Preclinical development of an anti-cancer bispecific antibody targeting VEGF and DLL4, ABL001

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Abstract

Several angiogenesis inhibitors targeting the vascular endothelial growth factor (VEGF) signaling pathway have been used for cancer treatment. However, VEGF inhibitors alone are known to promote tumor invasion and metastasis by increase of intratumoral hypoxia in some preclinical and clinical studies. Emerging reports suggest Delta-like-ligand 4 (DLL4) is a promising target to augment the effects of VEGF inhibitors. Simultaneous blockade of VEGF/VEGFR and DLL4/Notch signaling pathways leads to more potent inhibition of tumor progression by a synergic anti-angiogenic activity in various mouse xenograft models. We developed a novel bispecific antibody, ABL001 (previous code name: HD105), targeting VEGF and DLL4. ABL001 bispecific antibody is composed of an anti-VEGF antibody (bevacizumab-similar) backbone C-terminally linked with a DLL4-targeting single-chain variable fragment. This bispecific antibody competitively inhibited the binding of ligands to their receptors, i.e., VEGF to VEGFR2 and DLL4 to Notch1, resulting in a conspicuous inhibition of endothelial cell proliferation and DLL4-induced Notch1-dependent activation. In addition, ABL001 more efficiently inhibited the tumor progression of A549 lung cancer and ovarian patient-derived xenograft models than an anti-VEGF antibody or anti-DLL4 antibody alone. In a GLP nonclinical monkey toxicity study, ABL001 was well-tolerated only with a mild adverse histopathological finding of liver sinusoidal dilatation. In order to perform clinical phase I trial, ABL001 was produced by 1000-L GMP scale with 1 g/L of titer and 65% of purification yield, which showed the same activities and qualities compared to the internal reference standard. In conclusion, ABL001 is being developed as a therapeutic bispecific antibody for cancer treatment.

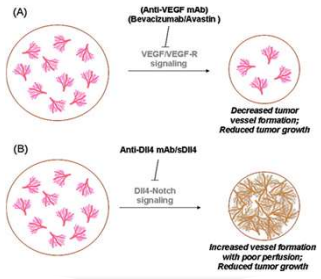
Background & Rationale

Medical Unmet Needs

Anti-VEGF therapy results in more invasive and metastatic tumor phenotype in clinical studies

- Regression of VEGF-dependent tumor vessels
- Formation of normalized, other VEGF-independent tumor vessels
- Can not destroy all tumor vessels
- **Resistant to anti-VEGF therapy**

Regulation of Angiogenesis by DLL4/Notch



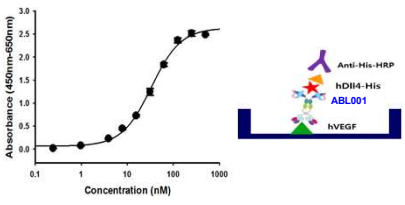
DLL4/Notch signaling pathway

- Different molecular mechanism of action compared to anti-VEGF therapy

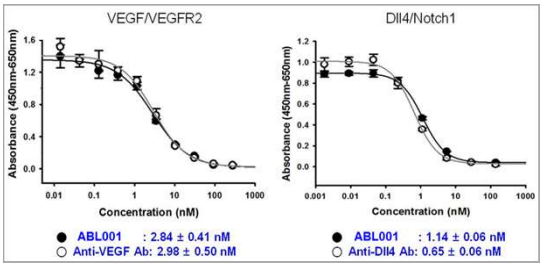
Binding Affinity of ABL001

Biacore assay	
Human DLL4	K_D
ABL anti-DLL4 Ab	3.6 nM
ABL 001	30 nM
Human VEGF	K_D
ABL anti-VEGF Ab	0.06 nM
ABL 001	0.126 nM

ELISA (dual-antigen capture)



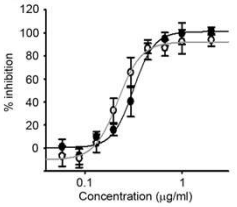
Competitive Inhibition of ABL001



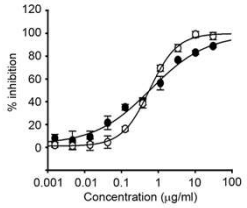
Competitive ELISAs demonstrated that the ABL001 bispecific antibody inhibited the interaction between VEGF/VEGFR2 and Dll4/Notch1 in a dose-dependent manner

Cell-based Assays of ABL001

HUVEC proliferation



Luciferase gene expression

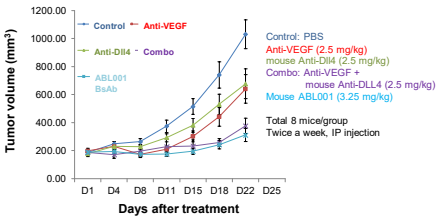


ABL001 inhibited VEGF-dependent HUVEC proliferation and Dll4-induced Notch-1-dependent activation of luciferase in SKOV-3-RBP-J_K luciferase cells in a dose-dependent manner.

