CT111, a novel trispecific PD-1 x CTA-4 x VEGF single-domain antibody, synergistically targets exhausted T cells and promotes cooperative antitumor effects

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Introduction

Background: Dual PD-1 and CTLA-4 targeting immunotherapy shows enhanced therapeutic efficacy and is approved across multiple advanced solid tumor indications. In recent clinical trials, combined treatment of anti-PD-(L)1 with anti-VEGF have also been shown to improve clinical outcomes and overcome treatment resistance to either drugs alone. Bispecific antibodies targeting PD-(L)1/CTLA-4 and PD-(L)1/VEGF synergistically potentiates antitumor activity in preclinical models, and more recently, demonstrated enhanced clinical efficacy and tolerability. We developed a de novo, multi-targeting PD-1 x CTLA-4 x VEGF single domain trispecific antibody, CT111, with enhanced synergistic anti-tumor activity by combining the effects of dual immune checkpoint and VEGF blockade.

CT111 is a Novel VHH-based Trispecific Antibody Against Three Clinically-Validated Targets

MOA design for CT111 is to effectively block and re-invigorate multiple anti-tumor processes within the cancer-immunity cycle as a single efficacious therapeutic.

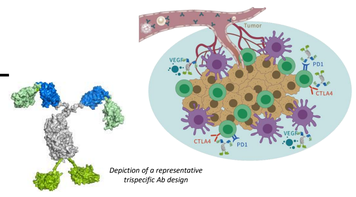
Development of CT111

Ab discovery campaigns VHHMAb® platform

Triage of 52 trispecific designs

- anti-PD-1 VHHs
- anti-CTLA-4 VHHs
- anti-VEGF VHHs

- top functional VHHs selected
- Ab structure
- linker design



Depiction of a representative trispecific Ab design

	CT111
Structure	• Comprises novel humanized PD-1, CTLA-4 and VEGF VHH hits selected for strong functional activities, low immunogenicity, absence of potential sequence liabilities, and optimal biophysical properties • IgG1 Fc backbone with mutations to remove effector functions • Bivalent design for each target • Linker optimized • Homodimer Ab for ease of manufacturing
Traits	• High binding affinities and blocking activities against each target (comparable or superior to clinical BMKs) • Leads selected based on in vitro functional cell-based assays vs BMKs • High expression and optimal developability • Top CT111 leads selected from 52 designs

CT111 is Highly Differentiated with:

1. Unique and More Potent Anti-VEGF Blocker

Anti-VEGF synergizes with PD-(L)1 inhibition to significantly enhance clinical efficacy. To our knowledge, current anti-VEGF bi/trispecifics, such as AK112, HC010, CS2009, and others in clinical development, incorporate bevacizumab's Fab domain. In contrast, our anti-VEGF VHH is unique and functionally more potent. This may translate to better VEGF inhibition as demonstrated in the HCC827 tumor model compared to AK112.

2. CTLA-4 and PD-1 Synergy

Clinical data strongly supports rationale for synergistically combining anti-PD-(L)1 and anti-CTLA-4 especially as a multispecific Ab, such as cadonilimab (AK104) for certain tumor indications. CT111 comprises novel and highly functional anti-PD1 and anti-CTLA-4 VHHs that are equivalent or superior to clinical BMKs as demonstrated in immune cell assays that are sensitive to PD-1 and CTLA-4 inhibition.

3. Smaller Multispecific Antibody Size

CT111 as a VHH-based Ab is significantly smaller than Fab- or scFv-based Abs (VHH being 70% and 40% smaller as antigen-binding units, respectively). In totality as a bivalent trispecific, CT111 (140 kDa) is significantly smaller than AK104/AK112 bispecific (200 kDa) and PD-1xCTLA-4xVEGF trispecifics, HC010 (230 kDa) and CS2009 (200 kDa). This could lead to improved tumor penetration, enhanced by anti-VEGF-mediated vascular normalization, allowing for deeper diffusion and more effective therapeutic efficacy.

