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

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INTRODUCTION

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Combination treatments using anti-PD-1/PD-L1 antibodies with other VEGF antagonists have shown enhanced clinical antitumor 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.

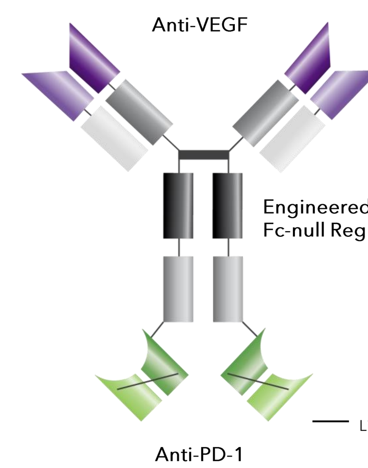
METHODS

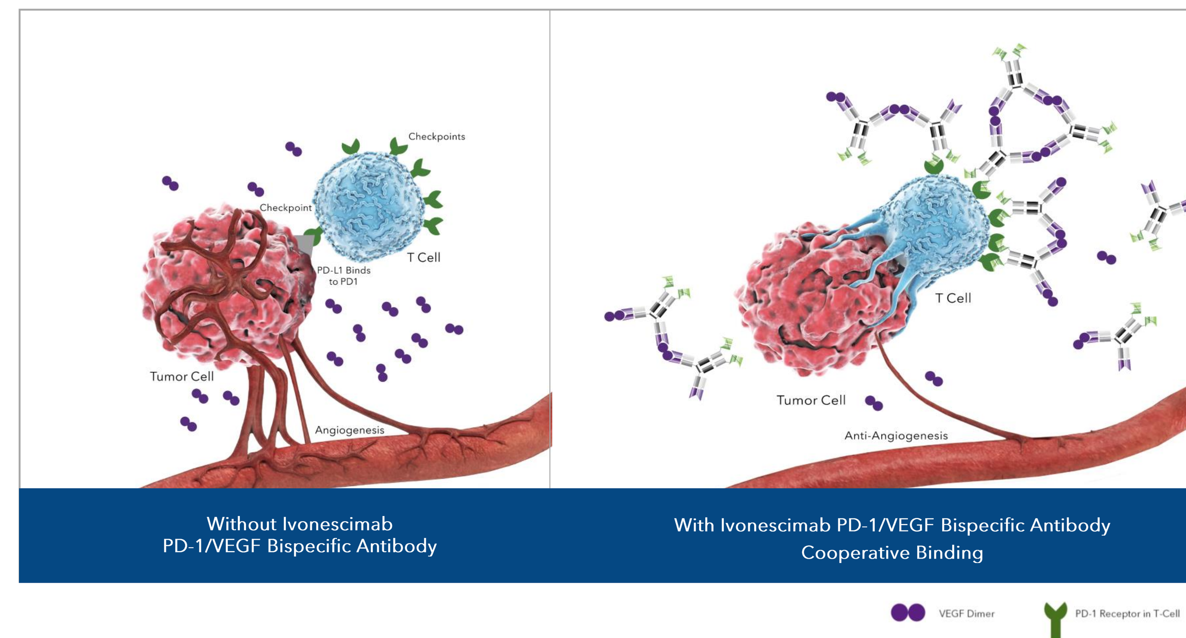
Binding activity of ivonescimab to PD-1/VEGF was assessed by ELISA. Blockade of PD-1/VEGF signaling pathways were determined in luciferase reporter cell assays. Ivonescimab-VEGF complex formation was detected by SEC-HPLC. Cooperative binding of ivonescimab-VEGF complex to PD-1 or ivonescimab-PD-1 complex 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). Moreover, antitumor activity of ivonescimab combination with anti-CD73 (AK119) and anti-CD47 (AK117) were investigated in MC38-hPD-L1/hCD73 and MDA-MB-231 cells xenograft models, respectively. 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. Additionally, ivonescimab enhanced anti-tumor response in combination with anti-CD47 (AK117) or anti-CD73 (AK119) in mouse models. 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.[13,14]

