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Abstract # 527

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Preclinical dose-pharmacokinetic-efficacy modeling of botensilimab using a mouse surrogate of the Fc-enhanced anti-CTLA-4 antibody

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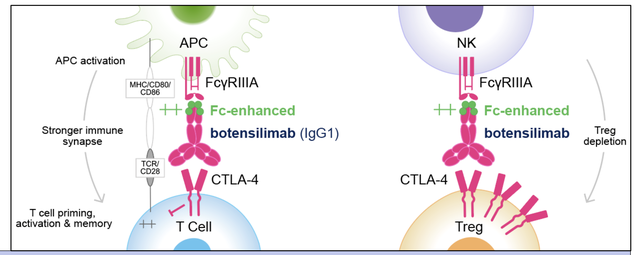
Background and rationale

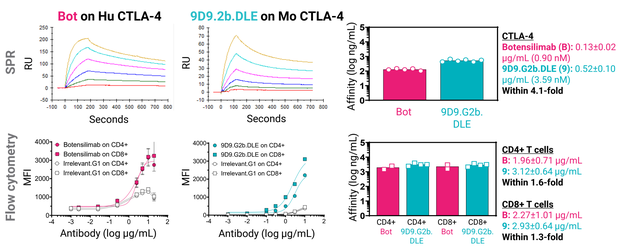
- While CTLA-4 antagonist antibodies were the first immune checkpoint inhibitors demonstrating durable clinical responses[1], their expansion to new indications has been limited.
- Preclinical murine studies have demonstrated that anti-CTLA-4 activity depends on co-engaging Fcγ receptor (FcγR)-dependent mechanisms to promote T-cell priming, Treg depletion, and myeloid activation[2,3].
- These findings extended to patients treated with **botensilimab**, an Fc-enhanced anti-CTLA-4 antibody, with clinical activity across multiple poorly immunogenic and treatment-refractory cancers, including microsatellite stabile (MSS) colorectal cancer (CRC)[4].
- To support the clinical development of **botensilimab**, we modelled efficacious doses and exposure parameters using a mouse tumor model treated with a pharmacologically matched mouse surrogate antibody (botensilimab^{ms}).

Botensilimab is an Fc-enhanced CTLA-4 antagonist monoclonal IgG1 antibody that enhances FcγR-dependent mechanisms to promote anti-tumor immunity

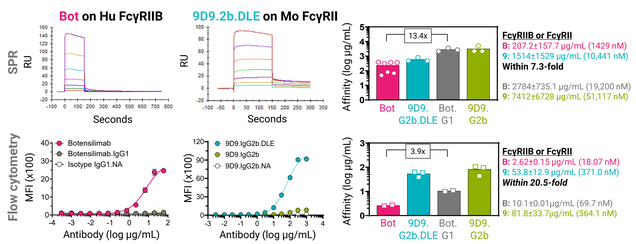


Botensilimab^{ms} (9D9 mouse IgG2b DLE) is a close pharmacologically-matched surrogate of botensilimab

