Rapid screening of novel OX40 agonistic antibodies for cancer immunotherapy

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ABSTRACT

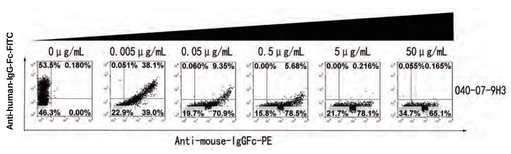
OX40, also known as TNFRSF4 or CD134, is a member of the tumor necrosis factor receptor superfamily and an important co-stimulator of T cell response. OX40L expressed at high levels on activated antigen presenting cells (APCs) can result in multimerization of OX40 receptors, leading to strong T cell activation. OX40 also plays important role in memory T cell formation. The highest levels of OX40 are found on effector and central memory CD4+ T cells and regulatory T cells (Treg). Given the duality of OX40 in both stimulating effector T cell activity and suppressing Treg activity, OX40 agonists have been widely explored for cancer immunotherapy.

Here we developed a novel OX40 stimulating antibody, YH002, that can effectively inhibit tumor growth. In vitro studies demonstrated that YH002 bound specifically and potently to human/monkey OX40. YH002 was able to dose-dependently potentiate the activation of human OX40 on reporter cells in the presence of FcγRIIB-expressing accessory cells, and showed ADCC activity in vitro. YH002 demonstrated robust anti-tumor efficacy against MC38 tumors in OX40 humanized knockin mice (B-hOX40), and Treg depletion was noted by tumor-infiltrating lymphocyte analysis.

In conclusion, YH002 showed anti-tumor activity by stimulating effector T cells and Treg depletion. Furthermore, we showed that YH002 worked in concert with Pembrolizumab in double humanized mouse model of B-hPD-1/hOX40. The combination of YH002 and Ipilimumab demonstrated synergistic effect in double humanized mouse model of B-hCTLA4/hOX40. In toxicology analysis, the MTD of a single dose of YH002 in cynomolgus monkeys was considered to be 200 mg/kg/day. Cynomolgus monkeys were tolerated to repeated i.v. infusions of YH002 to at 10, 30, or 90 mg/kg for 29 days (QW×5), and the HNSTD was considered to be 90 mg/kg.

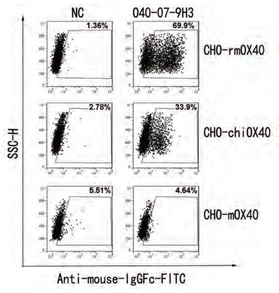
RESULTS AND DISCUSSIONS

Fig 1. Ligand competition of the anti-OX40 antibody



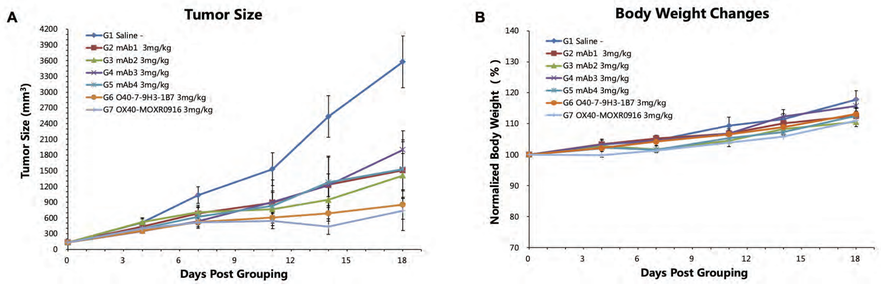
hOX40L-hFc (Y axis) was used to compete various amount of anti-human OX40 antibodies (X-axis) preincubated with CHO-hOX40 cells. Binding was analyzed by FACS.