MECHANISM OF ACTION





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[4,7,10]
- Inhibiting VEGF can help improve the effect of immunotherapy by modulating the tumor microenvironment[7]
- Enhancing the 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]

RESULTS

VEGF-A and PD-1 are Highly Co-expressed in Various Human Tumors

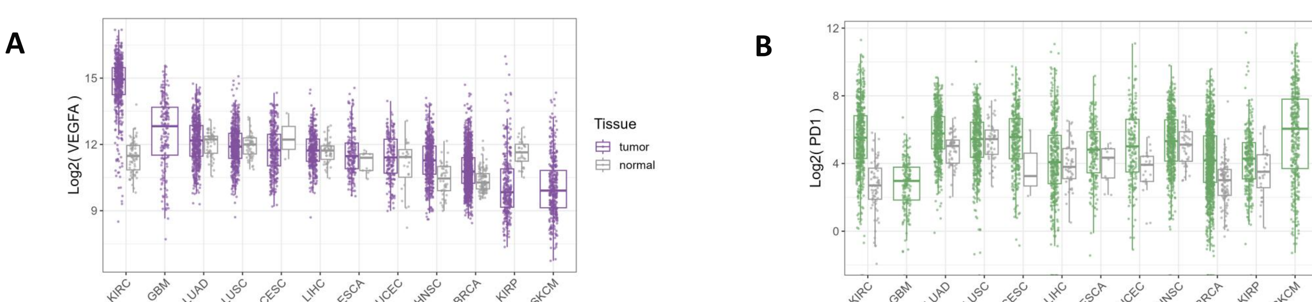


Fig 1. VEGF-A and PD-1 are highly co-expressed in various human tumors.
VEGF **(A)** and PD-1 **(B)** mRNA expression data originated from The Cancer Genome Atlas (TCGA).
KIRC=Kidney renal clear cell carcinoma, GBM=Glioblastoma multiforme, LUAD=Lung adenocarcinoma, LUSC=Lung squamous cell carcinoma, CESC=Cervical squamous cell carcinoma and endocervical adenocarcinoma, LIHC=Liver hepatocellular carcinoma, ESCA=Esophageal carcinoma, UCEC=Uterine Corpus Endometrial Carcinoma, HNSC=Head and Neck squamous cell carcinoma, BRCA=Breast invasive carcinoma, KIRP=Kidney renal papillary cell carcinoma, SKCM=Skin Cutaneous Melanoma

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.

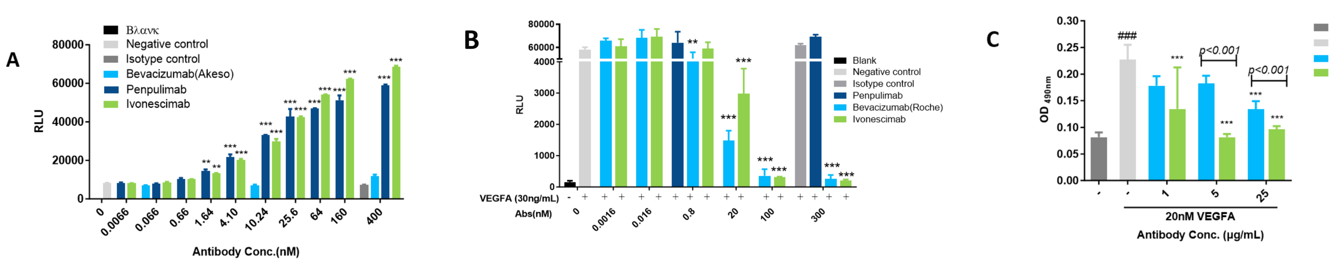


Fig 2. Ivonescimab blocks PD-1/PD-L1 or VEGF/VEGFR signaling and inhibits VEGF-induced HUVECs proliferation in a concentration-dependent manner.
(A) Ivonescimab blocks PD-1/PD-L1 signaling. Luminescence signals in the co-culture of PD-L1 aAPC/CHO-K1 cells and PD-1 effector cells (NFAT-RE-Luc) were measured 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

