CT111, a PD-1 x CTLA-4 x VEGF trispecific antibody, demonstrates potent anti-tumor efficacy through synergistic immune checkpoint blockade and VEGF inhibition with cooperative avidity

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Abstract #976

Introduction

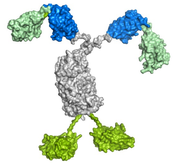
Background: PD-1, CTLA-4, and VEGF are clinically validated targets with complementary roles in suppressing anti-tumor immunity[1]. PD-(L)1/VEGF and PD-(L)1/CTLA-4 bispecific antibodies show promising clinical benefits for certain solid tumors, and several on-going studies explore a tri-targeting multi-drug combination approach. Given the avidity and co-operative binding advantages for multispecific antibodies, combining anti-PD-1 (restore effector T cell activity), anti-CTLA-4 (T cell priming/proliferation), and anti-VEGF (inhibit tumor angiogenesis) may provide better biological synergy and clinical benefits. In this study, CT111, our lead PD-1 x CTLA-4 x VEGF trispecific antibody, was characterized to gain mechanistic insights into its functional attributes in synergistically blocking PD-1/CTLA-4-mediated immunosuppression and VEGF-induced tumor angiogenesis.

CT111 is a differentiated PD-1 x CTLA-4 x VEGF trispecific antibody

CT111 is engineered with balanced potency across three targets to achieve effective engagement and deliver efficacy and safety at a clinically practical dose

Relative potency

VEGF > PD-1 ≈ CTLA-4 (Fc-silenced)



Potent VEGF blockade that amplifies PD-1/CTLA-4 engagement in the presence of VEGF

- VEGF VHH potency is ~4-5x higher than bevacizumab, enabling tri-targeting at clinically relevant exposures at a single dose level*
- Cooperative VEGF binding significantly increases avidity to PD-1 and CTLA-4, translating to enhanced ligand blockade and dual checkpoint activity
- CT111 binds VEGF homodimer without higher-order multimerization, which may mitigate aggregation in compartments with high local VEGF concentration[2,3]

Balanced, high-affinity PD-1 and CTLA-4 arms to support dual checkpoint synergy and tolerate target heterogeneity

- Coverage of single-positive or imbalanced expression across key TIL subsets (e.g. CD8+ TILs, Treg, T_{RM}, T_{conv})
- Maximize effect in heterogeneous patient TIL pools by engaging PD-1-dominant effector T cells and CTLA-4-dominant Tregs
- Intercept transient, rapidly recycled CTLA-4 via high-affinity binding and sustained occupancy

Fc-silenced IgG1 to deliver checkpoint blockade with reduced toxicity

- Fc-mutations to significantly reduce ADCC/ADCP/CDC
- Lower risk of Fc-mediated irAEs (e.g. colitis, dermatitis, hypophysitis), especially for CTLA-4[4,5]
- Target CTLA-4 on Tregs without depletion, preserving peripheral tolerance

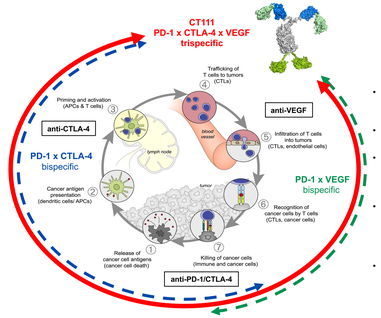
Size for manufacturability and tumor penetration

- All-VHH trispecific homodimer (138 kDa) is significantly smaller and simpler than competitor multispecifics
- Translates to improved manufacturability/developability
- Smaller size may enhance tumor penetration; combined with potent anti-VEGF vascular normalization, CT111 can diffuse more effectively in the TME

* Clinical dosing of bevacizumab is 5-15 mg/kg Q2-3W versus pembrolizumab ~2-3 mg/kg (200 mg Q3W)

CT111 to effectively re-invigorate the cancer immunity cycle

CT111 engages multiple nodes of the cancer-immunity cycle across major T-cell subsets in different compartments



PD-1 x CTLA-4

- Targets two distinct and complimentary T cell pathways to promote T cell activation and enhance effector response
- Re-invigorates exhausted TILs
- Enhances T cell priming to mount effective and long-term immune response

PD-1 x VEGF

- Adds anti-angiogenic therapy with PD-1 checkpoint inhibition
- Re-invigorates PD-1+ exhausted TILs
- Normalizes tumor vasculature and promote immune cell infiltration
- Counters VEGF-mediated immunosuppression of immune cells (e.g. DCs, MDSCs)
- VEGF-driven avidity to enhance anti-PD-1 effects

CT111 combines PD-1/CTLA-4 checkpoint blockade with VEGF inhibition for dual-target synergy via VEGF-driven avidity

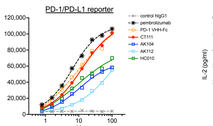
Cancer-immunity cycle by Mellman et al., 2013[1]

CT111 demonstrates potent PD-1, CTLA-4, and VEGF binding and functional activities

- CT111 showed high-affinity binding to human and cynomolgus PD-1, CTLA-4, and VEGF, comparable to clinical benchmark and parental VHH Abs.
- CT111 effectively blocked PD-1/PD-L1 and CTLA-4/CD80 signaling with potencies matching or exceeding clinical competitors in cell-based assays.
- VEGF VHH inhibited VEGF/VEGFR2 signaling ~4-5x more potently than bevacizumab and its multispecific formats (AK112, HC010).