Fig 2. Cross-species reactivity of the anti-OX40 antibody



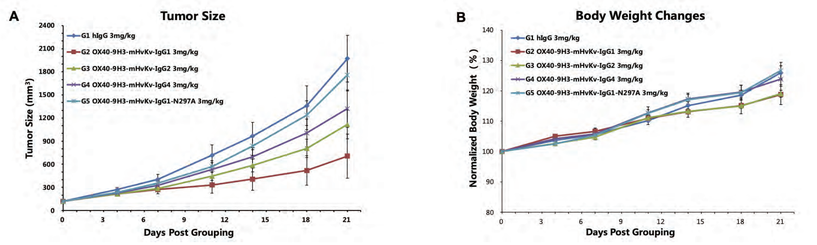
CHO cells overexpressing rhesus monkey (rm), murine (m) or chimeric (chi,human and mouse) OX40 were stained with anti-OX40 antibody.

Fig 3. Functional *in vivo* anti-hOX40 antibody screening in B-hOX40 mice



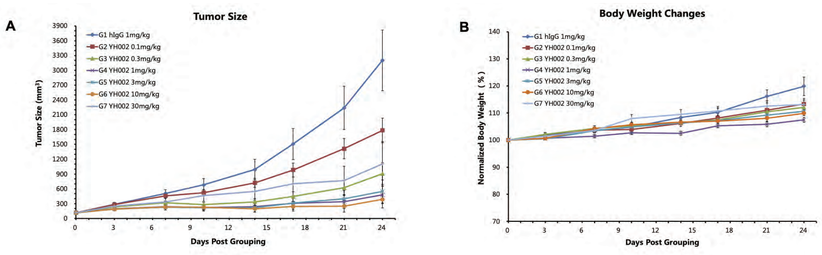
Gender and aged matched B-hOX40 mice (female, 5-7-weeks-old, n=5)were inoculated with MC38 cells subcutaneously. When the average tumor size reached to about 100mm3, mice were treated with monoclonal murine antibodies every three days for 6 times. Data of tumor volume (A) and normalized body weight (B) were expressed as mean ± SEM.

Fig 4. Modulation of YH002 anti-tumor activity by IgG subtypes in MC38/B-hOX40 mice



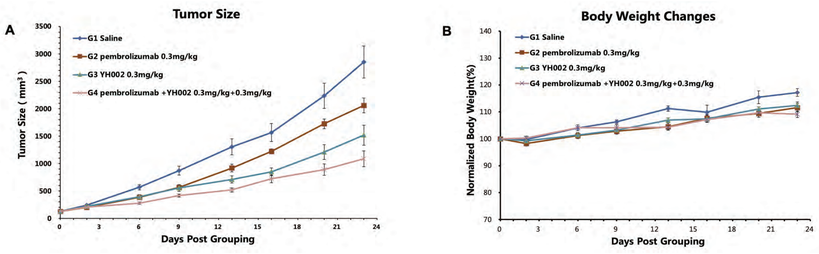
Chimeric antibodies were generated by YH002 into various human IgG expression vectors. Recombinant antibodies were purified from the supernatant of transfected CHO cells. These antibodies were screened in the subcutaneous MC38/B-hOX40 model (female, 7-week-old, n=8 per group). Data of tumor volume (A) and normalized body weight (B) were expressed as mean ± SEM.

Fig 5. Dose range and tolerability of anti-OX40 antibody YH002 in B-hOX40 mice



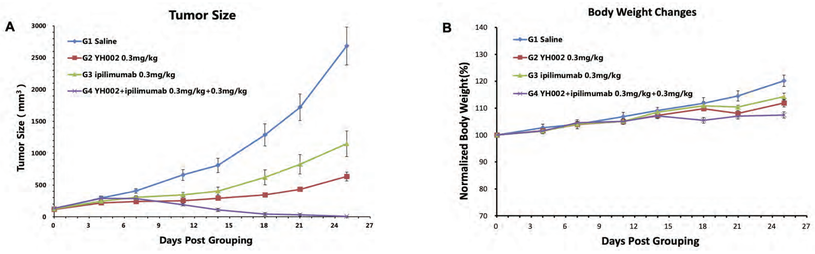
B-hOX40 mice (female, 8-week-old, n=8) were inoculated with MC38 cells subcutaneously. When the average tumor size reached to about 100mm3, mice were treated with anti-hOX40 antibody at the indicated dose twice a week for 6 times. Data of tumor volume (A) and normalized body weight (B) were expressed as mean ± SEM.