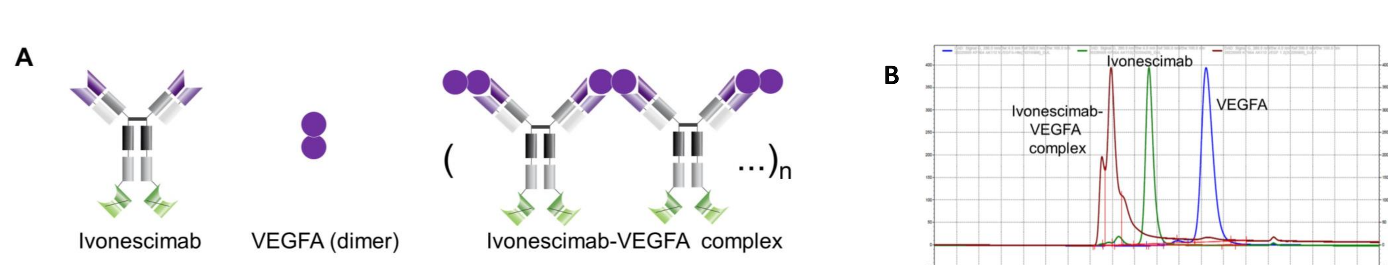
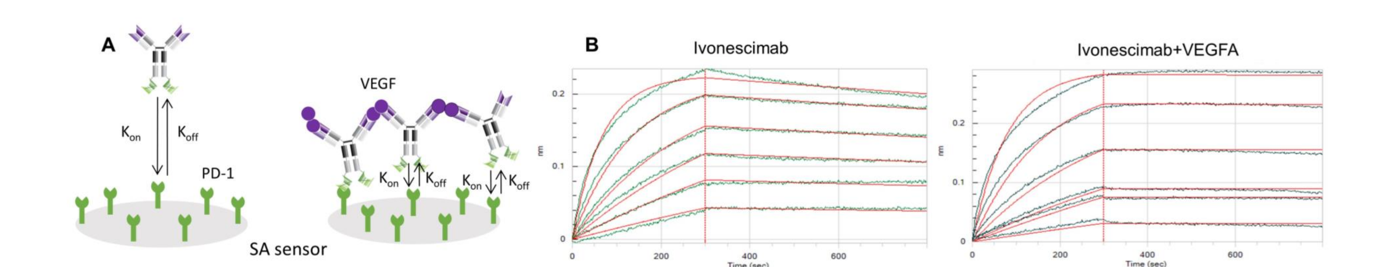
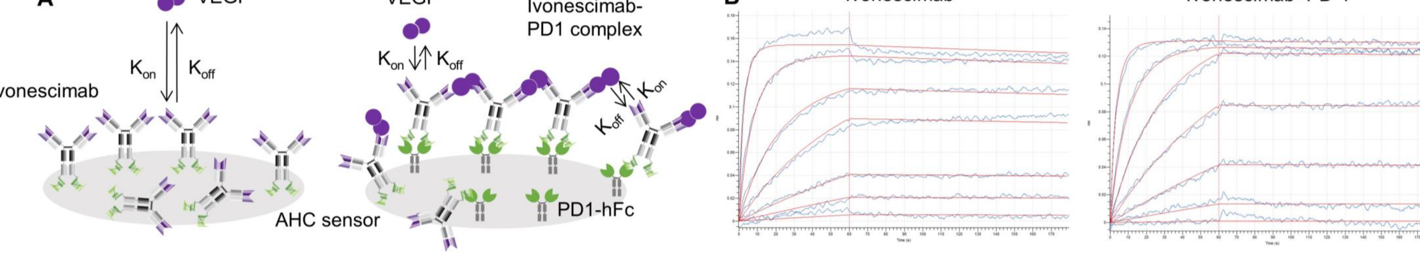


