Generation of Wnt- and mitogenic receptor binding bispecific antibodies to target cancer stem cells (C21)

Berina Eppink[1,4], Rob Roovers[1,4], Bram Herpers[2,4], Wim de Lau[3,4], Carina Clements[1,4], Vanessa Zondag van der Zande[1,4], Abdul Basmeleh[1,4], Willem Bartelink[1,4], Marc van de Wetering[3,4,5], Robert Vries[3,4,5], Leo Price[2,4], John de Kruif[1,4] and Mark Throsby[1,4].

[1]Merus B.V. Utrecht, The Netherlands; [2]OcellO B.V. Leiden, The Netherlands; [3]Hubrecht Institute; Utrecht, The Netherlands; [4]suppresSTEM consortium, European Community FP7, grant agreement number 601876; [5]Hubrecht Organoid Technology (HUB), Utrecht, The Netherlands.

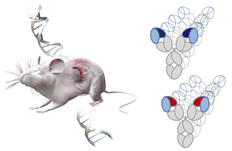
See also: www.suppresstem.eu

Introduction

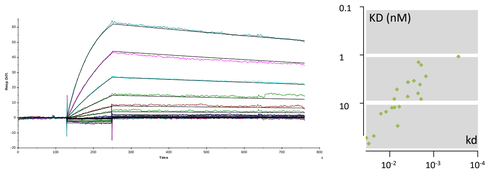
- Cancer stem cells (CSC) have the ability to self-renew over long periods of time to initial and sustain both primary and metastatic tumors.
- Recent evidence suggests that while conventional chemotherapy and current targeted therapies kill differentiated and differentiating cells that form the bulk of tumors, self-renewing cancer stem cell are less sensitive to these therapeutic approaches.
- We have generated large bispecific antibody panels that bind surface expressed proteins associated with the WNT pathway (LGR4, LGR5, ZNRF3 and RNF43) and RTK targets EGFR and HER3 to specifically target cancer stem cells and shut down important growth and differentiation pathways.

Merus Biclonics® technology platform

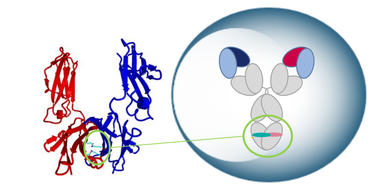
MeMo® common light chain mice



- MeMo® mice generates robust immune responses after immunization.
- MeMo® produces high affinity human antibodies that all share a common light chain (cLC).

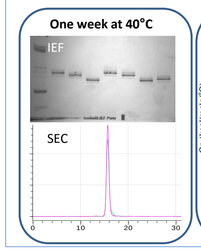


Bispecific IgG engineering

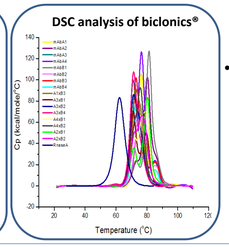


- cLC antibodies directly from MeMo® prevent heavy/light chain mispairing.
- CH3 regions were engineered to generate bispecific human IgG_1 molecules.
- Biclonics® can be efficiently produced in a single cell.
- Biclonics® are unaltered in accelerated stability assays and all have near wild type IgG melting temperatures.

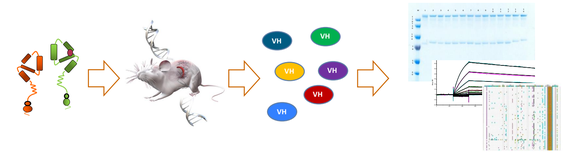
One week at 40°C



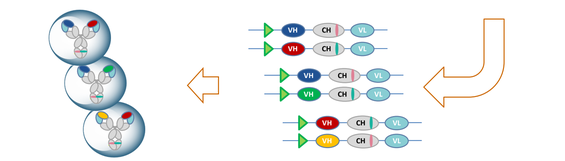
DSC analysis of biclonics®



Summary of CSC Biclonics discovery



4 New Targets — **54** MeMo® mice immunized — **499** new wnt target specific Fab arms — **53** Fabs selected after characterization

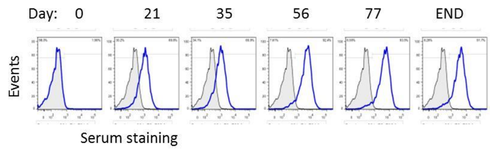


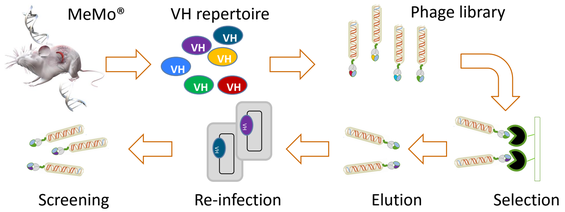
545 novel Bispecific antibodies produced in replicates using high throughput automated liquid handling.