Fig 6. Combination of YH002 with pembrolizumab in MC38 model



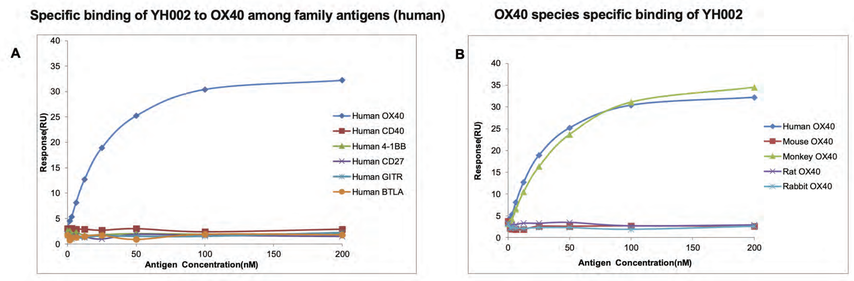
B-hPD-1/hOX40 mice (female, 7-8-week-old, n=8) were inoculated with MC38-hPD-L1 cells subcutaneously. When the average tumor size reached to about 100mm3, mice were treated with YH002 and pembrolizumab at the indicated dose once a week for 3 times. Tumor volume were measured with a caliper. Data of tumor volume (A) and normalized body weight (B) were expressed as mean ± SEM.

Fig 7. Combination of YH002 with ipilimumab in MC38 model



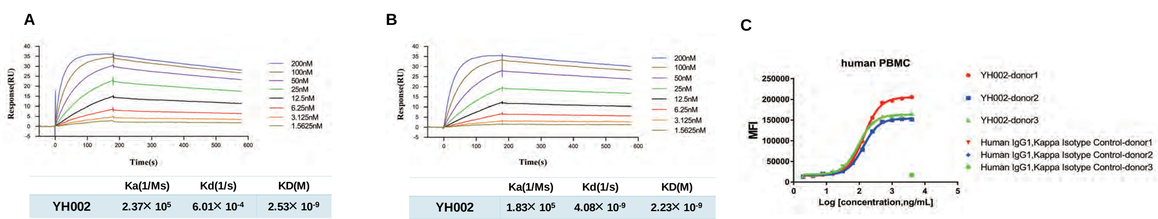
B-hCTLA4/hOX40 mice (female, 7-8 week old) were inoculated with MC38 cells subcutaneously. When the average tumor size reached to about 100 mm3, mice were treated with YH002 and ipilimumab at the indicated dose twice a week for 6 times. Tumor volume were measured with a caliper. Data of tumor volume (A) and normalized body weight (B) were expressed as mean ± SEM.

Fig 8. Specific binding of YH002 to OX40 among family antigens (human)



YH002 (1 μg/mL) was tested for binding specificity to members of functionally and structurally related family of OX40 (A) and recombinant OX40 from different species (B) with surface plasmon resonance (SPR) technology.