Fig 3. Ivonescimab forms soluble complexes with VEGF.
(A) Diagram representing ivonescimab, VEGF and proposed ivonescimab-VEGF complex structure. **(B)** Ivonescimab-VEGFA complex formation determined by SEC-HPLC. Ivonescimab were premixed with 2x VEGFA and then analyzed on SEC-HPLC (Red color). Ivonescimab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

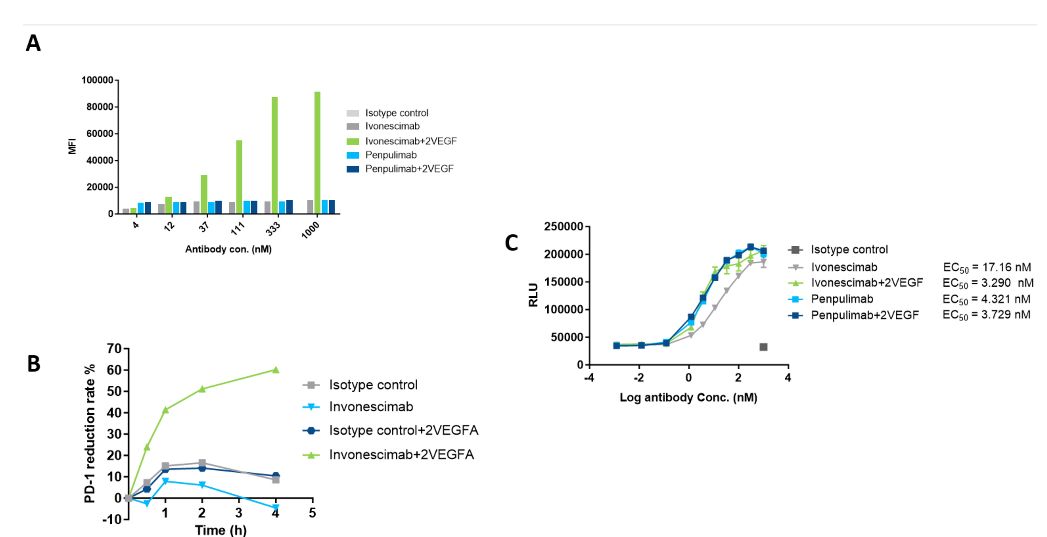


Fig 6. Enhanced binding of ivonescimab-VEGF soluble complexes to PD-1-expressing cells results in the reduction of cell surface PD-1 protein level and better potency on blockade of PD-1/PD-L1 signaling.
(A) Binding of ivonescimab and anti-PD-1, penpulimab, +/-VEGF on PD-1 transfected Jurkat cells via 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.

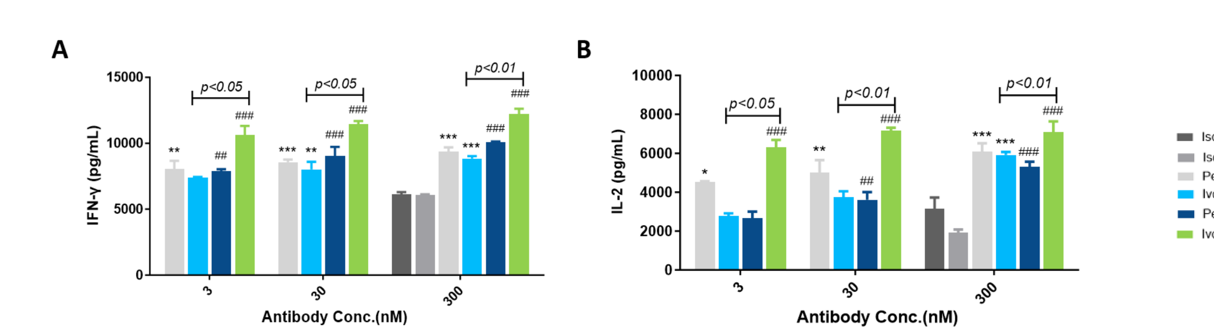


Fig 7. Ivonescimab-VEGF complexes enhance T cell activation.
(A) IFN-γ and **(B)** IL-2 production in co-culture of hPBMCs and Raji-PD-L1 cells were analyzed by ELISA. Compared with the isotype control, *p<0.05, **p<0.01, ***p<0.001; compared with the isotype control+VEGF, ##p<0.01, ###p<0.001.