62 VH cloning
53 WNT arms
8 HER arms
1 mock arm

WNT target immunization & phage antibody selection

- MeMo® mice were immunized with DNA (by tattooing shown left) or recombinant protein with adjuvant.
- Serum taken from mice was screened regularly for appearance of a target specific serum titer (example shown below).

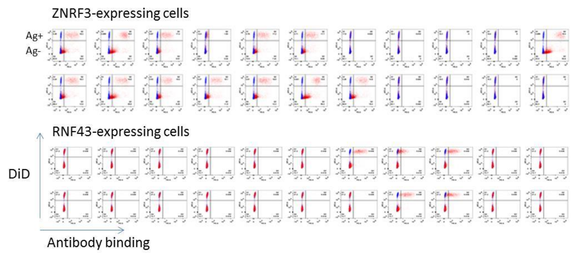




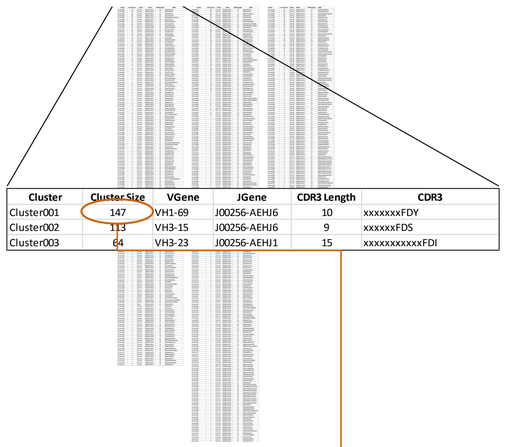
- From RNA extracted from lymphoid organs, phage antibody display libraries were made from which antigen-specific Fabs were isolated by phage display.

Screening of WNT immune cLC phage libraries

- Antigen-specific cLC Fab's from immune libraries of WNT target immunized MeMo® mice were identified by FACS based screening for binding to cells expressing the target.

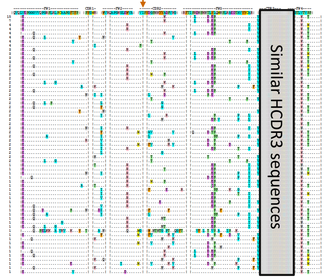


- Selection outputs screened positive for binding to the relevant WNT target were sequenced to establish VH identity and yielded 100^{nds} of unique and related sequences.



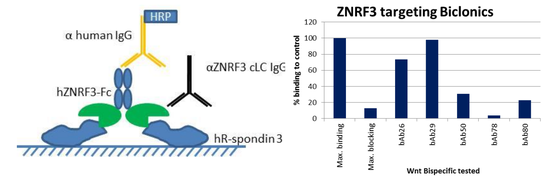
Cluster	Cluster Size	VGene	JGene	CDR3 Length	CDR3
Cluster001	147	VH1-69	J00256-AEHJ6	10	xxxxxxxFDY
Cluster002	113	VH3-15	J00256-AEHJ6	9	xxxxxxFDS
Cluster003	64	VH3-23	J00256-AEHJ1	15	xxxxxxxxxxxFDI

- Discovery process for the four WNT targets yielded very large clusters of related antibodies from which candidates with optimal affinity and biophysical characteristics could be selected (e.g. pI variants, lack of post translation of modifications, similarity to germline).



WNT antibody panel characterisation

- Multiple assays were performed to differentiate (bin) WNT specific cLC Biclonics (expressed as bispecific with a mock non-binding Fab arm).
- For example, the panel was characterised for the ability to block the WNT receptor interaction with the ligand R-Spondin (below).



- Combined data set used to select candidates for initial functional screening based on stability, affinity, sequence diversity, epitope diversity & ligand blocking activity (table of attributes given below).