Fig 9. Affinities of YH002 to human and cynomolgus monkey OX40

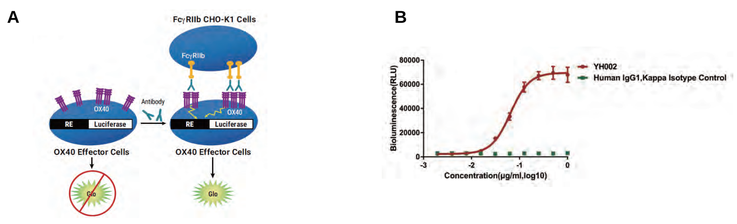


	Ka(1/Ms)	Kd(1/s)	KD(M)
YH002	2.37× 10^5	6.01× 10^{-4}	2.53× 10^{-9}

	Ka(1/Ms)	Kd(1/s)	KD(M)
YH002	1.83× 10^5	4.08× 10^{-9}	2.23× 10^{-9}

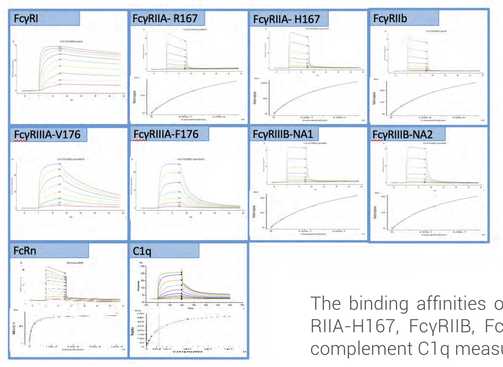
Binding kinetics of YH002 to human (A) and cynomolgus monkey OX40 (B) proteins by SPR. Binding of YH002 to OX40 on activated (anti-CD3/CD28) human PBMC（C） and cynomolgus monkey PBMCs (D).

Fig 10. YH002 activated human OX40 reporter activity



YH002 induced T cell activation reporter activity in vitro after incubation with Jurkat-Luc-hOX40 in the presence of CHO-K1-FcγRIIB cells for 6 hours at 37°C. Data were expressed as mean ± SEM.

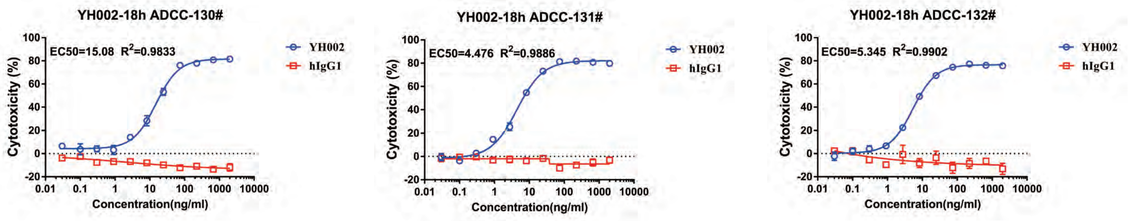
Fig 11. Affinities of YH002 to Fcγ receptors and C1q



	Affinity model	ka(1/Ms)	kd(1/s)	KD(M)
FcγRI	Kinetics	2.83ï 10^5	1.50ï 10^{-3}	5.31ï 10^{9}
FcγRIIA-R167	Affinity	—	—	3.30ï 10^{-6}
FcγRIIA-H167	Affinity	—	—	1.96ï 10^{-6}
FcγRIIB	Affinity	—	—	7.60ï 10^{-6}
FcγRIIIA-V176	Kinetics	2.38ï 10^5	9.20ï 10^{-3}	3.87ï 10^{-8}
FcγRIIIA-F176	Kinetics	6.18ï 10^5	8.90ï 10^{-2}	1.44ï 10^{-7}
FcγRIIIB-NA1	Affinity	—	—	4.03ï 10^{-6}
FcγRIIIB-NA2	Affinity	—	—	1.88ï 10^{-6}
FcRn	Affinity	—	—	4.62ï 10^{-7}
C1q	Affinity	—	—	7.34ï 10^{-8}