Ivonescimab Combination Therapy Demonstrates Anti-tumor Response in Mice

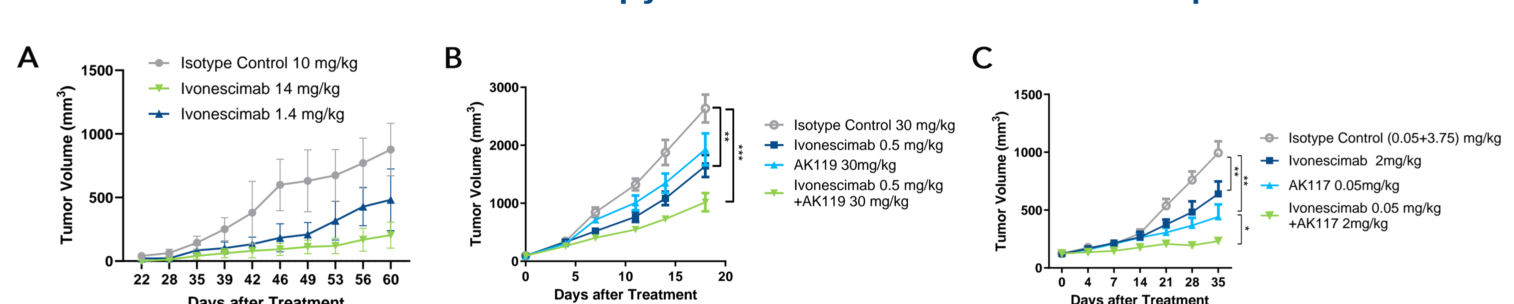


Fig 8. Ivonescimab demonstrates anti-tumor response in both single/combination treatment in mice.
(A) The tumor growth curves of different groups in mice with subcutaneous at the right hind flank with HCC827 cells. PBMCs and ivonescimab or isotype control anti-HEL mixture were administered on day 0. Different doses of antibodies were then continuously intravenously injected on day 7, 14, 21, 28, 35. **(B)** The tumor growth curves of different groups in B6-hPD1/hPD-L1/hCD73 mice with subcutaneous MC38-hPDL1/hCD73 tumor. MC38-hPDL1/hCD73 cell (a mouse colon cancer cell line expressing human PD-L1 and human CD73) suspension was inoculated subcutaneously to the right forelimb of mouse. On the 7th day after inoculation, mice were randomly grouped into 4 groups, and each mice was intraperitoneally injected with antibody on D0, D3, D7, D10, D14, D17, respectively. **(C)** The tumor growth curves of different groups in SCID/Beige mice with subcutaneous MDA-MB-231 tumor. Mice were inoculated subcutaneously at the right hind flank with MDA-MB-231 cells. Mice were randomly grouped into 4 groups, and intraperitoneally injected with activated PBMC. On the day of grouping, AK117 (anti-CD47 mAb developed by Akesobio) was intraperitoneally injected twice a week for four weeks, and ivonescimab was intraperitoneally injected once a week for four weeks. The tumor diameter and weight of the animals were monitored. Data are expressed as mean ± SEM and analyzed using one-way ANOVA. Compared with the isotype control, *p<0.05, **p<0.01, ***p<0.001.