CT111 Exhibits Strong Binding Affinity to Human and Cynomolgus IO Targets

A Ligand Binding Kinetics (BLI)

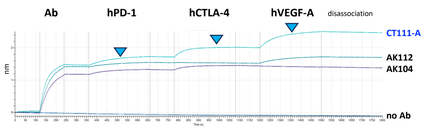
	human PD-1			cyno PD-1		
	KD (M)	ka (1/Ms)	kdis (1/s)	KD (M)	ka (1/Ms)	kdis (1/s)
pembrolizumab	8.7E-09	3.9E+05	3.4E-03	2.8E-09	5.3E+05	1.5E-03
PD-1 VHH-Fc Ab1	5.1E-09	9.6E+04	1.0E-03	7.1E-09	1.1E+05	7.7E-04
PD-1 VHH-Fc Ab2	1.1E-08	1.1E+05	1.2E-03	7.7E-09	1.2E+05	9.6E-04
AK112	4.4E-09	1.1E+05	4.8E-04	6.7E-09	1.1E+05	7.2E-04
CT111-A	3.7E-09	1.3E+05	4.9E-04	3.8E-09	1.3E+05	4.8E-04
CT111-B	2.0E-09	1.2E+05	2.4E-04	2.7E-09	1.2E+05	3.2E-04
CT111-C	5.1E-09	1.1E+05	5.5E-04	4.7E-09	1.1E+05	5.4E-04

	human CTLA-4			cyno CTLA-4		
	KD (M)	ka (1/Ms)	kdis (1/s)	KD (M)	ka (1/Ms)	kdis (1/s)
ipilumimab	2.9E-09	3.1E+05	9.2E-04	2.4E-10	6.0E+05	1.5E-04
CTLA-4 VHH-Fc Ab1	2.5E-09	3.4E+05	8.4E-04	5.9E-10	3.0E+05	1.8E-04
CTLA-4 VHH-Fc Ab1	6.3E-09	3.2E+05	2.0E-03	1.2E-09	3.3E+05	4.1E-04
CT111-A	1.6E-10	3.8E+05	6.0E-05	5.8E-10	5.4E+05	3.1E-04
CT111-B	1.8E-09	3.7E+05	6.6E-04	4.1E-10	5.0E+05	2.0E-04
CT111-C	1.6E-10	3.7E+05	5.8E-05	7.2E-10	5.2E+05	3.7E-04

	human VEGF		
	KD (M)	ka (1/Ms)	kdis (1/s)
bevacuzimab	1.2E-12	4.7E+04	<1.0E-07
aflibercept	6.0E-11	9.6E+05	5.7E-05
VEGF VHH-Fc Ab1	2.4E-10	1.8E+05	4.2E-05
AK112	1.9E-12	3.1E+04	<1.0E-07
CT111-A	2.2E-10	2.8E+05	6.2E-05
CT111-B	9.1E-12	2.5E+05	2.3E-06
CT111-C	6.5E-11	8.4E+05	5.5E-05

CT111 Binds Sequentially and Maintains Binding to All Three Targets

B Sequential Binding (BLI)



- **(A and C) Selected CT111 leads show high-affinity binding to all 3 human and cyno targets comparable to clinical benchmarks, as well as VHH-Fc mAbs from which binders were derived, indicating proper integration within the trispecific structures.**
- **CT111-A binds sequentially to all three targets, indicating that the trispecific antibody can engage all targets simultaneously.**

CT111 Binds to CHO Human and Cyno PD-1 and CTLA-4 Cells

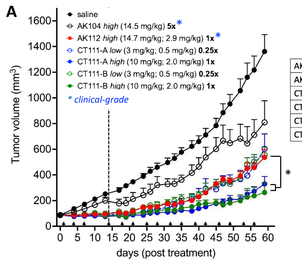
C Cell Binding Affinities

	human PD-1		cyno PD-1	
PD-1 cell binding	EC50 (nM)	Bmax	EC50 (nM)	Bmax
pembrolizumab *	0.6	180,579	1.1	212,012
PD-1 VHH-Fc Ab 1	1.3	243,739	2.6	393,641
PD-1 VHH-Fc Ab 2	1.2	246,831	3.0	389,873
AK104 *	1.8	229,509	3.9	353,689
AK112 *	7.3	208,223	13.0	414,811
CT111-A	3.2	168,022	7.9	406,513
CT111-B	3.8	222,921	3.5	315,738
CT111-C	4.2	224,461	2.8	309,728

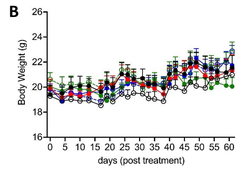
	human CTLA-4		cyno CTLA-4	
CTLA-4 cell binding	EC50 (nM)	Bmax	EC50 (nM)	Bmax
ipilumimab	1.9	6,607	0.7	4,476
CTLA-4 VHH-Fc Ab 1	0.5	6,308	0.3	6,785
CTLA-4 VHH-Fc Ab 2	0.9	5,626	0.7	5,955
AK104 *	44	5,054	27	6,496
CT111-A	1.6	5,686	1.8	5,623
CT111-B	1.9	6,367	1.5	8,001
CT111-C	3.8	7,242	1.9	7,075