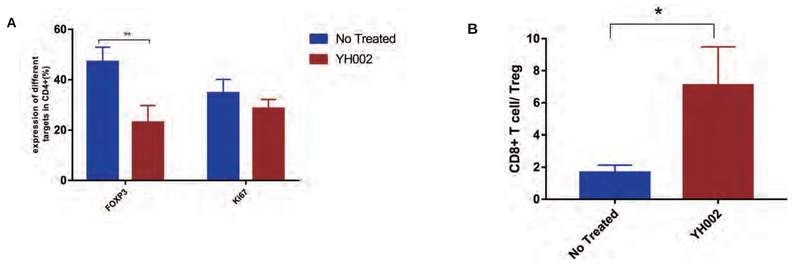
The binding affinities of YH002 to recombinant human Fc receptors (FcγRI, FcγRIIA-R167, FcγRIIA-H167, FcγRIIB, FcγRIIIA-V176, FcγRIIIA-F176, FcγRIIIB-NA1, FcγRIIIB-NA2 and FcRn) and complement C1q measured by SPR.

Fig 12. YH002-mediated ADCC



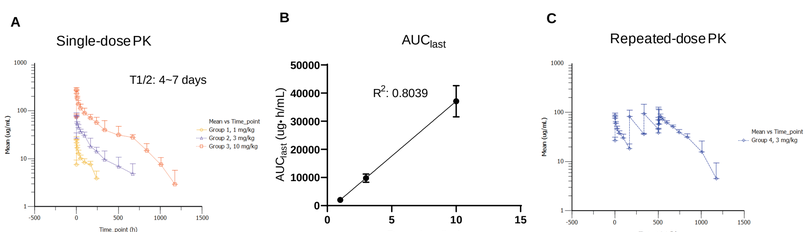
ADCC activity was measured 18 hours after coincubation of varied amount of YH002 with PBMCs (effector, E), isolated from three healthy donors and pre-cultured in RPMI 1640 medium for 1 hour at 37 C , and CFSE-stained Jurkat-hOX40 cells (target, T) (E:T= 10:1). The cells were then stained with zombie NIR dyes and analyzed by FACS. ADCC was indicated by cytotoxicity (%) (cytotoxicity% = (1- live cells in treated group/live cells in untreated group) × 100%).

Fig 13. YH002 treatment decreased Treg and increased CD8/Treg ratio in MC38/B-hOX40 mice



Murine colon cancer MC38 tumor-bearing B-hOX40 mice (3F/group, 6-week-old) received YH002 (ip. Q2D x 2) or no treatment as control. Tumor-infiltrating lymphocyte analysis by flow cytometry was conducted 48 h after the last dose. Data were expressed as mean ± SEM. *P<0.05, **P<0.01

Fig 14. YH002 PK in cynomolgus monkey



PK analysis of YH002 in cynomolgus monkey. A. Single-dose PK analysis of YH002 in cynomolgus monkeys (3/sex/group) received i.v. infusion at single doses of 1, 3 or 10 mg/kg. B. Dose proportionality of YH001 exposure. C. Repeated dosing PK analysis of YH002 in cynomolgus monkeys (3/sex/group) received i.v. infusion at repeat doses of 3 mg/kg YH002 once a week (QW x 4). Values were expressed as mean ± SEM.

YH002 was well tolerated in cynomolgus monkey

Single-dose toxicity study showed MTD to be 200 mg/kg/day
Repeated-dose toxicity (QW x 5, 29 days) was tolerated up to 90 mg/kg.
HNSTD was considered 90 mg/kg.

CONCLUSIONS

- We generated an anti-human OX40 antibody YH002, a humanized IgG1, using rapid *in vivo* efficacy-based screening in B-hOX40 mice.
- YH002 binds specifically to human and monkey OX40 with similar affinity.
- YH002 showed strong anti-tumor efficacy in syngeneic murine tumor models, both as single agent and in combination with pembrolizumab and ipilimumab.
- YH002 promoted ADCC *in vitro*. Pharmacodynamic studies show that it reduces Treg and increased CD8/Treg ratio in tumor in murine models.
- YH002 exposure showed dose linearity in cynomolgus monkey with half life between 4-6 days.
- YH002 was well tolerated in monkey.
- Our preclinical investigation of YH002 demonstrates Biocytogen's capability in discovering clinical candidates using its robust antibody discovery platform.