome immune evasion against TGF-β- and VEGF-enriched tumors. Specifically, several reports from clinical data suggest that VEGF-induced endothelial cell anergy (ECA) acts as a vascular immune checkpoint in TME immune response, and the activation of ECA is associated with worse outcomes. Herein, we demonstrate that TU2218, a *first-in-class*, orally available inhibitor against ALK5 and VEGFR2 can recover the downregulated endothelial adhesion molecules, i.e., ICAM-1 and VCAM-1, and suppress ECA. In this work, TU2218 completely recovered the expression of ICAM-1 and VCAM-1 on VEGF-induced ECA in HUVECs. The restored level of ICAM-1 and VCAM-1 at 1 μM TU2218 was equivalent to the activity of combined treatment of 1 μM Vactosertib (ALK5 inhibitor) and 25 μg/ml Ramucirumab (VEGFR2 inhibitor). 1 μM of Vactosertib alone, however, did not show such restoration. These results indicate that VEGF-induced ECA is mediated by both VEGFR2 and TGF-β signal, thereby validating the superiority of dual target strategy for ALK5 and VEGFR2 over a single target in overcoming ECA. We further tested if TU2218 could restore VEGF-induced decrease of Jurkat adhesion to HUVECs, considering the close relationship between the expression of adhesion-molecules of endothelial cell surface and the adhesion of lymphocytes to endothelium. TU2218 recovered the number of Jurkat adhering to VEGF-elicited HUVEC monolayer in a dose-dependent manner, but Vactosertib did not. Furthermore, the activity of TU2218 on Jurkat adhesion was reversed by VCAM-1 neutralizing antibody. Therefore, our results demonstrate that TU2218 improves Jurkat adhesion by restoring VCAM-1 expression. Finally, the *in vivo* translatability of TU2218 in overcoming ECA was confirmed with B16F10-bearing mice, a well-defined immune desert model, after treatments of anti-PD1 antibody, TU2218, or combined regimen for 15 days. TU2218 combined with an anti-PD1 antibody significantly suppressed tumor growth by c.a. 74 % compared to vehicle, thus being superior to a single treatment (e.g., tumor growth inhibition (TGI) 44% for TU2218, TGI 45% for anti-PD1). In this combination, TU2218 increased the number of both CD31⁺VCAM-1⁺ and IFNγ⁺CD8⁺ T cells in the tumor. We conclude that TU2218 leads not only to the enhancement of T cell-traffic toward TME, but also to the conversion of immune balance favorable to anti-PD1 therapy. The Phase 1b trial of TU2218 combined with pembrolizumab is underway for advanced solid cancers (NCT05204862).

Expected MoA of TU2218



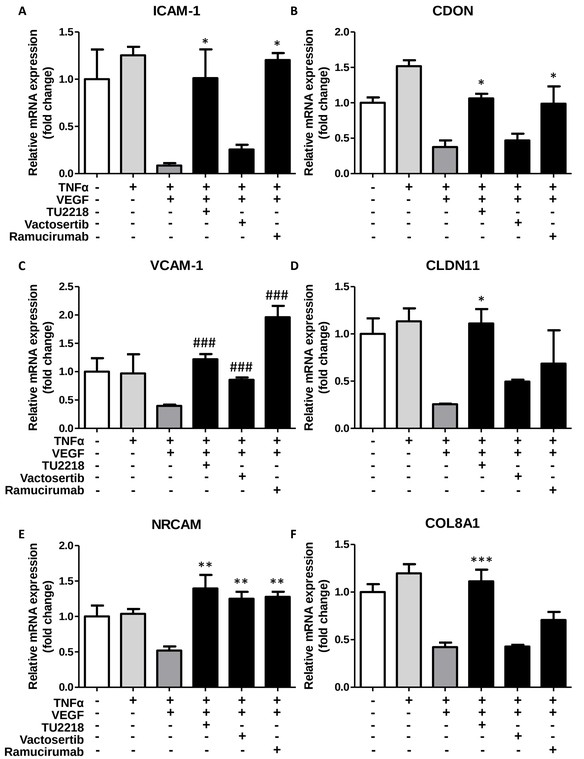
Immune evasion mechanism in TGFβ/VEGF enriched context vs. Immune response to tumor-immune microenvironment by TU2218, Changing the immune balance toward favorable status to anti-PD1 antibody drugs.