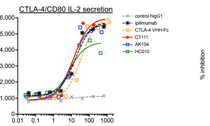
A

Ab	human PD-1 EC50 (nM)	cyno PD-1 EC50 (nM)
pembrolizumab	0.59	1.1
parental PD-1 VHH-Fc	1.2	3.0
CT111	3.2	2.9
AK104	1.8	3.9
AK112	7.3	13



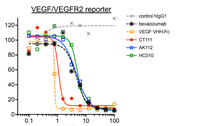
B

Ab	human CTLA-4 EC50 (nM)	cyno CTLA-4 EC50 (nM)
ipilimumab	1.9	0.73
parental CTLA-4 VHH-Fc	0.48	0.32
CT111	1.6	1.8
AK104	44	27



C

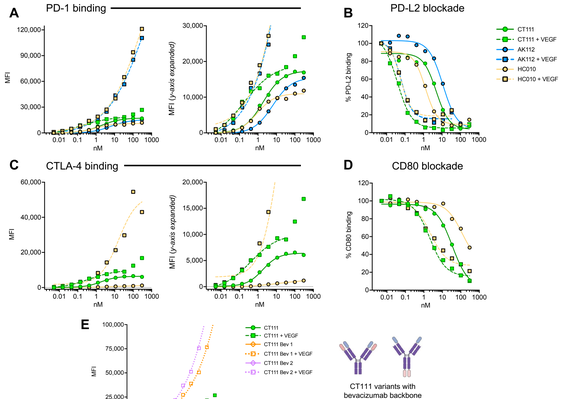
Ab	human VEGF 165	
	KD (M)	kon (1/Ms)	koff (1/s)
bevacizumab	1.17E-12	4.65E+04	4.10E-07
AK112	1.90E-12	3.10E+04	1.15E-07
affibercept	6.20E-11	9.95E+04	5.73E-05
parental VEGF VHH-Fc	2.41E-10	1.76E+05	4.24E-05
CT111	2.23E-10	2.80E+05	6.23E-05



Target binding and functional activities of CT111 versus benchmark Abs. (A) PD-1 binding affinities on CHO cells expressing human or cynomolgus PD-1. Lower panel: anti-PD-1 activity measured as reversal of PD-L1/PD-1-mediated suppression of TCR/NFAT reporter signaling in effector reporter cells. (B) CTLA-4 binding affinities on CHO cells expressing human or cynomolgus CTLA-4. Lower panel: anti-CTLA-4 activity determined by measuring IL-2 secretion in CD80/CD28 co-culture assay with CTLA-4-Fc protein. (C) VEGF kinetics by BLI using the 1:1 fitting model. Lower panel: VEGF-blocking activity measured as inhibition of VEGF/VEGFR2 signaling in NFAT-luciferase reporter cells.

VEGF-driven avidity increases CT111 PD-1 / CTLA-4 engagement and PD-L2 / CD80 blockade

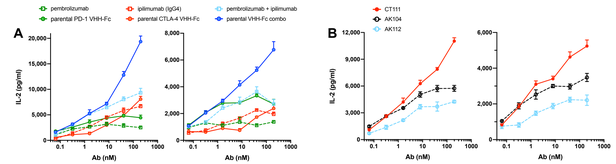
- CT111 complexes with VEGF homodimer and demonstrates strong avidity to PD-1 and CTLA-4 on cells with 12- and 5-fold increased affinity, respectively.
- Avidity translates to enhanced PD-L2 and CD80 inhibition by 129- and 16-fold, respectively.
- The VEGF-binding VHH mediates this avidity, analogous to bevacizumab-based multispecifics, without extensive formation of higher-order Ab/VEGF multimers reported for bevacizumab and related multispecific formats.
- Bevacizumab-based CT111 variants with the same PD-1 and CTLA-4 VHHs showed similarly high, non-saturating cell-surface loading on PD-1 cells.
- CT111 may support VEGF-driven avidity without higher-order aggregation to amplify PD-1/CTLA-4 engagement in VEGF-high TME.



