Generation of immuno-modulatory receptor binding bispecific antibodies to modulate tumor immunity (B088)

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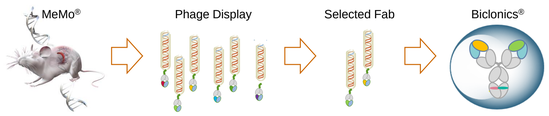
Introduction

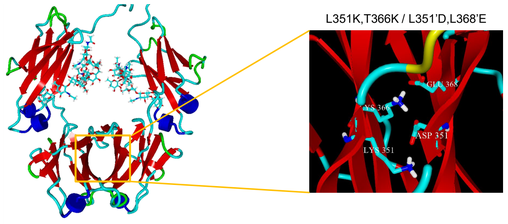
- Monoclonal antibodies blocking CTLA-4 or PD-1 axis have been shown to induce durable clinical responses in a subset of melanoma, NSCLC, renal cell carcinoma and urothelial carcinoma patients.
- Based on clinical and preclinical data it is expected that combinations of antibodies blocking inhibitory and/or costimulatory molecules could benefit patients that do not respond to existing immunotherapies.
- Dual blockade of immuno-modulatory receptors (iMODs) has been shown to increase immune-related toxicity.
- Bispecific antibodies may be ideally suited to address dual blockade of iMODs, as they can potentially exert functional activities that cannot be reproduced by monoclonal antibody combinations, and can more selectively target specific cell populations, which could reduce safety liabilities in patients.
- We report here the generation and characterization of common light chain (cLC) Fab panels against PD-1, PD-L1, LAG-3, CD137, OX40 and TIM-3 for combination in a full length IgG1 bispecific antibody format and present the functional evaluation of some of these combinations in *in vitro* functional assays using human cells.

Merus technology platform

- MeMo® is a proprietary mouse line that generates a wide variety of human antibodies characterized by a common light chain (cLC).
  - MeMo® generates a robust immune response after immunization (protein, DNA or cells).
  - Target specific human Fab fragments are selected via phage display technology from the repertoire generated in MeMo®.
  - With the Biclonics® technology platform bispecific IgG molecules can be generated with VH sequences isolated from MeMo®



- Biclonics® technology platform
  - CH3 regions are engineered to generate bispecific human IgG1 molecules.
  - Biclonics® can be efficiently produced in a single cell.

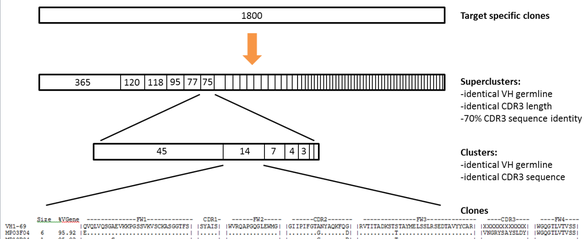


From Fab panels to bispecific antibodies

- cLC antibody panels were generated for 6 iMOD targets: PD-1, OX40, TIM-3, LAG-3, CD137 and PD-L1 ⇒ per target >1000 clones were selected.
- Before generation of bispecific antibody combinations, Fab clones representative for each panel were identified based on VH sequence diversity (see below for VH sequence diversity analysis)
- Thereafter representative clones were further binned on binding characteristics (for examples of binning results see below and to the right).

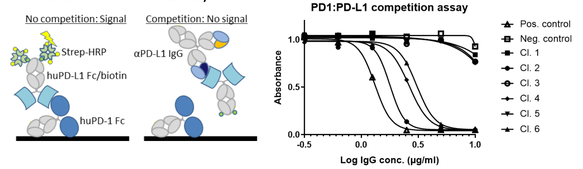
VH sequence diversity analysis

- All binding clones within each panel were grouped based on VH germline and CDR3 amino acid similarity. Representative clones from each group were chosen for further characterization.