TU2218, ALK5/VEGFR2 dual inhibitor

Drug	Enzyme activity(IC_{50} nM)		Cellular activity(IC_{50} nM)	
	ALK5	VEGFR2	ALK5	VEGFR2
TU2218	1.2	4.9	101	52.5

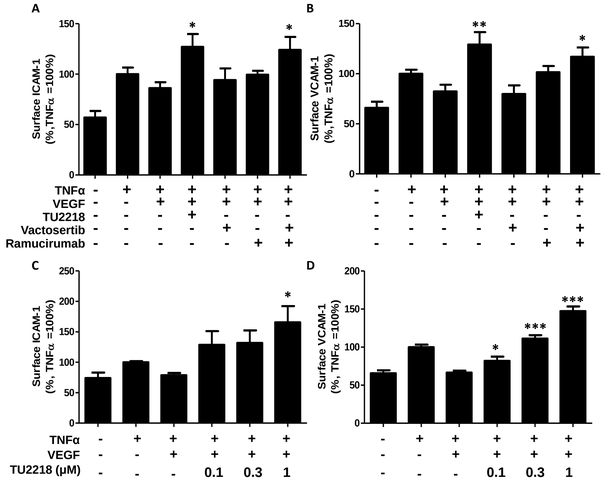
TU2218 is a highly potent, orally available dual inhibitor against ALK5 and VEGFR2. Cellular activity was determined by the IC_{50} value for phosphorylation of SMAD2 and VEGFR2 with stimulation of TGF-β and VEGF, respectively. Phosphorylation of SMAD2 and VEGFR2 were analyzed by flow cytometry or immunoblotting using whole blood culture or HUVECs.

6 DEGs on TU2218-treated endothelial anergy



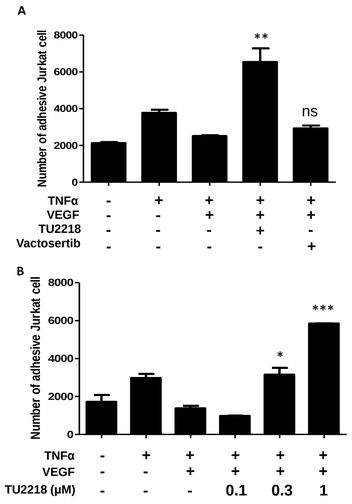
Six differentially expressed genes were down-regulated on VEGF-induced endothelial cell anergy and recovered by TU2218. Relative level of mRNA was quantified by RT-PCR from HUVECs with indicated treatment condition. GAPDH was used as housekeeping and fold change was calculated with comparison to vehicle. (A, B, D, E, F) One-way ANOVA with Tukey's multiple comparison test was used to compare to TNFα+VEGF stimulation *: p ≤ 0.05, **: p ≤ 0.01, ***: p ≤ 0.001. (C) Two-tailed t-test was used to compare to TNFα+VEGF stimulation ###: p ≤ 0.001.

Restoration of adhesion molecules by dual inhibition of ALK5/VEGFR2 on VEGF-induced anergy



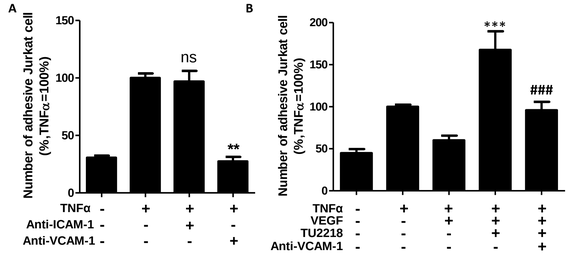
TU2218 significantly restored VEGF-induced decrease of surface ICAM-1 and VCAM-1 on HUVECs compared to Vactosertib(ALK5 inhibitor) or Ramucirumab(anti-VEGFR2 monoclonal antibody). HUVECs were treated by indicated condition. Fluorescence intensity of ICAM-1 and VCAM-1 on HUVECs were quantified by FACS. **A**. Relative ratio of surface ICAM-1. **B**. Relative ratio of surface VCAM-1. *: p ≤ 0.05 vs. TNFα+VEGF (Two-tailed t-test) **C**. Relative ratio of surface ICAM-1. **D**. Relative ratio of surface VCAM-1. *: p ≤ 0.05, ***: p ≤ 0.001 vs. TNFα+VEGF (One-way ANOVA, Tukey)

