CADONILIMAB, AN ANTI-PD1/CTLA4 BI-SPECIFIC ANTIBODY WITH FC EFFECTOR NULL BACKBONE

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Background Tumor infiltrating lymphocytes co-express PD-1 and CTLA-4 at much higher levels compared to normal tissues and peripheral blood cells, thus anti-PD1/CTLA4 bi-specific antibody with a preferential tumor tissue enrichment over normal tissue would contribute to enhanced efficacy and safety. Currently available anti-PD1 and anti-CTLA4 antibodies used in combination therapy are of residual bindings to FcγRs, which mediate antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), leading to compromise on efficacy and safety. Moreover, activated macrophage in tumor microenvironment plays key role in mediating immune suppression by secreting proinflammatory cytokines, such as IL-6. Cadonilimab, also known as AK104, is an IgG1 scaffold Fc-engineered antibody, which is designed to eliminate binding to FcγRs and C1q, and subsequently minimize lymphocyte loss, and antibody dependent cytokine release from macrophage, which associate with irAEs and poor prognosis in immunotherapy.¹

Methods PD-1 and CTLA4 antigen co-binding activity of Cadonilimab was determined by ForteBio and assay of co-culture Cadonilimab with Hoechst33342-labelled Jurkat cells expressing PD-1 and CHO-K1-CTLA4 cells. Binding kinetics of Cadonilimab to C1q, FcγRIa, FcγRIIa_H131, FcγRIIa_R131, FcγRIIIa_V158 and FcγRIIIa_F158 were measured by ForteBio. ADCC, ADCP and CDC activities were determined in cellular assays. IL-6 and IL-8 from macrophage were detected in a assay of human macrophage and CHO-K1-PD1-CTLA4 cells co-culture.

Abstract 289 Figure 1 AK104 crosslinks cells expressing CTLA-4 and PD-1. AK104 can crosslink cells expressing CTLA-4 with cells expressing PD-1. CTLA-4-expressing CHO-K1 cells were plated into the plates. Then the mixture of AK104 or control antibodies with Hoechst 33342 labelled PD-1-expressing Jurkat cells were added into the plates and incubated for 20min. After the incubation, suspended Jurkat cells were removed, and the crosslinking between PD-1 and CTLA-4 expressing cells was analyzed microscopically.

Abstract 289 Figure 2 ADCC, CDC and ADCP activities of AK104. (A) Antibody-dependent cell-mediated cytotoxicity (ADCC) activities of AK104 (hG1), a version of Cadonilimab with wildtype IgG1 scaffold and Cadonilimab were determined by measuring lactate dehydrogenase (LDH) release from 293T-CTLA4-PD1 cells. (B) Complementary-dependent cell-mediated cytotoxicity (CDC) activities of AK104 (hG1) and Cadonilimab were determined by measuring LDH release from 293T-CTLA4-PD1 cells. (C) Antibody-dependent cellular phagocytosis (ADCP) activities of AK104 (hG1) and Cadonilimab were studied by examining phagocytosis of CHO-K1-PD1-CTLA4 cells by murine bone marrow derived macrophages. Data are expressed as mean ± SEM of two independent experiments.

Abstract 289 Figure 3 IL-8 and IL-6 secretion induced by AK104. Effects of Fc engineering of cadonilimab on the release of inflammatory cytokines. (A) IL-8 and (B) IL-6 by HPMMs in the presence of IFN-γ. Data are expressed as mean ± SEM of two independent experiments.

Abstract 289 Table 1 Affinity of AK104 to FcγRs and C1q. Affinity of cadonilimab to FcγRIa, FcγRIIa_H131, FcγRIIa_R131, FcγRIIIa_V158, FcγRIIIa_F158 and C1q.

FcγR/C1q	Antibody	KD (M)	kon (1/Ms)	SE (kon)	kdis (1/s)	SE (kdis)	Rmax (nm)
FcγRIa	Cadonilimab	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	AK104(hG1)	5.92E-09	3.06E+05	8.35E+03	1.81E-03	5.75E-05	0.53-0.62
FcγRIIa_H131	Cadonilimab	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	AK104(hG1)	2.22E-08	3.83E+05	4.03E+04	8.49E-03	7.44E-04	0.96-1.63
FcγRIIa_R131	Cadonilimab	4.20E-08	3.72E+05	4.19E+04	1.56E-02	8.99E-04	0.16-0.36
	AK104(hG1)	1.43E-08	3.58E+05	3.31E+04	5.10E-03	5.87E-04	0.66-1.34
FcγRIIIa_V158	Cadonilimab	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	AK104(hG1)	1.77E-07	1.21E+05	1.64E+04	2.14E-02	3.71E-03	1.56-1.61
FcγRIIIa_F158	Cadonilimab	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	AK104(hG1)	2.21E-07	1.12E+05	1.39E+04	2.47E-02	3.43E-03	0.39-0.64
C1q	Cadonilimab	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	AK104(hG1)	2.53E-09	2.05E+06	2.10E+05	5.17E-03	5.81E-04	0.05-0.18

Results Cadonilimab binds to the antigens PD-1 and CTLA-4 simultaneously, and as shown in figure.1, Cadonilimab crosslinks cells expressing CTLA-4 with those expressing PD-1 (figure 1). Cadonilimab exhibited no binding to FcγRIa, FcγRIIa_H131, FcγRIIIa_V158, FcγRIIIa_F158 or C1q (table 1), eliciting no apparent ADCC, ADCP or CDC (figure 2).

Cadonilimab induced no remarkable IL-6 or IL-8 release by human macrophage compared with combination of nivolumab and ipilimumab (figure 3). Clinical trials of Cadonilimab as monotherapy, in combination with chemotherapy or tyrosine kinase inhibitor, such as Lenvatinib and Anlotinib, to treat metastatic cervical cancer (NCT04380805), gastric adenocarcinoma/gastroesophageal junction adenocarcinoma (NCT04728321), non-small cell lung cancer (NCT04647344) and hepatocellular carcinoma (NCT04444167) are ongoing, and a promising efficacy and acceptable safety profile were observed.

Conclusions Cadonilimab, an IgG1 antibody with Fc-engineering, exhibits neither Fc effector functions including ADCC, ADCP, CDC, nor activating macrophage to secrete IL-6 or IL-8. Possible tumor tissue preferential retention of Cadonilimab over conventional anti-PD-1 and anti-CTLA-4 antibodies noted above could potentially lead to better safety profile.

REFERENCE

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