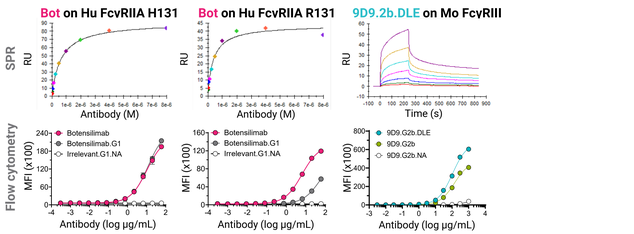
Comparable CTLA-4 binding affinities



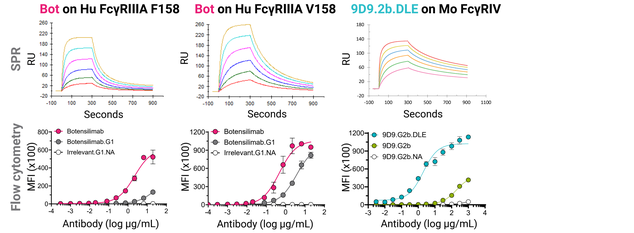
Comparable FcγRIIB binding affinities



Comparable FcγRIIA binding affinities†



Comparable FcγRIIIA binding affinities††



Ratios of activitory-to-inhibitory receptor binding

Interaction	$KD_{Act.}/KD_{Inh.}$ (µg/mL) SPR	1/Ratio SPR	$KD_{Act.}/KD_{Inh.}$ (µg/mL) FC	1/Ratio FC
Botensilimab FcγRIIA(Avg)/FcγIIB	274.6/207.2	0.75	2.90/2.62	0.90
9D9.G2b.DLE FcγRII/FcγRII	525.1/1514	2.88	72.6/53.8	0.74

Interaction	$KD_{Act.}/KD_{Inh.}$ (µg/mL) SPR	1/Ratio SPR	$KD_{Act.}/KD_{Inh.}$ (µg/mL) FC	1/Ratio FC
Botensilimab FcγRIIA(Avg)/FcγRIIB	0.81/207.2	255.8	1.03/2.62	2.54
9D9.G2b.DLE FcγRIV/FcγRII	0.11/1514	13,793	1.17/53.8	46.0

Legend. (SPR) Antibody affinities determined by Biacore using a dose range (0.5 – 8000 nM) of recombinant human or mouse CTLA-4-His or indicated FcγR flowed on captured botensilimab or 9D9 by surface plasmon resonance (SPR) in 2+ experiments. Steady-state or 1:1 binding kinetic models used. (flow cytometry) Human PBMC or mouse splenocytes stimulated with anti-CD3 plus anti-CD28 activator beads for 2 days and immunostained with lineage markers, permeabilized & fixed, and then immunostained with a dose range of botensilimab or 9D9.G2b.DLE followed by secondary antibody detection. †Mouse FcγRIII akin to human FcγRIIA[5]. ††Mouse FcγRIV akin to human FcγRIIIA[6]. Abbreviations: 9 – 9D9.2b or 9D9.2b.DLE, Act. – Activating receptors, Avg – Average, B – Botensilimab or botensilimab.G1, Bot – Botensilimab (IgG1 Ser(S)-239-Asp(D), Ala(A)-330-Leu(L), and Ile(I)-332-Glu(E)), 9D9.G2b.DLE – mouse IgG2b Ser-245-Asp, Ser-336-Leu, and Ile-338-Glu FC – Flow cytometry, G1 – human IgG1, G2b - mouse IgG2b, Hu - human, Inh. - inhibitory receptors, KD – binding affinity, M – molar, Mo – mouse, nM – nanomolar, NA – N297A, nM – nanomolar, RU – response units, SPR – surface plasmon resonance, µg/mL – microgram per milliliter.

Dose-PK-Efficacy relationship of botensilimab^{ms} (9D9.G2b.DLE)

Design: Single administration IP in subcutaneous CT26 BALB/c

List of studies

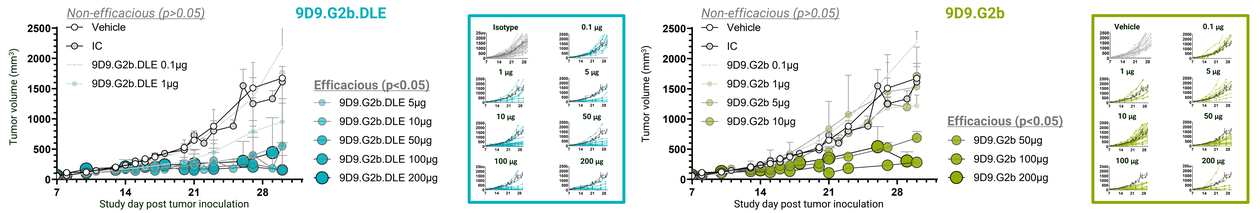
Exp #	Test articles	Doses (µg)	Treatment day (d)	Last measure (d)	Group size (n)
1	vehicle, IC, 9D9.G2b, 9D9.G2b.DLE	200	7	29	10
2	vehicle, IC, 9D9.G2b, 9D9.G2b.DLE	5, 10, 50, 100	7	30	10
3	IC, 9D9.G2b, 9D9.IgG2b.DLE	10, 200	8	29	10
4	vehicle, IC, 9D9.G2b, 9D9.G2b.DLE	10	9	37	10
5	vehicle, IC, 9D9.G2b, 9D9.G2b.DLE	0.1, 1, 10, 50, 100	8	30	9
6	9D9.G2b, 9D9.G2b.DLE	200	9	68	50

