Poster B123
Abstract #35333

Mechanism of Action of Ivonescimab (AK112/SMT112): A First-in-Class Tetravalent Bispecific Antibody with Dual Blockade of PD-1 and VEGF that Promotes Cooperative Biological Effects

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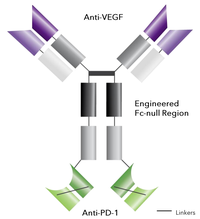
INTRODUCTION

Combination treatments using anti-PD-1/PD-L1 antibodies with other VEGF antagonists have shown enhanced clinical anti-tumor activities.[1] The expression of PD-1 and VEGF are found to be frequently upregulated and co-expressed in solid tumors. Importantly, VEGF promotes tumor angiogenesis and suppresses anti-tumor immune response.[2,3] Consequently, we characterized the mechanism-of-action of a novel first-in-class anti-PD-1/VEGF bispecific antibody, ivonescimab, designed to simultaneously inhibit PD-1-mediated immunosuppression and block tumor angiogenesis in the tumor microenvironment.

MECHANISM OF ACTION

Ivonescimab: First-in-Class PD-1/VEGF Bispecific Antibody in Clinical Development

Brings two validated mechanisms in oncology[4,5,6] into ONE novel tetravalent molecule.



Designed to Optimize the Balance of Anti-tumor Activity and Safety[7,8]

Cooperative Binding

- Presence of VEGF increases binding of PD-1 by >10-fold in-vitro[9]
- VEGF dimer leads to potential interconnection of multiple ivonescimab molecules, which may lead to increased binding of T-cells in-vitro[9]

Potential to accumulate higher levels of ivonescimab in the tumor microenvironment vs. healthy tissue

- Higher levels of PD-1 & VEGF expression in the TME[7-9]

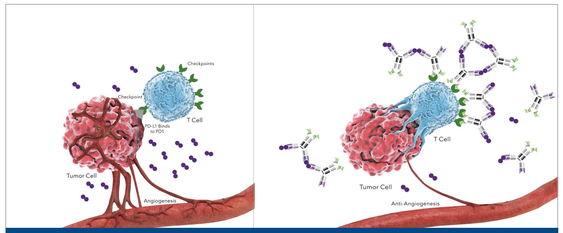
Simultaneous interaction of PD-1 & VEGF blockades have the potential to drive synergistic anti-tumor activity[5,7,10]

- Inhibiting VEGF can help improve the effect of immunotherapy by modulating the tumor microenvironment[7]
- Enhancing PD-1 blockade helps activate T-cells[5]

Engineered Fc-null region could lead to reduced adverse events

- Via reduction of ADCC, ADCP, and CDC in-vitro[10,11] and no meaningful infusional cytokine release (IL-6 and TNF-α) in patients[10]
- Humanized IgG1 bispecific antibody[7]

$T_{1/2}$ of 6-7 days[12] of ivonescimab provides blockade of both targets and with its affiliated clearance, could potentially lead to a favorable safety profile[7,8]



METHODS

Binding activity of ivonescimab to PD-1/VEGF was assessed by ELISA. Blockade of PD-1/VEGF signaling pathways were determined in luciferase reporter cell assays. Ivonescimab-VEGF complex formation was detected by SEC-HPLC. Cooperative binding of ivonescimab-VEGF complex to PD-1 or ivonescimab-PD-1 complex to VEGF was measured by Octet BLI. The enhanced PD-1 blockade bioactivity of ivonescimab with VEGF was evaluated in hPBMC and engineered cell-line co-culture/luciferase-reporter cell assays. Anti-tumor activity of ivonescimab was investigated in hPBMC-humanized SCID/Beige mice implanted with HCC827 (mEGFR lung adenocarcinoma). Immuno-safety was assessed by FcγR binding, ADCC, ADCP assays, and reported clinical irAEs.

RESULTS

Ivonescimab displayed strong binding activity to human PD-1 and VEGF alone or simultaneously, effectively blocking interactions with ligands and the downstream signaling effects. In presence of VEGF, ivonescimab forms soluble complexes with VEGF dimers, leading to over 10-fold enhanced binding affinity (K_D) of ivonescimab to PD-1. The avidity increase was consistent with reduced cell surface PD-1 expression on human T-cell lines, increased potency on blockade of PD-1/PD-L1 signaling and subsequent enhanced T cell activation in-vitro. Likewise, PD-1 binding enhanced ivonescimab binding to VEGF which was associated with enhanced VEGF-signaling blockade. Furthermore, ivonescimab treatment demonstrated statistically significant dose-dependent anti-tumor response in hPBMC-humanized murine HCC827. Finally, ivonescimab contains Fc-silencing mutations abrogating FcγRI/IIIa binding and showed significantly reduced ADCC, ADCP activities and cytokine release in-vitro. Clinically, this is consistent with the safety profile in Phase 1/2 studies of ivonescimab in advanced solid tumors.[7,8,12,13,14]

RESULTS

VEGF-A and PD-1 are highly co-expressed in various human tumors

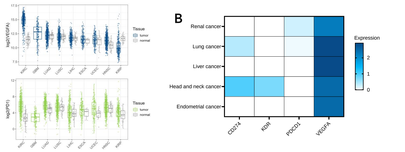


Fig 1. **VEGF-A and PD-1 are highly co-expressed in various human tumors.** A, mRNA expression data originated from The Cancer Genome Atlas (TCGA). B, IHC protein expression in tumor tissues (source: ProteinAtlas).