** clinical-grade*

CT111 Demonstrates Superior Anti-Tumor Activity Compared to AK104 and AK112 in the HCC827 NSCLC HSC Humanized Tumor Model



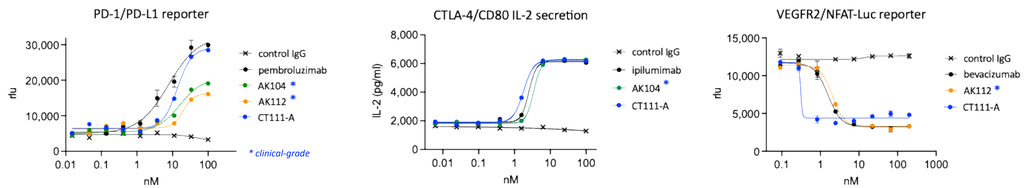
	% TGI (day 59)
AK104 **high, 5x**	43%
AK112 **high, 1x**	65%
CT111-A **low, 0.25x**	59%
CT111-A **high, 1x**	81%
CT111-B **low, 0.25x**	63%
CT111-B **high, 1x**	86%



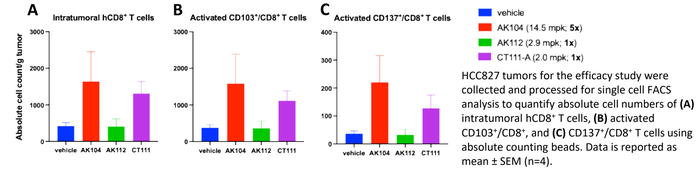
(A and B) Immunodeficient NOG-EXL female HSC-humanized mice were inoculated with 2e6 HCC827 cells (NSCLC) in the right flank. Treatments were initiated when tumors reached 80-100 mm³ and mice were treated at dose level 1 (day 0 to 14; *dotted line*) and decreased to dose level 2 for the remainder of the study (BIW x 8 weeks). **x** represent molar-equivalent dose level 2 relative to CT111 at 2 mpk. **(A)** Tumor volumes with % TGI at day 59, **(B)** Body weights are reported as mean ± SEM (n=6). Statistical analysis was performed using unpaired two-tailed t-test. * = p< 0.05.

- **Both CT111-A and -B at high dose showed superior anti-tumor efficacy compared to AK104 (5x dose) and AK112 (1x dose). CT111 at 1/4th lower dose levels were equivalent to AK112. No significant BWL observed.**

CT111 Effectively Blocks PD-1, CTLA-4, and VEGF Mediated Interactions Compared to Benchmark Antibodies in Cell-Based Assays



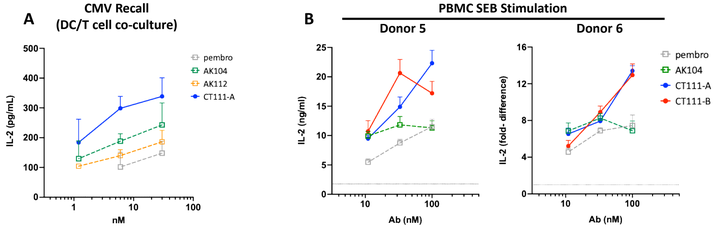
CT111 Mediates Increased CD8⁺ T Cell Infiltration, Activation, and Decreased Intratumoral Treg Accumulation



HCC827 tumors for the efficacy study were collected and processed for single cell FACS analysis to quantify absolute cell numbers of **(A)** intratumoral hCD8⁺ T cells, **(B)** activated CD103⁺/CD8⁺, and **(C)** CD137⁺/CD8⁺ T cells using absolute counting beads. Data is reported as mean ± SEM (n=4).

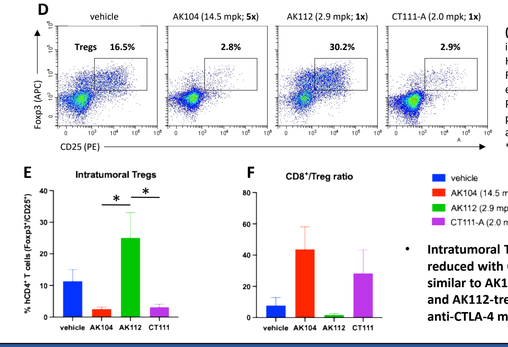
- **CT111-A at 1x dose mediated increased number of activated CD8⁺ TILs compared to vehicle and AK112-treated tumors. Increased CD8+ TIL infiltration was similarly observed with PD-1/CTLA-4 AK104 at 5x dose.**

CT111 Shows Superior Functional Activity in Primary Immune Assays



(A) Autologous mature monocyte-derived dendritic cells and T cells were isolated and generated from CMV⁺ human donors and co-cultured at a DC-to-T cell ratio of 1:10 in the presence of CT111 at different concentrations with CMV pp65 peptide pool for 5 days. **(B)** PBMC from health human donors were isolated and cultured in the presence of CT111 at different concentrations and 200 ng/ml Staphylococcal enterotoxin B (SEB) for 5 days. Following immune cell activation, supernatants were collected and analyzed for IL-2 secretion. Data represents mean ± SEM (n=5).