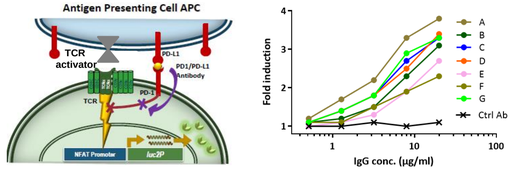


Ligand blocking ability/functionality

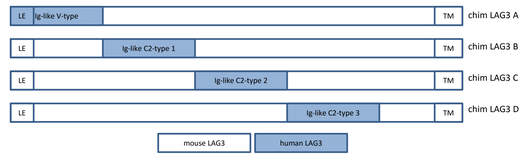
- iMOD panels were binned on the ability to prevent ligand-receptor interaction with in-house developed ELISA based assays and cell-based reporter assays.
- Figure below shows results (right panel) obtained for representative clones within the PD-L1 antibody panel tested as monovalent/bispecific IgG in an ELISA based PD-1:PD-L1 binding assay (left panel), and compared to a bivalent control antibody.

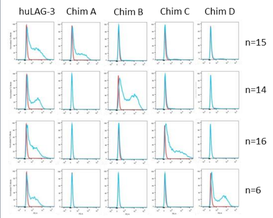


- Figure below shows results (right panel) obtained for representative PD-1 blocking clones within the PD-L1 antibody panel tested as monovalent/bispecific IgG in the PD-1:PD-L1 reporter assay (left panel).



Domain specificity





- Domain specificity of the CD137, OX40 and LAG-3 panels were determined using human/mouse chimeric expression constructs in FACS.
- The figure above shows the constructs generated for LAG-3.
- Representative obtained binding patterns are shown on the left with the number of identified clones indicated, which shows the panel diversity.

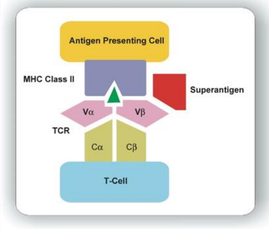
iMOD antibody panel binning overview

- The table below shows an overview of the different VH sequence groups, and the corresponding binding characteristics of each clone for part of the PD-L1 panel.

Clone information					FACS data				PD-1 blocking data		Affinity rank
Ab no.	Super cluster	Cluster	CDR3 length	VH pI	Ag- cells	huPD-L1	moPD-L1	cyPD-L1	blocks PD-1	Ab./Bench IC50	Ab./Bench EC50
1	1	Cluster004	10	6.51	242	3486	460	4059	yes	ND	3.4
2	1	Cluster001	10	6.49	247	5109	831	4601	yes	2.3	1.9
3	2	Cluster009	12	9.23	259	2579	473	355	no	ND	1.6
4	2	Cluster009	12	7.98	250	2243	469	333	no	1.8	2.5
5	3	Cluster002	9	8.64	280	2674	291	4633	yes	ND	3.6
6	3	Cluster002	9	8.99	252	2952	649	3320	yes	2.2	2.1
7	4	Cluster032	11	7.9	247	3969	1038	3575	yes	2.7	2.2
8	4	Cluster073	11	7.91	254	4227	2476	3880	yes	2.0	1.9
9	5	Cluster019	12	8	250	3887	1053	3746	yes	2.5	3.1
10	5	Cluster007	12	8.96	261	3198	475	3189	low	ND	3.6
11	6	Cluster005	15	8.01	247	5143	453	4150	yes	1.3	1.3
12	6	Cluster005	15	8.6	245	3489	438	3602	yes	2.3	2.6
13	7	Cluster010	13	8.57	252	2387	445	1166	no	ND	2.8
14	7	Cluster016	13	5.28	250	2406	446	961	yes	ND	2.4
15	8	Cluster008	11	7.85	244	2484	2887	2886	low	0.7	4.9
16	8	Cluster124	11	7.91	249	4022	1344	4521	yes	2.9	1.6
17	9	Cluster038	10	8.59	247	2118	452	2633	yes	ND	2.9
18	9	Cluster023	10	8.64	273	1609	308	3118	yes	ND	4.0
19	10	Cluster017	10	6.47	250	4664	1225	4398	yes	2.0	2.0
20	10	Cluster017	10	8.64	258	4178	891	4010	yes	2.6	2.6
21	11	Cluster100	11	8.67	269	296	306	345	low	ND	11.8
22	11	Cluster043	11	8.74	235	3874	920	3043	yes	2.3	3.4
23	12	Cluster131	11	6.83	254	4705	2328	5576	yes	ND	1.3
24	13	Cluster027	12	8.6	243	3916	453	4461	no	5.5	2.1
25	13	Cluster151	12	8.05	253	4473	467	4043	no	2.0	1.6
26	14	Cluster069	9	7.92	247	1176	440	2541	no	ND	12.2
27	15	Cluster015	8	8.62	271	3550	514	3591	yes	3.5	3.3
28	15	Cluster015	8	8.03	273	3533	499	4193	yes	ND	2.4
29	16	Cluster035	12	9.43	260	4085	446	3714	yes	2.0	2.4
30	16	Cluster041	12	9.47	274	2758	312	3904	yes	ND	1.2
31	17	Cluster104	11	8.66	248	3007	451	334	no	ND	3.5
32	18	Cluster097	12	6.49	242	2497	446	3038	low	ND	3.7
33	18	Cluster021	12	7.93	244	2121	437	2156	low	ND	6.3
34	19	Cluster098	10	8.6	263	3025	639	3134	low	ND	3.7
35	19	Cluster057	10	8.03	256	1162	566	2453	yes	ND	6.6
36	20	Cluster044	10	6.11	258	2307	450	2251	no	ND	9.2
37	20	Cluster086	10	6.4	247	5506	1118	4475	no	1.7	1.9
38	21	Cluster047	10	8.6	270	4821	503	4752	no	1.9	1.6
39	21	Cluster091	10	7.95	249	3479	444	3157	no	ND	2.6
40	22	Cluster037	14	7.93	249	2762	458	5121	yes	ND	2.1
41	22	Cluster037	14	6.51	288	2967	437	3411	yes	ND	2.6
42	23	Cluster142	13	7.99	249	3547	472	3760	no	ND	2.2
43	24	Cluster067	17	5.21	245	4466	442	3769	yes	2.5	2.5
44	24	Cluster085	17	7.94	254	2449	452	2639	yes	ND	2.6
45	25	Cluster052	10	8.04	250	932	496	1825	no	ND	6.9
46	25	Cluster052	10	8.64	292	514	310	1209	no	ND	11.5
47	26	Cluster053	15	8.66	278	2958	479	3279	yes	ND	3.7
48	26	Cluster053	15	8.64	249	3052	500	4711	yes	ND	3.4
49	27	Cluster175	13	8.05	246	3908	469	3380	yes	ND	1.8
50	27	Cluster090	13	8.6	244	3476	464	4414	yes	ND	1.9

