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

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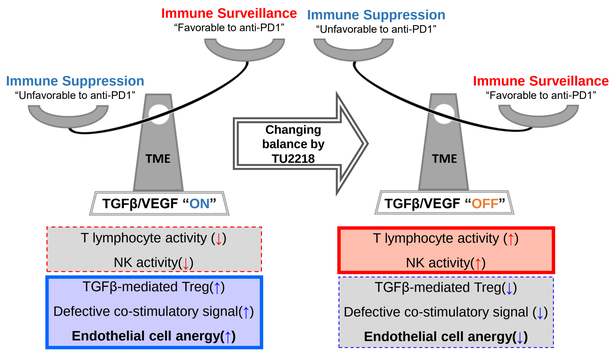
Abstract

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



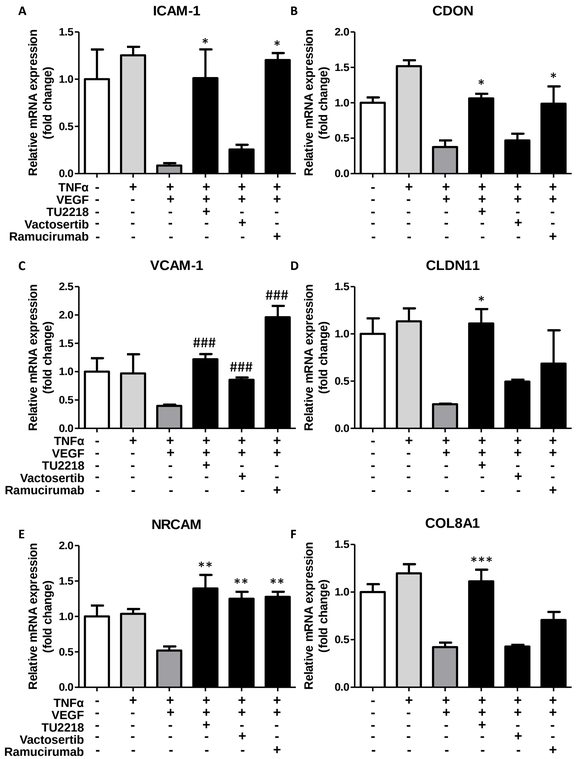
Immune evasion mechanism in TGFβ/VEGF enriched context vs. Immune response to tumor-immune microenvironment by TU2218, Changing the immune balance toward favorable status to anti-PD1 antibody drugs.

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

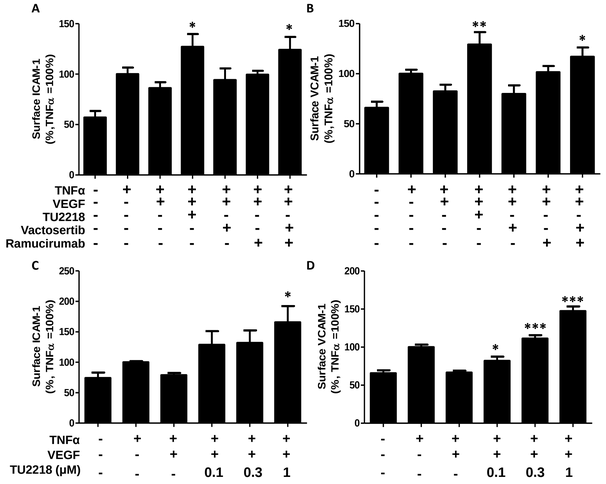
TU2218 is a highly potent, orally available dual inhibitor against ALK5 and VEGFR2. Cellular activity was determined by the IC_{50} value for phosphorylation of SMAD2 and VEGFR2 with stimulation of TGF-β and VEGF, respectively. Phosphorylation of SMAD2 and VEGFR2 were analyzed by flow cytometry or immunoblotting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