Improvement of lymphocyte adhesion by TU2218 against VEGF-induced anergy



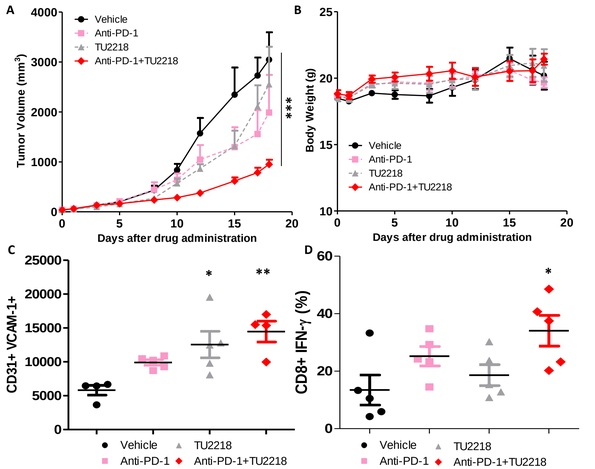
TU2218 significantly improved VEGF-induced decrease of lymphocyte adhesion to endothelial cell. The number of adhesive Jurkat was quantified by counting the remaining Jurkat after co-culture with HUVECs. Before co-culture, HUVECs were plated as monolayer cells and treated by indicated conditions. Jurkat cells were tagged by fluorescence(CFSE) **A**, The number of adhesive Jurkat on HUVECs treated by TNFα, VEGF and TU2218 or Vactosertib. One-way ANOVA with Tukey's multiple comparison test was used to compare to TNFα+VEGF stimulation **: p ≤ 0.01, ns: not significant. **B**, The number of adhesive Jurkat on HUVECs treated by indicated concentration of TU2218. One-way ANOVA with Tukey's multiple comparison test was used to compare to TNFα+VEGF stimulation *: p ≤ 0.05, ***: p ≤ 0.001

Normalization of Vascular-Immune crosstalk via VCAM-1



Blocking VCAM-1 directly inhibited the activity of TU2218 on Jurkat-HUVEC adhesion. **A**, Relative ratio of adhesive Jurkat on HUVECs with ICAM-1 or VCAM-1 neutralizing antibodies. **: p ≤ 0.01, ns: not significant vs. TNFα (One-way ANOVA, Tukey) **B**, Relative ratio of adhesive Jurkat on HUVECs with TU2218 and VCAM-1 neutralizaing antibody. ***: p ≤ 0.001 vs. TNFα+VEGF, ###: p ≤ 0.001 vs. TNFα+VEGF+TU2218 (One-way ANOVA, Tukey)

Antitumor activity of combination with TU2218 and anti-PD1 on immune-desert tumor models



Antitumor activity of combination with TU2218 and anti-PD1 antibody in B16F10 syngeneic mouse model. **A**, Tumor volume at indicated time points. Data are shown as mean + SEM. ***: p ≤ 0.001 vs. vehicle (Two-way ANOVA). **B**, Mean body weight + SEM for each treatment group. **C**, Fluorescence intensity of CD31+VCAM1+ cell in tumors. * p ≤ 0.05, **: p ≤ 0.01 vs. vehicle (One-way ANOVA, Tukey) **D**, Percent of CD8+IFNγ+ T cells in tumors. *: p ≤ 0.05 vs. vehicle (One-way ANOVA, Tukey)

Conclusion

- TU2218 normalizes VEGF-induced endothelial anergy for potentiating cancer immunity.
- Combination of TU2218 and anti-PD1 is valid therapeutic strategy that can enhance tumor-infiltrating lymphocytes(TILs) on immune-desert context.
- The ongoing phase1/2 study is further evaluating safety and effective clinical dose of TU2218 in combination with pembrolizumab in patients with advanced solid tumors(NCT05204862).