Ivonescimab binds to and antagonizes PD-1 and VEGF interactions with PD-L1 and VEGFR, respectively

Table 1. Ivonescimab binds to dual antigens, PD-1 and VEGF, and blocks binding to PD-L1 and VEGFR2, respectively

Antibody	EC50 (nM) of antigen binding activity		EC50 (nM) of competitive binding activity	
	PD-1[a]	VEGF[b]	PD-L1 to PD-1[c]	VEGFR2 to VEGF[d]
Ivonescimab	0.06	0.036	1.216	5.324
Bevacizumab	NA	0.035	NA	5.086
Nivolumab	0.044	NA	0.449	NA

Note: a, a human PD-1 extracellular domain with mFc fusion protein was coated onto the plate in this assay; b, 6× His-tagged VEGF protein was coated onto the plate; c, serial dilutions of antibodies with 0.3 μg/ml hPD-L1 mFc fusion protein were added to hPD-1 hFc fusion protein coated plates. Bound PD-L1 was detected by anti-mouse IgG; d, serial dilution of antibodies with 0.02 μg/ml human VEGFR2-mFc-biotin were incubated together onto human VEGF coated plates. Bound VEGFR2 was detected by HRP-labeled Streptavidin.

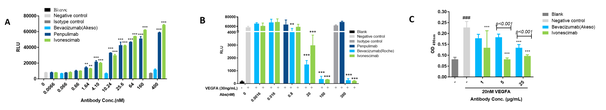


Fig 2. **Ivonescimab blocks PD-1/PD-L1 or VEGF/VEGFR signaling and inhibits VEGF-induced HUVECs proliferation in a concentration-dependent manner.** (A) Ivonescimab blocks PD-1/PD-L1 signaling. Luminescence signals in the co-culture of PD-L1 aAPC/CHO-K1 cells and PD-1 effector cells (NFAT-RE-Luc) were measured using the Steady-Glo Luciferase assay (Promega). RLU, relative light units. (B) Ivonescimab blocks VEGF/VEGFR signaling in VEGF-mediated reporter assay. Luminescence signals in the 293T-KDR-NFAT-Luc cells (Promega) treated with VEGF alone or VEGF with different concentration of antibodies as indicated were assessed by Steady-Glo Luciferase assay. (C) Ivonescimab inhibits VEGF-mediated HUVECs proliferation. HUVEC cells were cultured in the presence of 20 nM VEGF and various conc of antibodies for 3 days. At the end of 3 days, proliferation was determined by the addition of MTT to the assay plates and OD measurements at 490 nm.

Through cooperative binding, VEGF binding to ivonescimab enhances affinity to PD-1, and PD-1 binding enhances affinity to VEGF

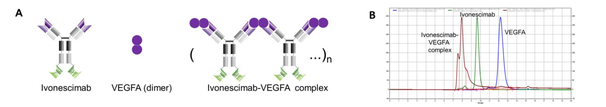
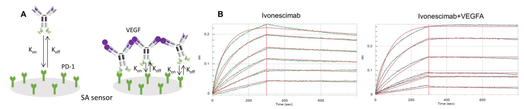
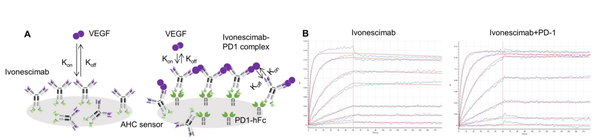


Fig 3. **Ivonescimab forms soluble complexes with VEGF.** (A) Diagram representing ivonescimab, VEGF and proposed ivonescimab-VEGF complex structure. (B) Ivonescimab-VEGFA complex formation determined by SEC-HPLC. Ivonescimab were premixed with 2x VEGFA and then analyzed on SEC-HPLC (Red color). Ivonescimab alone (Green color) and VEGFA alone (Blue color) were also analyzed on SEC-HPLC as references. The results were merged.



