A New Generation of Fully Human anti-CTLA-4 Heavy Chain Only Antibody

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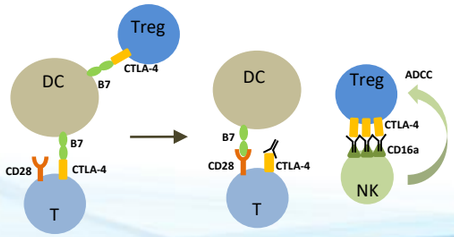
Abstract

Anti-CTLA-4 Abs are one of three types of immune checkpoint inhibitors (alongside anti-PD-1 and anti-PD-L1 Abs) with proven monotherapy value in cancer immunotherapy. Despite demonstrated efficacy, their broad application in both mono- and combination therapies are limited by their safety profiles. Evidence from animal models suggests that the efficacy of anti-CTLA-4 antibodies is at least partially due to depletion of Treg cells via ADCC function, while the clinically adverse events are directly correlated with systemic exposure of the drugs.

Given the limited usability of current anti-CTLA-4 therapies, we set out to develop the next generation of the anti-CTLA-4 antibody with potentially better efficacy and safety profile. A fully human heavy chain only Ab (HCAb) HBM4003-2 was generated using the patented Harbour HCAb transgenic mouse platform. HBM4003-2 specifically binds to CTLA-4 and blocks its binding to B7.1/B7.2. It was designed to have an elevated ADCC function that showed superior ability to deplete Treg cells both in vitro and in vivo. In addition, HBM4003-2 also showed relatively more potent anti-tumor activity than other CTLA-4 antibodies, partly driven by the remarkable depletion in the levels of intra-tumoral Treg cells. Furthermore, owing to its shorter serum half-life, HBM4003-2 resulted in lower systemic drug exposure in vivo at efficacious doses, thereby suggesting a potentially significant improvement in its safety profile in clinical applications.

Thus, HBM4003-2's enhanced efficacy and safety profile presents it as an excellent candidate for the development of NextGen of the already proven anti-CTLA4 therapy to benefit patients across the world.

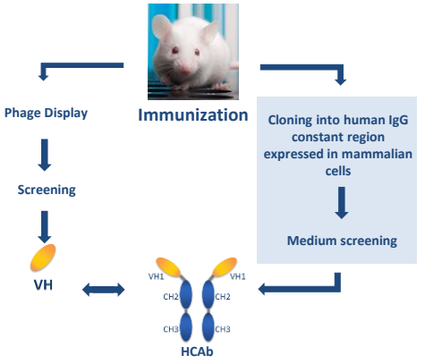
Mode of Action



Key Points

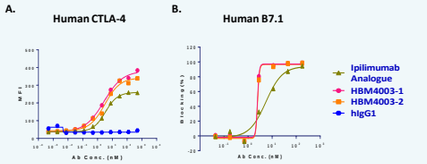
- **HBM4003-2 is a next generation anti-CTLA4 HCAb with novel MoA on Treg depletion.**
- **HBM4003-2 HCAb shows a significant Treg depletion and T cell activation *in vitro* and *in vivo*. It shows more potent tumor growth inhibition and prolonged survival in mouse model.**
- **HBM4003-2 shows a very promising safety profile in monkeys bringing potential clinical benefits.**

Heavy Chain Only Ab Generation



Results

HBM4003-2 HCAb Binds to CTLA-4 and Blocks Its Ligands

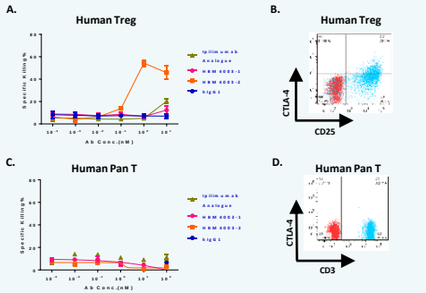


*HBM4003-1: human IgG1 ; HBM4003-2: human IgG1-eADCC

(A) HBM4003 HCAbs bind to human CTLA-4 on CHOK1-human CTLA-4 cells. (B) HBM4003 HCAbs block human B7.1/B7.2-Biotin binding to human CTLA-4 proteins.

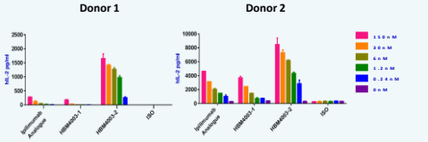
Results

HBM4003-2 HCAb Depletes CTLA-4+ Treg Efficiently by PBMC



(A). HBM4003-2 HCAb induces potent human Treg depletion by human PBMC in ADCC killing assay. (B). Flow cytometry of CTLA-4 surface expression on in vitro differentiated human Treg cells. (C). HBM4003-2 HCAb do not induce human Pan T cell depletion by human PBMC in ADCC killing assay. D). Flow cytometry of CTLA-4 surface expression on human Pan T cells.