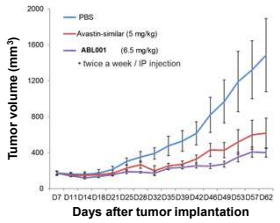
- HUVEC proliferation assay
  - ● ABL001: 1.58 ± 0.08 nM
  - ○ Anti-VEGF Ab: 1.49 ± 0.04 nM
- Luciferase assay
  - ● ABL001: 0.62 ± 0.23 nM
  - ○ Anti-DLL4 Ab: 0.58 ± 0.03 nM

In vivo Xenograft Assays

A549 lung cancer xenograft model



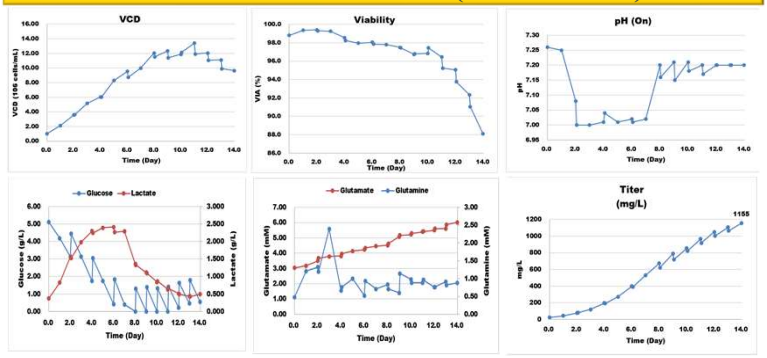
Ovarian cancer patient xenograft model



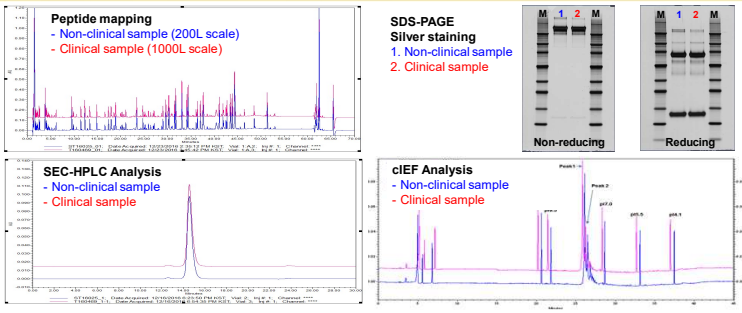
Summary of Nonclinical Study

Items	Observations	Items	Observations
Mortality	No deaths	Coagulation	No test item-related changes
Clinical Signs	No test item-related clinical signs	Clinical Chemistry	- Increased triglyceride (TG) in both sexes at 50 mg/kg - But within an acceptable range
Body Weight	No test item-related effects	Urinalysis /Urine Chemistry	No test item-related changes
Food Consumption	No test item-related changes	Organ Weight	No test item-related changes
Ophthalmology	No test item-related changes	Macroscopic Findings	No test item-related changes
Electrocardiography	No test item-related changes	Microscopic Findings	Test item-related changes were found in the liver, femur/marrow, and thymus
Hematology	- Absolute lymphocyte count (LYMA): a trend of increasing In both sexes at 50 mg/kg (test item-related, non-adverse & reversible)	Anti-Drug Antibody Analysis	Production of anti-ABL001 antibodies in monkeys

Production of ABL001 (1000L Scale)



Quality Control of ABL001



Conclusion & References

A bispecific antibody, ABL001 (previous code name: HD105)

- simultaneously binds to VEGF and DLL4, which play important roles in tumor angiogenesis.
- competitively inhibits the binding of ligands to their receptors, i.e., VEGF to VEGFR2 and DLL4 to Notch1, resulting in a conspicuous inhibition of endothelial cell proliferation and DLL4-induced Notch1-dependent activation.
- more efficiently inhibits the tumor progression of A549 lung cancer and ovarian patient-derived xenograft models than an anti-VEGF antibody or anti-DLL4 antibody alone.
- is well-tolerated in the GLP nonclinical monkey toxicity study.
- can be produced by 1000-L GMP scale with 1 g/L of titer and 65% of purification yield, which showed the same activities and qualities compared to the internal reference standard.
- is being developed as a therapeutic bispecific antibody for cancer treatment.

References

1. Yin L. et al. Biochemical Pharmacology 2010, 80:690-701.
2. Miles KM. et al. PLoS One 2014, 9: e112371.
3. Lee D. et al. MAbs. 2016, 8(5):892-904.

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