Fixed antigen	Antibody	VEGFA-his (nM)	K_D (M)	k_{on} (1/ms)	k_{dis} (1/s)
PD1-his, 200 nM	Ivonescimab	0	7.15E-10	2.94E+05	2.10E-04
	Ivonescimab + VEGF	50-1.56	3.83E-11	2.51E+05	9.62E-05

Fig 4. **VEGF promotes cooperative binding of ivonescimab to human PD-1.** (A) Diagram representing the binding profile of ivonescimab to PD-1 in the presence/absence of VEGF. (B) Ivonescimab (50 nM) alone (left) or pre-incubated with human VEGF-His at same conc (right) and then diluted from 50 nM to 1.56 nM. The binding kinetics of ivonescimab alone or ivonescimab-VEGF to immobilized PD-1-His-biotin were determined by Octet BLI. The binding kinetic results show >18x increase in K_D, mainly driven by the slower dissociation rate (K_{dis}).



Fixed antigen	Analyte	K_D (M)	k_{on} (1/ms)	k_{dis} (1/s)
Ivonescimab	VEGFA-his[b]	1.96E-09	2.04E+05	4.01E-04
Ivonescimab+ PD1-hFc[a]		4.11E-10	1.60E+05	6.58E-05

Fig 5. **PD-1 enhances binding avidity of ivonescimab to human VEGF.** (A) Diagram representing the binding profile of ivonescimab to VEGF with or without PD-1. (B) Ivonescimab (7 nM) alone (left) or mixture of ivonescimab (7 nM) with PD-1-human Fc (PD-1-hFc, 7 nM) (right) were immobilized on the AHC sensor. The binding kinetics of serial dilution of human VEGF-his protein (1000 to 1.37 nM) to immobilized ivonescimab or ivonescimab-PD-1-hFc were determined by Octet BLI. The binding kinetic results show a >4x increase of affinity to VEGF in the presence of PD-1. a, ivonescimab was pre-incubated with PD1-hFc at same concentration (7 nM); b, VEGFA-his with three-fold serial dilution from 1000 nM to 1.37 nM.

Cooperative binding of VEGF enhances PD-1 blockade in cells

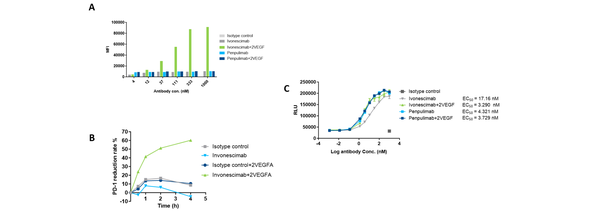


Fig 6. **Enhanced binding of ivonescimab-VEGF soluble complexes to PD-1-expressing cells results in the reduction of cell surface PD-1 protein level and better potency on blockade of PD-1/PD-L1 signaling.** (A) Binding of ivonescimab and anti-PD-1, penpulimab, +/-VEGF on PD-1 transfected Jurkat cells via FACS. Secondary antibody is mouse anti-hIgG Fc-Alexa Fluor 647. MFI, mean fluorescent intensity. (B) Cell surface PD-1 level on PD-1-expressing Jurkat cells, detected by FACS at different time points after ivonescimab treatment +/- VEGF. The reduction rates % were calculated from the decrease of surface PD-1 compared to its expression at 0 h. (C) Ivonescimab and penpulimab +/- VEGF blocked the interaction of PD-1 and PD-L1, leading to enhancement of luminescence in the co-culture of PD-L1 aAPC/CHO-K1 cells and PD-1 effector cells.

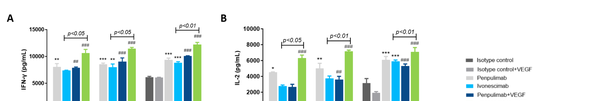


Fig 7. **Ivonescimab-VEGF complexes enhance T cell activation.** (A) IFN-γ and (B) IL-2 production in co-culture of hPBMCs and Raji-PD-L1 cells were analyzed by ELISA. Compared with the isotype control, *p<0.05, **p<0.01, ***p<0.001; compared with the isotype control+VEGF, ##p<0.01, ###p<0.001.

Ivonescimab monotherapy demonstrates anti-tumor response in mEGFR (ΔE746-A750) – NSCLC model

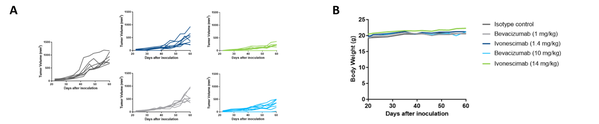


Fig 8. **Ivonescimab demonstrates anti-tumor response in treatment in mice model of mEGFR-NSCLC.** **(A)** Individual tumor growth curves of each treatment cohort and **(B)** the mean body weight curves of treatment cohorts in SCID/Beige mice with subcutaneous HCC827-hPBMC tumor. Mice were randomly grouped into 5 cohorts. Each mouse was inoculated SC at right hind flank with HCC827 and hPBMCs mixture. In this model, freshly isolated human PBMC were mixed with HCC827 cells to provide human immune cells. Treatments were via SC injection on day 0 and IV injection on days 7, 14, 21, 28, 35.