HBM4003-2 HCAb Shows Superior T Cell Activation



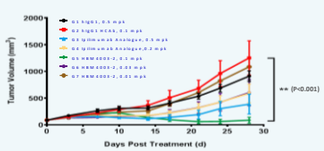
HBM4003 HCAbs induce IL-2 release in PBMC-SEB assay

High Binding Affinity of HBM4003-2 HCAb to Human/Cyno CTLA-4

Antibody	Antigen	ka (1/Ms)	kd (1/s)	KD (M)
Ipilimumab Analogue	hCTLA-4	1.23E+06	8.98E-05	7.32E-11
HBM4003-1		5.35E+06	2.29E-04	4.28E-11
HBM4003-2		5.40E+06	7.58E-05	1.40E-11
Ipilimumab Analogue	cynoCTLA-4	3.73E+06	1.29E-03	3.47E-10
HBM4003-1		5.21E+06	3.08E-04	5.91E-11
HBM4003-2		4.55E+06	1.10E-04	2.43E-11

Binding affinity of HBM4003 HCAb to human/cyno CTLA-4 proteins by BiaCore

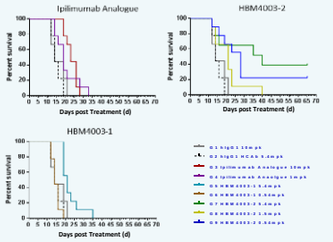
HBM4003-2 HCAb Reduces MC38 Tumor Growth and Extends Mice Survival Significantly



B.

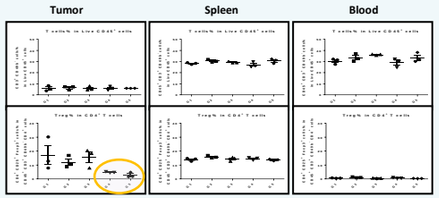
Increase of Life Span

Group		MST	ILS(%)	p value
G1	hIgG1, 10mpk	14		--
G2	hIgG1 HCAb, 5.4mpk	14	0.0	ns
G3	Ipilimumab Analogue, 10mpk	23	64.3	<0.01
G4	Ipilimumab Analogue, 1mpk	19	35.7	ns
G5	HBM4003-1, 5.4mpk	21	50.0	<0.05
G6	HBM4003-1, 0.54mpk	14	0.0	ns
G7	HBM4003-2, 5.4mpk	40	185.7	<0.05
G8	HBM4003-2, 1.5mpk	21	50.0	<0.05
G9	HBM4003-2, 0.54mpk	28	100.0	<0.05



(A). HBM4003 HCAbs reduce tumor growth in MC38 bearing human CTLA-4 KI mouse model. (B). HBM4003 HCAbs increase the life span of MC38 bearing human CTLA-4 KI mice.

HBM4003-2 HCAb Depletes TIL Treg Significantly in MC38



G1: hIgG1, 10 mpk; G4: HBM4003-2, 5.4 mpk; G2: Ipilimumab Analogue, 10mpk; G5: HBM4003-2, 1.5 mpk; G3: hIgG1 HCAb, 5.4 mpk;

Non-GLP Monkey Tox

Cynomolgus monkeys were administrated HBM4003-1 at doses of 10, 30 mg/kg or HBM4003-2 at doses of 7.5, 15 mg/kg via intravenous bolus injection once weekly for 4 weeks. All animals survived until scheduled necropsy. The HNSTD (Highest Non-Severely Toxic Dose) of HBM4003-1 and HBM4003-2 via intravenous bolus injection was considered to be 30 mg/kg and 15 mg/kg respectively.

Conclusions

Key Parameters	HBM4003-2	Ipilimumab Analogue
Blocking against hB7.1: EC50 (nM)	2.68	5.52
IL2 (pg/ml) @6nM Ab	1272	110
In vitro ADCC activity on Treg	Max killing: 53% EC50: 0.79nM	Max killing: 19% EC50: 7.6nM
In vivo efficacy: TGI	91% (0.1mpk)	33% (0.2mpk)
In vivo efficacy: ILS	185.7% (5.4mpk)	64.3% (10mpk)
TIL Treg depletion	73%	0

HBM4003-2 is a fully human HCAb monoclonal antibody that depletes Treg cells efficiently *in vitro* and *in vivo*, blocks CTLA-4 from binding to its ligand, thereby augmenting antitumor immune responses significantly.
Thus, we believe HBM4003-2's enhanced efficacy and safety profile makes it an excellent candidate for clinical development globally which is planned in the near future.

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