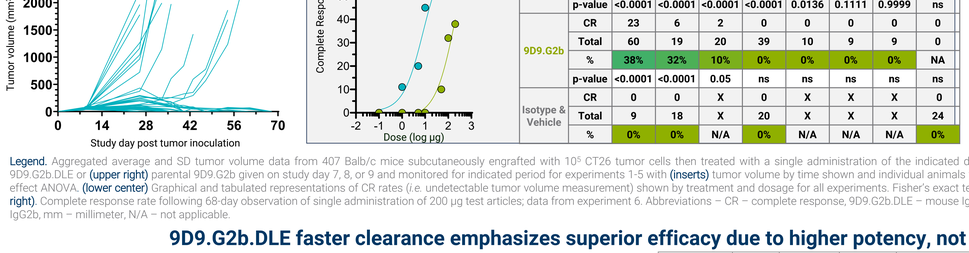
Treatment by test article, dose, and start of treatment

Demographics and Enrollment Conditions		n	%
Sex	Female	407	100.0%
Age	6-8 weeks	407	100.0%
Test article	9D9.G2b	166	40.8%
	9D9.G2b.DLE	170	41.8%
	Isotype + Vehicle	71	17.4%
Dose (µg)	0	24	5.9%
	0.1	19	4.7%
	1	18	4.4%
	5	20	4.9%
	10	99	24.3%
	50	40	9.8%
	100	57	14.0%
	200	130	31.9%
Start of treatment (d) †††	7	129	31.7%
	8	144	35.4%
	9	134	32.9%

Legend. (left) Tabulation of conducted studies with indicated dosages, day of treatment, day of last tumor measurement, and median group size. (right) Animal demographics and breakdown of proportion of test articles, dosages, and day of treatment across all included studies. Abbreviations: d – day, Exp - experiment, G2b – mouse IgG2b, IC – isotype control (mouse IgG2b), CR – complete response, DLE – Ser-245-Asp, Ser-336-Leu, and Ile-338-Glu, PD – progressive disease. †††Chi-square for start of treatment day 7 v day 8 v day 9 CR v PD p=0.2216.

Faster and stronger anti-tumor responses by 9D9.G2b.DLE compared to 9D9.G2b

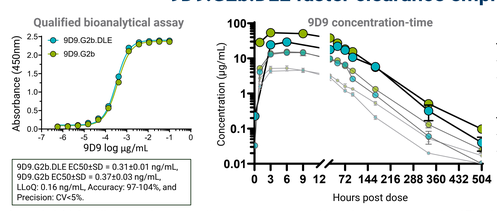




Article		200 µg	100 µg	50 µg	10 µg	5 µg	1 µg	0.1 µg	0 µg
9D9.G2b.DLE	CR	42	13	11	18	2	1	0	0
	Total	61	21	20	40	10	9	10	0
	%	69%	62%	55%	45%	20%	11%	0%	NA
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	0.0136	0.1111	0.9999	ns
9D9.G2b	CR	23	6	2	0	0	0	0	0
	Total	60	19	20	39	10	9	9	0
	%	38%	32%	10%	0%	0%	0%	0%	NA
	p-value	<0.0001	<0.0001	0.05	ns	ns	ns	ns	ns
Isotype & Vehicle	CR	0	0	X	0	X	X	X	0
	Total	9	18	X	20	X	X	X	24
	%	0%	0%	N/A	0%	N/A	N/A	N/A	0%

