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Cadonilimab, an anti-PD1/CTLA4 bi-specific antibody with Fc effector null backbone

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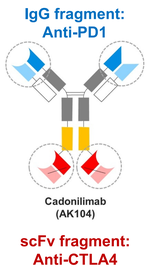
Introduction

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 [1].



Moreover, activated macrophage in tumor microenvironment plays a key role in mediating immune suppression by secreting proinflammatory cytokines, such as IL-6, and IL-8 [2-3]. 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 associated with irAEs and poor prognosis in immunotherapy [4].

Methods

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. 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 activity to cells expressing PD-1 or CTLA-4 was determined by FACS.

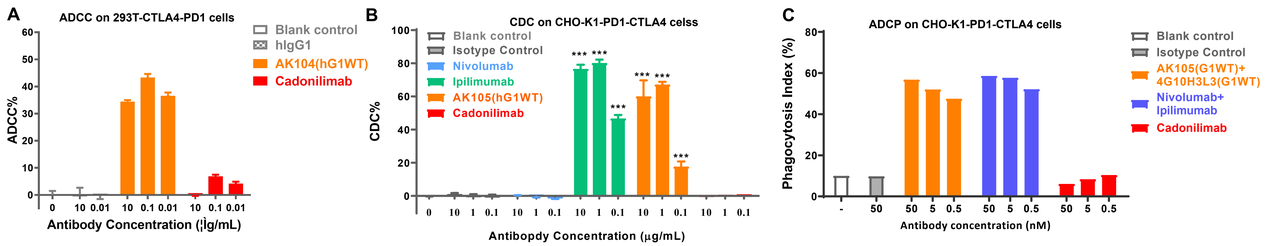
Results

Table 1. Affinity of Cadonilimab to FcγRs 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	3.13E+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

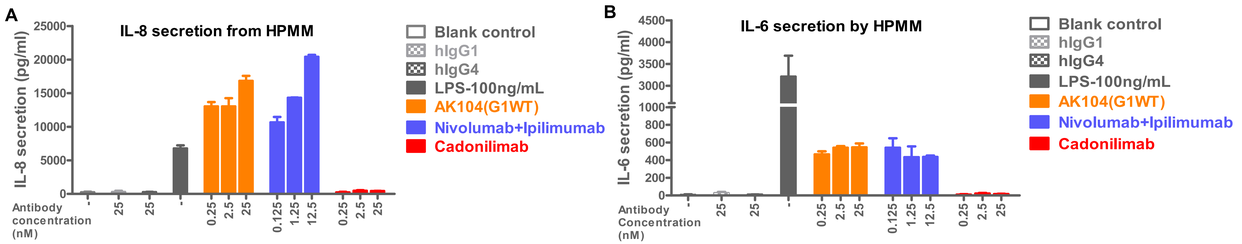
Results

Figure 1. Cadonilimab (AK104) that Remove binding to Fcγ receptors and C1q to avoid ADCC, CDC and ADCP



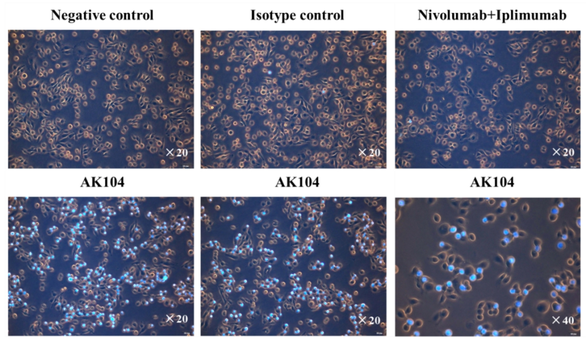
(A) ADCC activities of AK104 (hG1), a version of Cadonilimab with wildtype IgG1 backbone and Cadonilimab were determined by measuring lactase dehydrogenase (LDH) release from 293T-CTLA4-PD1 cells. (B) CDC activities of AK104 (hG1WT) and Cadonilimab were determined by measuring LDH release from CHO-K1-CTLA4-PD1 cells. (C) ADCP activities of AK104 (hG1WT) 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.

Figure 2. Cadonilimab (AK104) that Remove binding to Fcγ receptors to avoid the release of inflammatory cytokines



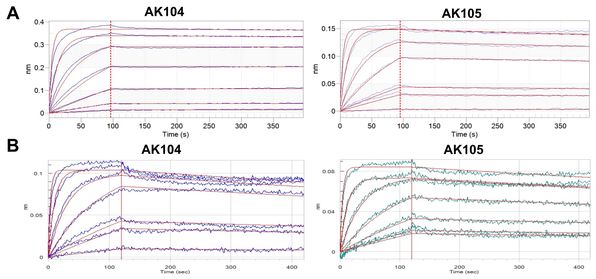
(A) IL-8 and (B) IL-6 by HPMMs in the presence of IFN-γ. Data are expressed as mean ±SEM of two independent experiments.

Figure 3. Cadonilimab (AK104) Target Binding in Trans : Cross-links 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.

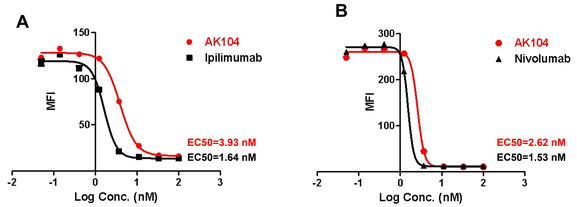
Figure 4. Cadonilimab (AK104) with Higher avidity of Binding than PD-1 Antibody on Surface with a High Density of PD-1 and CTLA-4



Fixed antigens (nM)			Antibody	KD (M)	kon(1/Ms)	koff(1/s)
A	PD1-mFc, CTLA4-mFc	50	**AK104**	**3.69E-11**	**1.06E+06**	**3.92E-05**
			AK105	1.98E-10	1.18E+06	2.33E-04
B	PD1-mFc	10	**AK104**	**3.48E-10**	**1.44E+06**	**5.01E-04**
			AK105	3.58E-10	1.22E+06	4.36E-04

Results

Figure 5. Cadonilimab (AK104) – Competitive Binding Activity Comparable to Nivolumab or Ipilimumab



(A) Competitive binding curve of AK104 and Ipilimumab with B7.1 to 293T-CTLA4 cells; (B) Competitive binding curve of AK104 and Nivolumab with PD-L1 to 293T-PD1 cells .

Conclusion

Cadonilimab, an IgG1 antibody with Fc-engineering, exhibits neither Fc effector functions including ADCC, ADCP, CDC, nor activating macrophage to secret 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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[4].Lo Russo G, Moro M, Sommariva M, Cancila V, Boeri M, Centonze G, et al. Antibody-Fc/FcR Interaction on Macrophages as a Mechanism for Hyperprogressive Disease in Non-small Cell Lung Cancer Subsequent to PD-1/PD-L1 Blockade. Clin Cancer Res 2019;25:989-999.

Acknowledgment

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