Fc Region of Ivonescimab is Designed to Reduce Interactions with FcγR That May Potentiate AEs/irAEs

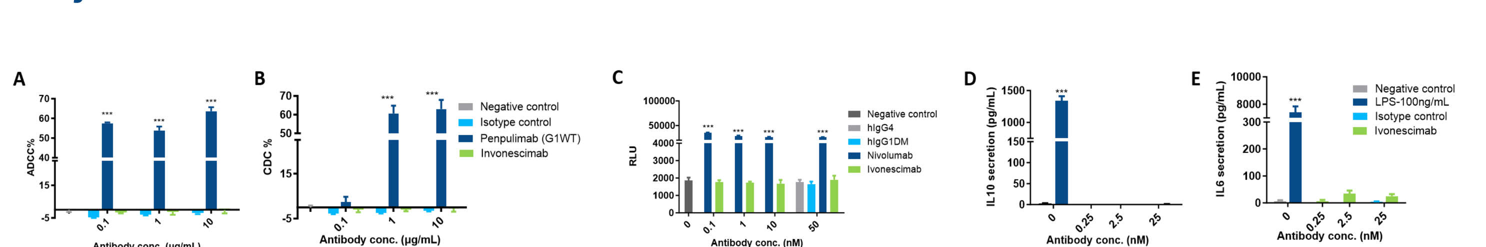


Fig 9. Ivonescimab manifests reduced ADCC, CDC, ADCP and ADCR activities
(A) ADCC of ivonescimab and penpulimab (G1WT) (an anti-PD-1 antibody with wildtype IgG1 backbone) were determined by measuring lactase dehydrogenase (LDH) release from CHO-K1-PD1 cells. **(B)** CDC of ivonescimab, nivolumab and penpulimab (G1WT) were determined by measuring LDH release from CHO-K1-PD1 cells. **(C)** ADCP activities of ivonescimab and nivolumab were measure 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 presented 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

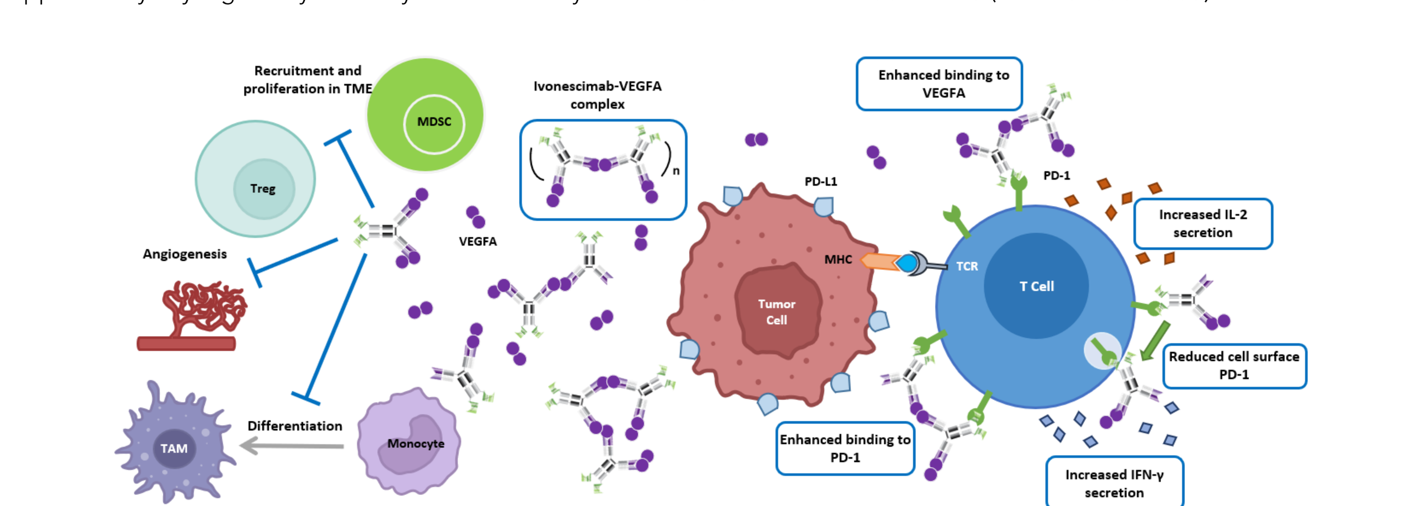
Table 3. Incidence of hemorrhages, hypertension, proteinuria and gastrointestinal perforation of ivonescimab in clinical trial

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).

CONCLUSIONS

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. 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 (trial NCT05899608).



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