

Supplemental Information

A novel bispecific c-MET/CTLA-4 antibody targeting lung cancer stem cell-like cells with therapeutic potential in human non-small cell lung cancer

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Table S1. Expression of CD166, CTLA-4 and c-MET in NSCLC samples

Case	Age	TNM	Clinical stage	Percentage of CD166 ⁺ cells	^a Normalized CTLA-4 amount $2^{-\Delta\Delta Ct}$	^a Normalized c-MET amount $2^{-\Delta\Delta Ct}$	Sphere formation
1	42	T1N0M0	IA	0.1%	3.3600	2.3657	No
2	35	T2N0M0	IB	0.3%	4.3423	5.2657	Yes
3	54	T1N1M0	IIA	0.6%	3.2454	3.2147	Yes
4	36	T2N1M0	IIA	0.7%	5.5938	4.1139	Yes
5	57	T2N2M0	IIB	1.1%	3.3579	2.4876	Yes
6	45	T3N0M0	IIIA	1.2%	4.2974	3.2014	Yes
7	48	T3N3M1	IV	0.8%	2.4732	3.1584	Yes
8	37	T2N2M0	IIIA	0.3%	3.4923	4.6957	Yes
9	46	T2N0M0	IB	0.5%	1.9832	6.3952	Yes
10	61	T2N3M0	IIIB	0.9%	5.0394	5.3461	Yes

^a Relative quantification was performed by the $2^{-\Delta\Delta Ct}$ method with the adjacent lung tissue sample as a calibrator. Data show the means from three independent analyses. Every independent analysis

was carried out after the RNA extraction step. Total RNA was poly-A tailed, reverse transcript, and then real-time PCR tested. ΔC_T obtained from real-time PCR was subject to paired t-test ($\Delta C_T = C_{T \text{ CTLA-4/c-MET}} - C_{T \beta\text{-actin}}$). The expression levels of CTLA-4/c-MET in tumor tissues were significantly higher than normal tissues ($P < 0.01$)

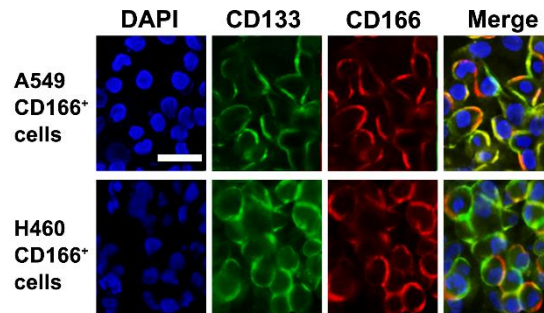


Figure S1, Expression of CD133 in CD166⁺ spheres by immunofluorescence staining. Scale bar, 100 mm.

Table S2. The affinity and specificity of BsAb-5

ID	Epitope group	Competitive Fab ELISA			EC50 (nM)	SPR (KD)
		0 nM	50 nM	5 nM		
BsAb-5	A	100.00%	17.83%	63.21%	17.91	1.79E-09