Legend. Aggregated average and SD tumor volume data from 407 Balb/c mice subcutaneously engrafted with 10^5 CT26 tumor cells then treated with a single administration of the indicated dose range of Fc engineered anti-mouse CTLA-4 (upper left) 9D9.G2b.DLE or (upper right) parental 9D9.G2b given on study day 7, 8, or 9 and monitored for indicated period for experiments 1-5 with (inserts) tumor volume by time shown and individual animals for 9D9.G2b.DLE and 9D9.G2b-treated mice. Two-way mixed-effect ANOVA. (lower center) Graphical and tabulated representations of CR rates (*i.e.* undetectable tumor volume measurement) shown by treatment and dosage for all experiments. Fisher's exact test versus all isotype and vehicle groups. (lower left and lower right). Complete response rate following 68-day observation of single administration of 200 µg test articles; data from experiment 6. Abbreviations – CR – complete response, 9D9.G2b.DLE – mouse IgG2b Ser-245-Asp, Ser-336-Leu, and Ile-338-Glu, G2b – mouse IgG2b, mm – millimeter, N/A – not applicable.

9D9.G2b.DLE faster clearance emphasizes superior efficacy due to higher potency, not drug exposure



Drug	Dose (µg)	Cavg (µg/mL)	Cmax (µg/mL)	Cmax/dose (µg²/mL)	Tmax (h)	$T_{1/2}$ (h)	$AUC_{0-\infty}$ (µg/mL*d)	Vz (mL)	Cl (mL/h)	Lambda z (1/h)
9D9.G2b.DLE	100	16.89	37.98	0.38	9	51.75	2837.1	2.63	0.035	0.013
	30	5.62	14.77	0.49	6	49.93	943.4	2.29	0.032	0.014
	10	1.66	4.62	0.46	9	49.98	279.0	2.58	0.036	0.014
9D9.G2b	100	23.23	53.86	0.54	3	53.65	3902.4	1.98	0.026	0.013
	30	6.51	15.38	0.51	6	52.61	1093.2	2.08	0.027	0.013
	10	2.10	5.29	0.53	6	60.14	352.5	2.46	0.028	0.012

Legend. (left) Qualified bespoke enzyme-linked immunosorbent bioanalytical assay in which CTLA-4-His was immobilized, incubated with 9D9, washed, detected with goat anti-mouse IgG antibody plus tetramethylbenzidine, and measured at 450 nm. (middle) Concentration time curve in plasma from Balb/c mice treated intraperitoneally with 10, 30, or 100 µg 9D9.G2b.DLE or 9D9.IgG2b once. Each dose given to 5 groups of 4 mice (group size = 4) with each limited to four of the following collection points 0.083, 1, 3, 6, 9, 24, 48, 72, 96, 168, 336, and 504 hours (h). Blood was collected by submandibular venous puncture in K2EDTA-coated tubes. (right) PK parameters were determined following a non-compartmental analysis. Abbreviations, $AUC_{0-\infty}$ - Area under curve to infinity, Cavg – average concentration, Cl –clearance, Cmax – maximum concentration, LLoQ – lower limit of quantitation, $T_{1/2}$ – half-life, Tmax – time of Cmax, Vz – volume.

Conclusions

9D9.G2b.DLE was determined to be a reasonable surrogate for botensilimab. Botensilimab^{MS} showed enhanced potency and efficacy as compared to unmodified 9D9.G2b. Exposure parameters for lowest efficacious doses were determined:

- 9D9.G2b.DLE lowest efficacious dose: **5 µg (Cavg=0.83 µg/mL)**
- 9D9.G2b lowest efficacious dose: **50 µg (Cavg=11.6 µg/mL)**

References and Correspondence

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