6 DEGs on TU2218-treated endothelial anergy



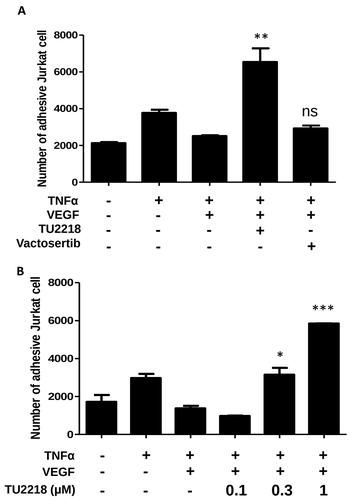
Six differentially expressed genes were down-regulated on VEGF-induced endothelial cell anergy and recovered by TU2218. Relative level of mRNA was quantified by RT-PCR from HUVECs with indicated treatment condition. GAPDH was used as housekeeping and fold change was calculated with comparison to vehicle. (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



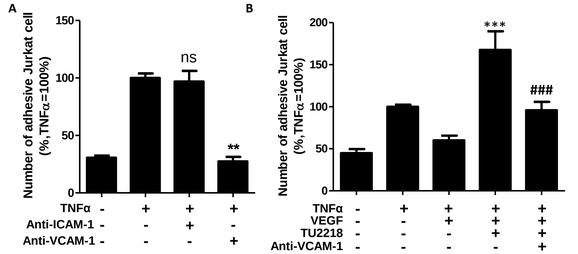
TU2218 significantly restored VEGF-induced decrease of surface ICAM-1 and VCAM-1 on HUVECs compared to Vactosertib(ALK5 inhibitor) or Ramucirumab(anti-VEGFR2 monoclonal antibody). HUVECs were treated by indicated condition. Fluorescence intensity of ICAM-1 and VCAM-1 on HUVECs were quantified by FACS. **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



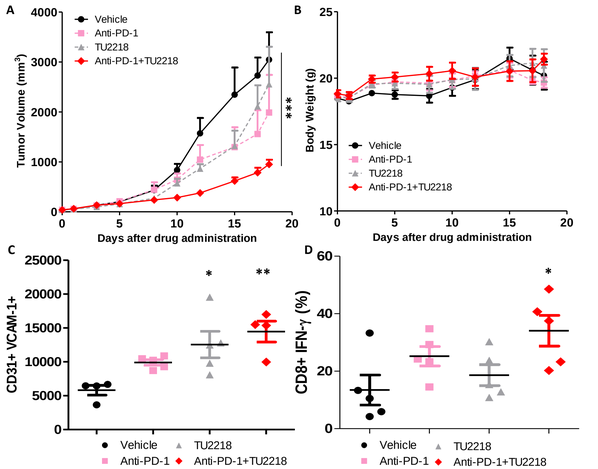
TU2218 significantly improved VEGF-induced decrease of lymphocyte adhesion to endothelial cell. The number of adhesive Jurkat was quantified by counting the remaining Jurkat after co-culture with HUVECs. Before co-culture, HUVECs were plated as monolayer cells and treated by indicated conditions. Jurkat 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).

TU2218, a dual inhibitor against ALK5/VEGFR2, increases anti-CTLA4 antitumor efficacy in syngeneic tumor models

5090

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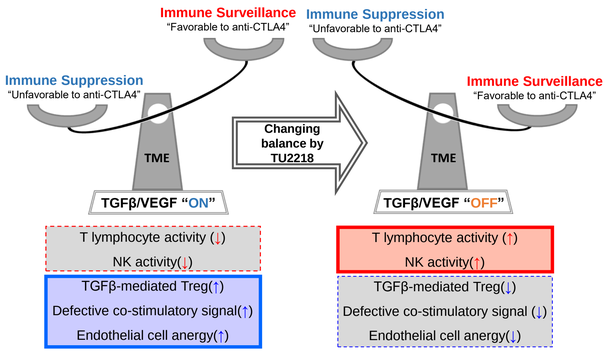
Abstract