RESULTS

Fc region of ivonescimab is designed to reduce interactions with FcγR that may potentiate AEs/irAEs

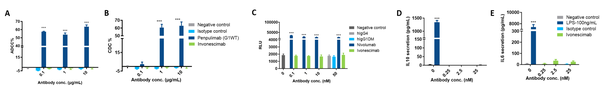


Fig 9. **Ivonescimab manifests reduced ADCC, CDC, ADCP and ADCR activities** (A) ADCC of ivonescimab and penpulimab (G1WT) (an anti-PD-1 antibody with wildtype IgG1 backbone) were determined by measuring lactase dehydrogenase (LDH) release from CHO-K1-PD1 cells. (B) CDC of ivonescimab, nivolumab and penpulimab (G1WT) were determined by measuring LDH release from CHO-K1-PD1 cells. (C) ADCP activities of ivonescimab and nivolumab were measured by reporter assay. Jurkat-NFAT-CD64-CD32H cells and CHO-K1-PD1 cells were cocultured for 5 hrs in the presence of ivonescimab or nivolumab. Effects of Fc engineering of ivonescimab on the release of inflammatory cytokines. Data are expressed as mean ± SEM (N=2-3) and analyzed using one-way ANOVA. Compared with the isotype control, ***p<0.001. (D) IL-10 and (E) IL-6 secretion by human peripheral monocyte-derived macrophages (HPMMs) in the presence of IFN-γ. Data are expressed as mean ± SEM (N=2-3) and analyzed using one-way ANOVA. Compared with the negative control, ***p<0.001.

Table 2. The incidence of immune-related adverse effects (irAEs) of ivonescimab in clinical trials

irAE	Ivonescimab[a] (%) N=282
Immune-mediated pneumonia	2.4
Immune-mediated colitis	0.6
Immune-associated hepatotoxicity	0.7
Hyperthyroidism	2.5
Hypothyroidism	6.7
Immune-mediated dermal toxicity	5.3

a: Data from AK112-201, AK112-202; Cut off 2022 Oct

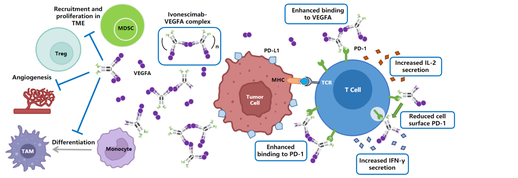
Table 3. Incidence of hemorrhages, hypertension, proteinuria and gastrointestinal perforation in clinical trials

Adverse events (Grade ≥3)	Ivonescimab (%) N=497
Hemorrhages	0.4
Hypertension	4
Proteinuria	0.6
Gastrointestinal perforation	0

Note: The safety data of ivonescimab was obtained from 6 clinical trials (AK112-101, AK112-102, AK112-103, AK112-201, AK112-202 and AK112-203).

CONCLUSION

Ivonescimab is a novel tetravalent anti-PD-1/VEGF bispecific antibody displaying unique cooperative binding to each of its intended targets consistent with increased in vitro functional bioactivities compared with bevacizumab or PD-1 inhibitors alone. Importantly, the Fc-null IgG1 design resulted in reduced FcγR interactions and minimal ADCC, ADCP activities, together with its half-life of 6-7 days, is consistent with its clinical immunosafety profile.[7,8,12] Ivonescimab is an investigational therapy that is not approved by any regulatory authority and is currently in Ph3 NSCLC trials in the US and EU (refer to TiP EORTC 2023 C#030, and trial NCT05899608).



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REFERENCES

1. Socinski M, et al. *N Engl J Med.* 2018;378(24):2288-2301
2. Carmeliet P. *Oncology* 2005;(Suppl 3):4-10
3. Ohm J., Carbone D. *Immunol Res* 2001;23(2-3):263-72
4. Maneggold C. et al. *J Thorac Oncol* 2017;12(2):194-207
5. Pardoll, D. *Nat Rev Cancer* 2012;12(4):252-64
6. Tamura R, et al. *Med Oncol* 2020;37(1):2
7. Zhao Y. et al. eClinicalMedicine 2023;62:102106
8. Zhou C. et al. *J Clin Oncol* 2022; 40(16_suppl):poster;9040
9. Zhong T. et al. *JITC* 2022;10(2):521
10. Data on File. [14, 15] Summit Therapeutics, Inc.
11. Huang Z. et al. *Front Immunol* 2022;13
12. Zhang L. et al. ASCO 2023 poster #9087
13. Benesova K. et al. *Ann Rheum Dis* 2022;81(12):1730-41
14. Martins F. et al. *Nat Rev Clin Oncol* 2019;16:563-580

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