						Affinity ELISA	Affinity FACS	R-Spondin blocking		40°C stability ELISA				Mouse cross reactivity FACS		
Ab nr.	Target	VH family	CDR3 length	Super-cluster	Cluster	ELISA (AUC)	FACS (AUC)	% binding remaining	R-Spondin blocking	OD_{450nm} Fresh	OD_{450nm} 4°C, 1wk	OD_{450nm} 40°C, 1wk	% 40°C/ 4°C	Stable at 40°C	Ag-	Ag+	Mouse Cross reactiv
1	LGR5	VH4-59	7	5	Cluster003	9.338	20843	91	No	0.829	0.606	0.309	51.0	Yes	282	326	No
2	LGR5	VH5-51	10	14	Cluster022	12.5	16024	96	No	1.816	1.63	1.739	106.7	Yes	653	568	No
3	LGR5	VH5-51	11	2	Cluster025	12.78	11199	92	No	1.92	1.738	1.529	88.0	Yes	451	392	No
4	LGR5	VH5-51	10	6	Cluster004	13.46	16095	97	No	1.74	1.722	1.741	101.1	Yes	1497	1139	No
5	LGR5	VH4-39	16	8	Cluster007	8.868	43203	59	Partial	1.115	1.005	0.876	87.2	Yes	308	3706	Yes
6	LGR5	VH4-59	18	9	Cluster014	13.87	24223	95	No	1.841	1.616	1.713	106.0	Yes	423	7559	Yes
7	LGR5	VH5-51	10	6	Cluster023	11.62	12767	88	No	1.682	1.603	1.858	115.9	Yes	991	706	No
8	LGR5	VH1-69	10	4	Cluster008	8.111	6847	98	No	0.732	0.601	0.325	54.1	Yes	268	359	No
9	LGR5	VH4-59	7	5	Cluster003	8.519	16260	85	No	0.686	0.532	0.325	61.1	Yes	276	316	No
10	LGR5	VH5-51	10	14	Cluster022	12.63	14966	96	No	1.82	1.598	1.627	101.8	Yes	869	669	No
11	LGR5	VH5-51	11	2	Cluster025	13.15	22628	94	No	1.72	1.506	1.681	111.6	Yes	715	650	No
12	LGR5	VH5-51	10	6	Cluster004	11.35	8779	95	No	1.679	1.596	1.27	79.6	Yes	360	373	No
13	LGR5	VH4-39	16	8	Cluster007	8.346	22890	62	Partial	0.885	0.785	0.58	73.9	Yes	324	4495	Yes
14	LGR5	VH1-69	10	4	Cluster010	7.799	6101	100	No	0.665	0.535	0.398	74.4	Yes	291	306	No
15	LGR5	VH5-51	11	2	Cluster025	12.15	24879	94	No	1.68	1.473	1.564	99.2	Yes	1113	981	No
16	LGR5	VH1-69	10	4	Cluster034	7.774	6007	111	No	0.533	0.431	0.235	54.5	Yes	281	348	No
17	LGR5	VH4-39	16	17	Cluster035	8.945	22197	80	Partial	0.722	0.655	0.401	61.2	Yes	432	2048	Yes
18	LGR5	VH5-51	11	2	Cluster029	9.293	24974	82	No	0.864	0.839	0.597	71.2	No	311	571	No
19	LGR5	VH1-69	12	1	Cluster013	8.963	40348	68	Partial	1.104	1.085	0.99	91.2	Yes	328	5073	Yes
20	LGR5	VH1-69	12	1	Cluster015	9.517	41734	63	Partial	1.091	1.074	0.975	90.8	Yes	285	5579	Yes
21	LGR5	VH1-69	12	1	Cluster012	9.642	40533	71	Partial	1.063	1.054	0.952	90.3	Yes	348	5514	Yes
22	LGR5	VH1-69	12	1	Cluster006	7.864	40187	59	Partial	1.048	0.985	0.867	88.0	Yes	379	4997	Yes
23	LGR5	VH1-69	12	1	Cluster016	8.956	40833	65	Partial	1.133	1.111	0.98	88.2	Yes	288	4529	Yes
24	LGR5	VH1-69	10	4	Cluster011	8.797	41872	95	No	1.085	1.05	0.964	91.8	Yes	531	5333	Yes
25	LGR5	VH1-69	10	4	Cluster031	8.001	19378	107	No	0.902	0.81	0.609	75.2	Yes	438	346	No
26	LGR5	VH1-69	12	1	Cluster040	9.264	44272	67	Partial	1.066	1.059	1.043	98.5	Yes	305	4758	Yes