Effect of VEGF pre-complexation on multispecific Ab binding and ligand blockade. (A and C) Binding of CT111 and bevacizumab-based multispecifics with or without pre-complexed VEGF to CHO-PD-1 (A) and CHO-CTLA-4 cells (C). Bevacizumab-based Abs (AK112, HC010) showed much higher, non-saturating MFI binding than CT111 (*left plots*), which exhibited markedly lower cell binding capacity (*right plots with same data; y-axis magnified*). The two highest non-saturating points after initial plateau were excluded from CT111 curve fitting. Pre-complexation with VEGF left-shifts the binding curves for all three Abs, indicating increased apparent affinity (lower EC_{50}). (B and D) Corresponding PD-L2 (B) and CD80 (D) blocking activities on CHO-PD-1 and CHO-CTLA-4 cells, respectively. Note: PD-L1 blocking activity for CT111 is also enhanced in the presence of VEGF *(data not shown)*. (E) Comparison of CT111 versus bevacizumab-based variants, CT111 Bev 1 and CT111 Bev 2 (different optimized designs illustrated). Similar non-saturating, high MFI cell-surface binding on CHO-PD-1 cells were observed at higher Ab/VEGF concentrations in contrast to CT111.

CT111 demonstrates superior potency over AK104 and AK112 in primary immune assays

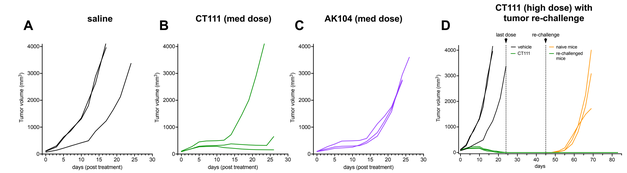
- CT111's component PD-1 and CTLA-4 VHHs, when combined, showed greater synergistic IL-2 secretion than pembrolizumab + ipilimumab combination in SEB-stimulated human PBMCs across donors.
- CT111 induced stronger IL-2 secretion than AK104 or AK112, demonstrating superior dual-checkpoint activity.



SEB-induced IL-2 production in human PBMCs. Human PBMCs from healthy donors were stimulated with SEB for 5 days in the presence of: (A) mono-targeting PD-1 and CTLA-4 Abs as single agents or as combination (CT111 parental VHHs vs pembrolizumab and ipilimumab IgG4 surrogate), or (B) multispecific Abs (CT111 vs clinical-grade AK104 and AK112). IL-2 in culture supernatants was measured as a functional readout for anti-PD-1/CTLA-4 synergy. Two representative donors, Donor 1 (*left*) and Donor 2 (*right*), are shown for panels (A) and (B).

CT111 outperforms AK104 and drives complete regression with durable memory

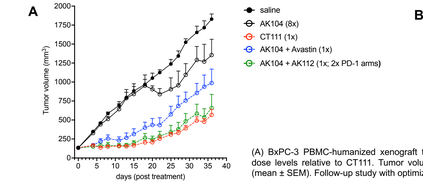
- CT111 anti-PD-1/CTLA-4 activity was evaluated in a syngeneic model validated for dual-checkpoint activity (VEGF arm is non-functional in this model).
- In this pilot dose-finding study, CT111 showed dose-response activity (medium and high shown) with potent tumor regression. Superior activity in vivo was observed compared to AK104 at equivalent medium dose.
- CT111-A at high dose achieved complete responses in 3/3 mice for up to 3 weeks and complete rejection upon tumor re-challenge without treatment (Day 83). No significant body weight loss was observed for all groups, indicating good tolerability at tested doses (data not shown).



Individual tumor growth curves showing anti-PD-1/CTLA-4 activity in a syngeneic mouse model. Transgenic BALB/c-hPD-1/hPD-L1/hCTLA-4 mice bearing CT26-PD-L1 tumors were treated twice weekly (BIW, i.p., n=3/group) with (A) saline, (B) CT111 at medium dose, or (C) AK104 (molar equivalent dose). (D) High-dose CT111 induced complete responses in 3/3 mice by Day 19-24 (final dose). After 3 weeks of no visible tumor re-growth, mice were re-challenged on the contralateral flank. Naive transgenic mice served as tumor-growth controls.

CT111 demonstrates strong antitumor activity versus clinical bispecific combinations in the PBMC-humanized BxPC-3 model

- CT111 was compared with tri-targeting clinical combinations, AK104+bevacizumab and AK104+AK112, in a pilot dose-finding study in the BxPC-3 PBMC-humanized xenograft (pancreatic adenocarcinoma; VEGF arm is functional).
- CT111 showed strong anti-tumor activity, comparable to AK104+AK112 at 1x molar dose for CTLA-4 and VEGF and 2x for the PD-1 arms of AK104 and AK112, and outperformed AK104+bevacizumab at arm-matched molar dosing.
- As a single tri-targeting agent, CT111 showed equivalent or better anti-tumor activity than the clinical bispecific combinations. A follow-up study is in progress.