The combination of pembrolizumab with low-dose ipilimumab shows substantial antitumor activity and manageable profile of toxicity in anti-PD-(L)1 antibody failure-setting (NCT02743819). This suggests a breakthrough for the absence of treatment option after relapsed/refractory anti-PD-(L)1 therapy. Accordingly, the anti-CTLA4 drug-based combination can be considered as a promising strategy for beneficial outcomes against resistance acquired from immunotherapy. Herein, we demonstrate that TU2218, a first-in-class, orally available inhibitor against ALK5 and VEGFR2, showed synergistic antitumor efficacy when combined with an anti-CTLA4 antibody in preclinical tumor models which is being accompanied by increasing ratio of CD8 T cell to regulatory T cell and enhanced immunological memory. In this work, antitumor efficacy of TU2218 combination with an anti-CTLA4 antibody was assessed with CT26-, 4T1-, B16F10- and WEHI-164-bearing mice. In the CT26 model, the combination of TU2218 with an anti-CTLA4 antibody significantly inhibited tumor growth up to 92% compared to vehicle, thus being superior to single treatments (e.g., tumor growth inhibition (TGI) 46% for TU2218, TGI 74% for anti-CTLA4). In this combination group, the complete regression (CR) rate was 75 % (i.e., six cases among eight mice), while single treatments showed lower CR rates (e.g., CR 10% (1/10) for TU2218, CR 30% (3/10) for anti-CTLA4). Meanwhile, the role of CD8+ T cell in antitumor activity was elucidated by in vivo depleting CD8+ T cell in mice treated with combination therapy. The depletion of CD8+ T cells reduced the antitumor response, which suggests the indispensable role of CD8+ T cells in the antitumor efficacy of TU2218 and anti-CTLA-4 antibody combination. In addition, the long-term immune-memory was evaluated by re-implanting tumor cells into both mice cured by combination therapy and age-matched tumor-naïve mice. In this case, 6 mice cured of original implantation with CT26 tumors showed complete resistance to the re-implantation of CT26 cells during an untreated period for 21 days, whereas all age-matched tumor-naïve mice have developed tumors after 10 days from cell-transplants. Importantly, we could confirm the positive correlation between the immunological memory-response of combination therapy and the increasing rate of effector memory CD4+ and CD8+ T cells in spleens compared to those of age-matched group. In 4T1-, B16F10- and WEHI-164-bearing mice, combination of TU2218 with an anti-CTLA4 antibody led to higher CR rate as well as enhanced inhibition of tumor growth. Overall, our findings showed that TU2218 plays multifaceted roles in inducing immune activation under combination with an anti-CTLA4 antibody, which may be attributed to the increased ratio of cytotoxic CD8 T cell to regulatory T cell and improvement of adaptive immunity with long-term immunological memory.

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-CTLA4 antibody drugs.

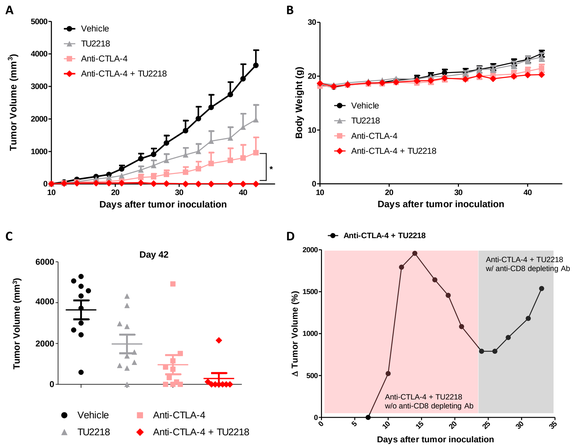
TU2218 synergizes with anti-CTLA4 antibody in syngeneic mouse tumor models

Syngeneic model		CT26		B16F10	WEHI164	4T1
Initiation tumor size (mean mm^3)		Unstaged	380 mm^3	40 mm^3	53 mm^3	53 mm^3
Treatment duration(day)		42	17	15	17	23
%TGI	TU2218	46%	n.t.[a]	44%	71%	15%
	Anti-CTLA4	74%	56%	41%	63%	17%
	Combination	92%	87%	67%	93%	50%
%CR	TU2218	10%(1/10)	n.t.[a]	n.d.[b]	30%(3/10)	n.d.[b]
	Anti-CTLA4	30%(3/10)	16.7%(1/6)	n.d.[b]	20%(2/10)	n.d.[b]
	Combination	75%(6/8)	40%(2/5)	n.d.[b]	80%(8/10)	n.d.[b]