27	RNF43	VH5-51	14	24	Cluster042	1.869	26991	107	No	1.326	1.12	0.525	46.9	No	399	884	No
28	RNF43	VH7-4-1	4	18	Cluster026	9.642	6150	100	No	0.709	0.594	0.285	48.0	No	474	6288	Yes
29	RNF43	VH3-13	14	1	Cluster050	10.14	83005	29	Yes	1.496	1.397	1.377	98.6	Yes	396	15900	Yes
30	RNF43	VH4-39	14	2	Cluster015	10.38	79575	71	Partial	1.525	1.396	1.335	95.6	Yes	525	22800	Yes
31	RNF43	VH3-13	14	1	Cluster003	8.931	83254	40	Yes	1.516	1.473	1.283	87.1	Yes	365	14400	Yes
32	RNF43	VH4-39	14	2	Cluster002	14.05	82972	71	Partial	1.512	1.523	1.529	100.4	Yes	445	59000	Yes
33	RNF43	VH3-13	14	1	Cluster047	12.52	82258	33	Yes	1.521	1.43	1.325	92.7	Yes	396	20500	Yes
34	RNF43	VH7-4-1	4	18	Cluster026	12.3	10550	100	No	0.949	0.795	0.356	44.8	No	530	8509	Yes
35	ZNRF3	VH7-4-1	11	6	Cluster004	9.608	32012	90	No	1.78	1.693	1.264	74.7	Maybe	415	45300	Yes
36	ZNRF3	VH7-4-1	11	12	Cluster017	11.29	29235	102	No	1.722	1.783	1.722	96.6	Yes	546	43400	Yes
37	ZNRF3	VH7-4-1	11	12	Cluster017	10.19	29680	93	No	1.799	1.673	1.705	102.0	Yes	480	46800	Yes
38	ZNRF3	VH7-4-1	11	5	Cluster005	10.89	38321	75	Partial	1.804	1.747	1.336	76.5	Maybe	398	53800	Yes
39	ZNRF3	VH5-51	13	26	Cluster048	0.5421	19142	96	No	1.762	1.659	1.5	90.4	Yes	367	37800	Yes
40	ZNRF3	VH5-51	13	26	Cluster048	10.85	19446	93	No	1.729	1.688	1.087	64.4	Yes	502	42100	Yes
41	ZNRF3	VH7-4-1	11	7	Cluster008	10.44	31884	90	No	1.656	1.562	0.928	59.4	Maybe	460	71500	Yes
42	ZNRF3	VH7-4-1	11	10	Cluster027	11.66	27470	85	No	1.523	1.587	0.973	61.3	Yes	501	47600	Yes
43	ZNRF3	VH7-4-1	11	9	Cluster039	7.763	12488	102	No	1.012	1.103	0.581	52.7	Yes	369	12300	Yes
44	ZNRF3	VH7-4-1	14	13	Cluster043	7.433	9295	105	No	1.09	0.948	0.695	73.3	Maybe	379	28200	Yes
45	ZNRF3	VH7-4-1	11	9	Cluster011	12.89	26933	86	No	1.776	1.714	1.776	103.6	Yes	646	43500	Yes
46	ZNRF3	VH7-4-1	11	7	Cluster014	12.04	46341	50	Yes	1.813	1.793	1.357	75.1	Yes	470	66800	Yes
47	ZNRF3	VH7-4-1	11	15	Cluster022	12.71	22920	104	No	1.683	1.641	1.272	77.1	Yes	542	11500	Yes
48	ZNRF3	VH7-4-1	14	13	Cluster032	12.2	24318	93	No	1.879	1.602	1.415	88.1	Yes	365	45400	Yes
49	ZNRF3	VH7-4-1	11	10	Cluster010	12.19	36923	82	No	1.821	1.434	1.137	79.3	Yes	674	54700	Yes

Conclusions

- WNT targeting Fab's were selected on the basis of their stability, affinity, R-Spondin blocking capacity and ortholog cross-reactivity.
- In total, 53 Fabs were selected (9xLGR4, 17xLGR5, 9xRNF43 and 18xZNRF3) for functional screening.
- These were combined with 8 different RTK (i.e. EGFR- and HER3-) targeting Fab's in bispecifics for functional testing.
- **See Poster C24 for the results of the initial functional screening.**