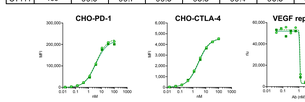
(A) BxPC-3 PBMC-humanized xenograft tumor growth curves. "x" denotes molar-equivalent dose levels relative to CT111. Tumor volumes are mean ± SEM. (B) Day 36 tumor weights (mean ± SEM). Follow-up study with optimized dose levels is in progress.

CT111 shows scalable high titer, yielding pure, stable, developable product

- CT111 showed excellent manufacturability with high expression titers (~3g/L) and high purity recovery.
- Formulation was optimized, and final material exhibited no apparent production liabilities.

	MW (kDa)	DNA plasmid	Titer 1L scale (g/L)	1st step purification ProA yield (mg)	1st step purification SEC purity (%)	Final yield (mg)	Final SEC purity (%)	nrCE-SDS (%)	HIC-HPLC (%)	HCP (ppm)	BVP	Tm (VHH) (°C)	T agg (°C)
CT111	138	1	3.09	2830	96.26	1670	99.64	98.15	96.4	30	2.55	64.2	63.7

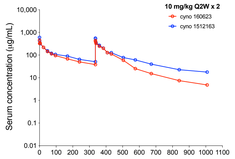
	% monomer integrity (aSEC)							Freeze/thaw stability	
	Accelerated thermostability testing								
	4°C		40°C			50°C		initial	3 cycles
	t=0	3w	1w	2w	3w	4w	2w	4w		
CT111	100	99.6	99.7	99.3	98.8	99.4	96.5	95.9	100	99.6



- CT111 showed no degradation or aggregation after 4 weeks at 40°C or 50°C with intact PD-1 and CTLA-4 binding on CHO cells and VEGF-reporter activity after 3-weeks at 40°C.
- CT111 was stable after three freeze-thaw cycles.

CT111 shows favorable PK profiles in cynomolgus monkeys

- CT111 exhibited IgG-like PK profiles at 10 mg/kg on repeat dosing in cynomolgus monkeys, with terminal half-lives ~8 days. Cmax were comparable across both doses indicating no accumulation.
- No ADA was detected in the two animals up to Day 42 (end of study).



Animal ID	Terminal t1/2 (day)	Dose	Cmax (µg/mL)	AUC(0-14d) (day*µg/mL)
160623	8.4	1	449	1283
		2	398	924
1512163	8.6	1	601	1471
		2	505	1133

Serum concentration-time profiles of CT111 in non-ADA positive male cynomolgus monkeys following administration of two IV doses of 10 mg/kg given 14 days apart. Individual PK profiles are shown (n=2 animals) with summarized PK parameters.

Conclusion

- CT111 is a potential best-in-class PD-1 x CTLA-4 x VEGF trispecific with differentiated mechanisms of action and balanced activities at a single optimized dose to effectively restore priming, lift T-cell exhaustion, and normalize the tumor vasculature, while avoiding Fc-mediated toxicity.
- Cumulative data[6] demonstrate key attributes for CT111:
  - Matched high-affinity binding to all three targets with potent ligand blockade (cell-based competition and reporter assays), simultaneous target engagement (BLI[6], VEGF-driven binding and blocking avidity to PD-1/CTLA-4), and translationally concordant cyno cross-reactivity
  - VEGF VHH is ~4-5x more potent than bevacizumab. Engages the VEGF homodimer to increase PD-1 and CTLA-4 binding, with concordant enhancement of PD-L1/PD-L2 and CD80 blockade, without higher-order multimeric aggregation. CT111 is uniquely differentiated from bevacizumab-based multispecifics and is designed for balanced tri-target engagement.
  - Potent, matched PD-1 and CTLA-4 activities with greater dual-checkpoint synergy in the SEB assay and PD-1/CTLA-4 transgenic tumor model compared to AK104. Achieves complete responses and durable tumor immunity on re-challenge, consistent with CTLA-4 role in priming and memory, with potential for longer-term benefit than PD-(L)1 x VEGF bispecifics.
  - Stronger anti-tumor activity than AK104 and AK112 across different models[6]. In the HCC827 model, CT111 increased effector T cell infiltration/activation (CD137, CD103) and decreased intratumoral Tregs compared to AK112[6].
  - IgG-like disposition and adequate systemic exposure in cynomolgus monkeys for IND-enabling toxicology study
  - Excellent manufacturability and developability traits: single-plasmid construct, high expression titer, streamlined purification, and high purity as demonstrated in two 1L transient scale-up productions
  - As drug product, CT111 is highly thermostable in 4-week studies at 40°C and 50°C. No changes were observed after three freeze-thaw cycles.
- CT111 has advanced into CMC and IND-enabling phases.

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