[a], not tested; [b], not detected

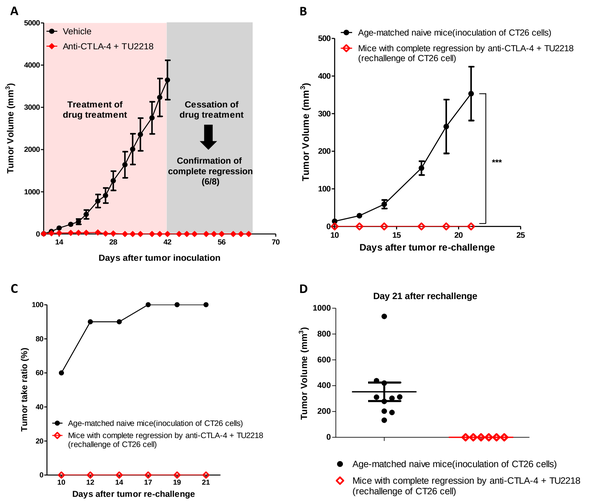
In vivo assessment of the antitumor activity of TU2218, anti-CTLA4 or the combination regimen in four syngeneic mouse tumor models. Efficacy is shown to tumor growth inhibition(%TGI) and complete regression(%CR) in the table. TU2218(50 mg/kg, bid) and anti-CTLA4(10 mg/kg, q3d) are administrated to oral and intraperitoneal route respectively for the indicated treatment duration. Long-term treatment(42 days) is performed to examine the sustained tumor-killing activity and immune memory.

Combination with TU2218 and anti-CTLA4 increases effective CD8 T cells in CT26 model



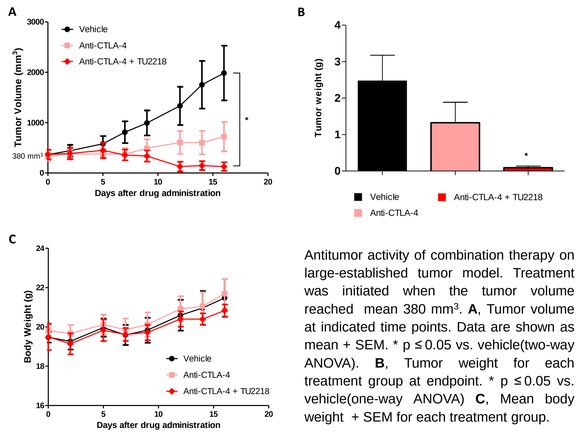
Antitumor activities of TU2218, anti-CTLA4 antibody and combination of TU2218 plus anti-CTLA4 antibody in CT26 syngeneic mouse tumor models. Mice were orally administrated 50 mg/kg of TU2218 twice a day, intraperitoneally injected with 10 mg/kg of anti-CTLA4 antibody every three days, subjected to a combination of both treatments, or subjected to non-treatment(vehicle). **A**, Tumor volume at indicated time points. Data are shown as mean + SEM. * $p \leq 0.05$ vs. anti-CTLA4 antibody alone(two-way ANOVA). **B**, Mean body weight + SEM for each treatment group. **C**, Individual tumor volume at endpoint, n=10 per group. **D**, To examine the immunological property in single individual, One mouse of TU2218 + anti-CTLA4 combination group was continuously administrated CD8 T depletion antibody since day 25(gray zone) after conformation of tumor regression(pink zone). And tumor volume was measured for indicated time point.