- **CT111-A and -B, as selected leads, showed strong synergy targeting PD-1 and CTLA-4 on activated T cells in CMV recall and SEB stimulation assays with multiple donors.**
- **CT111 mediates potent IL-2 secretion compared to AK104 across multiple donors in these assays.**



(D-E) Flow cytometry analysis of intratumoral CD25⁺/Foxp3⁺ Tregs in HCC827 treatment groups. **(D)** Representative FACS profiles for each treatment group are shown. **(E)** Percent Tregs within CD4⁺ T cell population, and **(F)** CD8+/Treg ratio are shown as mean ± SEM (n=4). * = p< 0.05, one-way ANOVA Tukey test

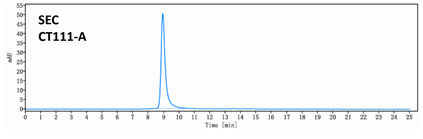
- **Intratumoral Tregs were concordantly reduced with CT111-A (1x dose) treatment similar to AK104 (5x dose), but not in vehicle and AK112-treated tumors, suggesting an anti-CTLA-4 mediated effect with CT111.**

CT111 Possesses Excellent Developability Characteristics

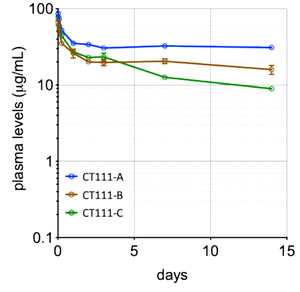
	Analytical SEC		Polyreactivity	Stability
	Monomer (%)	LMW species (%)	BVP score	Tm (°C)
CT111-A	99.37	nd	2.85	62.2

	Accelerated Stability (40°C) *	Freeze-Thaw Recovery (3 cycles) *	Plasma Stability Mouse/Human (1 week, 37°C)
CT111-A	102% recovered	102% recovered	>97% intact Ab recovered

Accelerated stability at 40°C and freeze-thaw Ab stability was measured by determining % recovery of monomer species by analytical SEC.
Plasma stability was assessed by incubating CT111-A in mouse and human plasma for 1 week at 37°C. Intact trispecific was determined by ELISA ligand bridging assay.



CT111 Leads Exhibit Favorable PK in BALB/c Mice



In vivo stability and PK evaluation of CT111 Abs at a single IV bolus at 4 mg/kg in BALB/c mice (n=3 groups of 3 mice per Ab group). Blood samples were collected via staggered serial bleeds distributed across 3 groups at 15 minutes; 2 and 6 hours; and 1-, 2-, 3-, and 14-days post-dose. (A) Time-concentration profiles are shown for CT111-A, -B and -C. Each data point represents mean ± SEM (n=3).

Conclusions

- CT111 is a novel VHH trispecific Ab that simultaneously and effectively targets PD-1, CTLA-4 and VEGF-A with high binding affinities to block cognate ligand interactions. Unlike VEGF-targeting multispecifics that is based on bevacizumab (e.g. AK112, HC010, CS2009), CT111 contains a more potent, de-novo anti-VEGF blocker.
- CT111 shows potent activity against PD-1 and CTLA-4 to reverse exhaustion and promote T cell activation in primary immune cell assays, and to block VEGF-mediated signaling.
- CT111 shows good biophysical attributes and developability with favorable PK in mice.
- CT111 shows superior anti-tumor activity compared to AK104 and AK112 in the HCC827 humanized tumor model. Post analysis shows increased CD8⁺ T cell infiltration and activation in tumors.
- Compared to AK112, CT111 shows unique attributes in decreasing Treg infiltration, similar to 5-fold molar excess of AK104, suggesting a role for targeting CTLA-4. Further investigation is in progress.
- **The data presented here demonstrate that CT111 is a unique, best-in-class PD-1 x CTLA-4 x VEGF IO-targeting trispecific antibody, making it a promising candidate for the treatment of solid tumors. IND-enabling studies are currently in progress.**

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