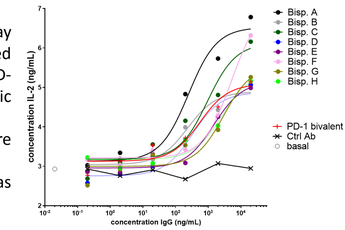
Functional evaluation bispecific antibodies

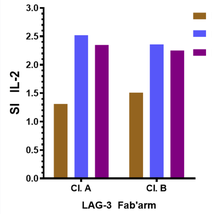
- The determined VH sequence and binding characteristics of the tested clones were used to select representative clones for generation of bispecific antibody combinations.
- Generated bispecific antibodies were tested in *in vitro* functional assays using human primary cells.



- The Figure on the left is a schematic representation of the *in vitro* SEB assay that was used, in which superantigen (SEB) activates T-cells by linking the T-cell receptor to MHC Class II molecules on antigen presenting cells.
- Increased T-cell activity by addition of (bispecific) antibodies is assessed by measuring the IL-2 levels in the medium.

- On the right, SEB assay results are shown obtained with a dilution series of PD-1xLAG-3 clones in bispecific IgG1 format.
- Used IgG samples were produced in 24 well format.
- A PD-1 control antibody was included as reference.





- On the left, SEB assay results are shown obtained with 4 LAG-3xPD-1 bispecific IgG1 samples.
- Bispecific IgG samples were generated in 24 well format with 2 different PD-1 and LAG-3 cLC Fab arms.
- A LAG-3 control antibody was included as reference.

Conclusions

- Large diverse common light chain Fab panels binding six different iMODs were generated for use in bispecific antibody combinations.
- Characterization and screening of large panels of bispecific IgG has been carried out in relevant functional assays.
- Merus' platform technologies allow for the screening of huge repertoires (both in number and in iMOD combination) of bispecific antibodies to identify potent immunomodulatory combinations.