Combination with TU2218 and anti-CTLA4 enhances tumor-specific memory immunity in CT26 model



Enhancement of systemic tumor-specific memory immune response by TU2218 plus anti-CTLA4 antibody. Mice that achieved complete response after TU2218 combined with anti-CTLA4 antibody treatment were evaluated for memory potentiality to combat repeated-tumor challenges. **A**, Complete regression of tumors was defined as undetectable tumor volume with additional drug cessation. **B**, Tumor volume at indicated time points. Data are shown as mean + SEM. *** $p \leq 0.001$ vs. age-matched naïve mice group(two-way ANOVA). **C**, Percent of tumorigenicity at indicated time point. **D**, Individual tumor volume at endpoint.

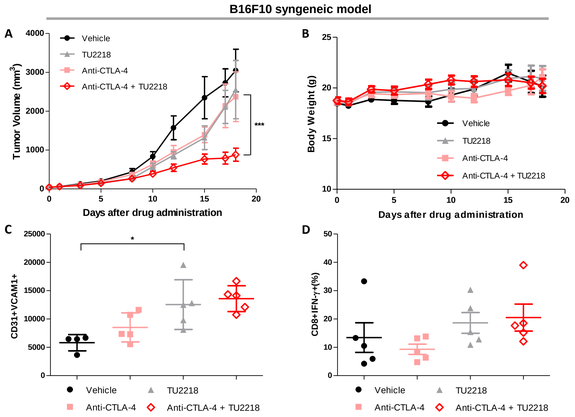
Combination with TU2218 and anti-CTLA4 is effective to large tumors in CT26 model



Antitumor activity of combination therapy on large-established tumor model. Treatment was initiated when the tumor volume reached mean 380 mm^3. **A**, Tumor volume at indicated time points. Data are shown as mean + SEM. * $p \leq 0.05$ vs. vehicle(two-way ANOVA). **B**, Tumor weight for each treatment group at endpoint. * $p \leq 0.05$ vs. vehicle(one-way ANOVA) **C**, Mean body weight + SEM for each treatment group.

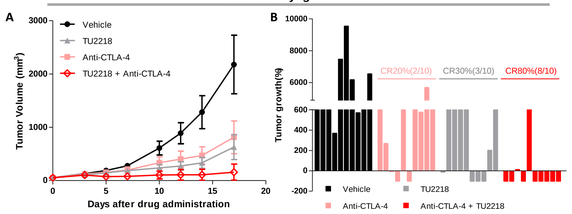
Combination therapy stimulates anti-tumor immunity in B16F10, WEHI-164 and 4T1 models

B16F10 syngeneic model



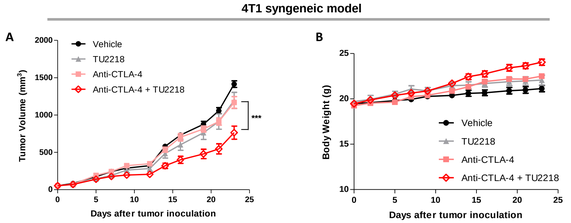
A, Tumor volume at indicated time points. Data are shown as mean + SEM. *** $p \leq 0.001$ vs. anti-CTLA4 antibody alone(two-way ANOVA). **B**, Mean body weight + SEM for each treatment group. **C**, Fluorescence intensity of CD31+VCAM1+ cell in tumors. * $p \leq 0.05$ vs. vehicle(one-way ANOVA, Tukey) **D**, Percent of CD8+IFNγ+ T cells in tumors.

WEHI-164 syngeneic model



A, Tumor volume at indicated time points. Data are shown as mean + SEM. **B**, individual tumor growth rate during 17 days

4T1 syngeneic model



A, Tumor volume at indicated time points. Data are shown as mean + SEM. *** $p \leq 0.001$ vs. anti-CTLA4 antibody or TU2218 alone(two-way ANOVA). **B**, Mean body weight + SEM for each treatment group.

Conclusions

- Combination with TU2218 and anti-CTLA4 antibody leads complete tumor regressions and sustained immunological memory.
- The ongoing phase1/2 study is further evaluating safety and effective clinical dose of TU2218 in patients with advanced solid tumors(NCT05204862).