

Fully Human Monoclonal Antibody Development In 60 Days





Strategic Alliance

Precision Antibody, a service division of A&G Pharmaceutical Inc is pleased to announce business alliance with **Trans Chromosomics**







A&G Pharmaceutical Inc/Precision Antibody

- Theranostic antibody development against breast & lung cancer
- Global leader in antibody development

Trans Chromosomics (TC)

- Global Leader Of Artificial Chromosome Engineering Technology
- Innovative Platforms For Biopharmaceuticals, Cell/Gene Therapy, And Xenotransplantation



CEO, Ginette Serrero Ph.D. Established: 1, June 2000 www.agpharma.com



President CEO, Mitsuo Oshimura Ph.D. Established: 17 December 2014 www.trans-chromo.com



President, Jun Hayashi Ph.D. Established: 1, June 2000 www.precisionantibody.com



Summary

- ➤ TC mAb[™] mice are not immuno-suppressed and develop strong titer against the immunogen in short period of time using PA technology.
- > PA generates fully human monoclonal Ab from TC mAb™ mice in about 60 days.
- > TC mAb™ is the mouse that expresses 100% human antibody.
- > The generated mAbs do not require further molecular manipulation such as constant region gene swapping.
- ➤ TC mAb[™] mice provides increased efficiency in the generation of Ag-specific mAb producing hybridoma clones; i.e., A large number of clones; Diverse epitope specificity.
- > Fully human mAb generated from TC mAb™ mice represent normal human IgG repertoire.



Case Study 1

Immunogen: ~90KDa protein with ~75% homology with mouse ortholog

Duration of the project: 60 days from immunization to expansion of antigen-specific clones.

Serum titer prior to fusion:

Serum Dilutions	1:1K	1:3K	1:10K	1:30K	1:100K	control
A ₆₂₀	3.40	2.90	1.66	0.64	0.10	0.04

Number of Ag-positive mAb producing clones by ELISA screening:

antigen: 30 ng/well 1° Ab: 100 ng/well

OD	# of Ag-positive clones (out of 1727 clones)
0.5-1.0	335
1.0-2.0	423
2.0->3.0	387
>3.0	183
Total Positiv	ve 1,228

~77% of clones were positive against the immunogen.

Case Study 2

Immunogen: ~50KDa protein no homology to mouse proteins

Duration of the project: 60 days from immunization to expansion of antigen-specific clones.

Serum titer prior to fusion:

Serum Dilutions	1:1K	1:3K	1:10K	1:30K	1:100K	control
A ₆₂₀	3.02	2.94	2.60	1.80	0.95	0.04

Number of Ag-positive mAb producing clones by ELISA screening:

antigen: 50 ng/well 1° Ab 100 ng/well

OD	# of Ag-positive clones (out of 1920 clones)
0.5-1.0	358
1.0-2.0	205
2.0-3.0	276
>3.0	118
Total Positiv	ve 957

~50% of clones were positive against the immunogen



Advantages of TC mAb ™ mice for the Development of Therapeutic mAbs

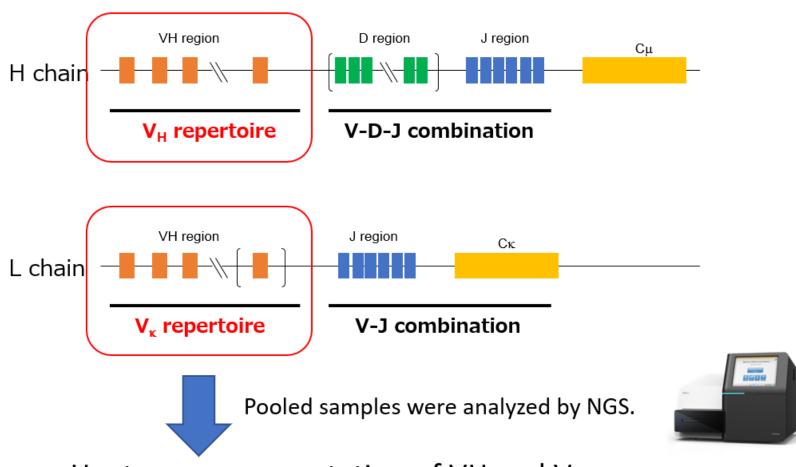
	Wild-type	Fully human IgG	
Animals	Balb/c, ICR, etc.	TC-mAb mice	
Immune response	Mouse IgG	Human IgG	
Antibody titer	Exellent	Exellent	
IgG subclass	mlgG1,2a, 2b, and 2c	hlgG1, 2, 3, 4	
Humanization	The entire region	Not necessary	
Ag-specific mAb generation	Normal	High ratio	
Size of spleen	Enlarged	Normal	
B cell development	Normal	Expanded	
Antigen-specific B cells	Normal	Expanded	
Ig-gene Repertoire	Normal mouse	Normal human	
Somatic hypermutation	Yes	Yes	
Length of CDR3H	Mouse (short)	Human (long)	

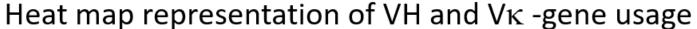
Summary

- ➤ TC mAb[™] mice represents normal human IgG repertoire.
- ➤ MABs generated from TC mAb™ mice are fully human, affinity matured and do not require any further molecular manipulations.



Normal Representation of Human Ab Repertoire by TC mAb™mice: Continued







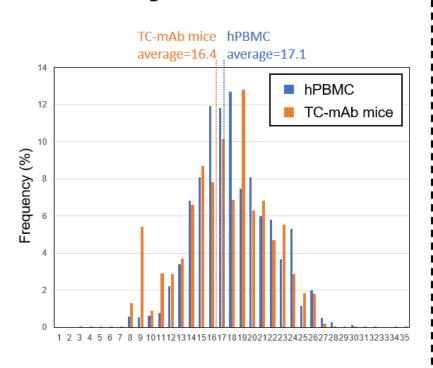
Normal Representation of Human Ab Repertoire by TC mAb™mice: Continued

VH gene usage (IgM&IgG) Vk gene usage VH gene usage (IgM & IgG) Vκ gene usage TC-mAb™ mice TC-mAb™ mice hPMBC Unimmunized Immunized hPMBC Unimmunized Immunized Reads 300,601 474,622 696,580 Enlarge IGHV1-2 301,138 243,321 486,580 Reads Enlarge IGHV1-3 IGHV1-8 IGKV1-5 IGHV1-18 IGKV1-6 IGHV1-24 IGKV1-8 IGHV1-38-4 IGKV1-9 IGHV1-46 IGHV2-/U IGHV2/OR16-5 IGHV3-7 IGHV3-9 IGKV3-15 ► IGKV3-20 IGHV3-11 IGKV3/OR2-268 IGHV3-13 IGHV3-15 IGKV3D-20 IGHV3-22 IGHV3-23 IGHV3-23D ► IGKV4-1 IGKV5-2 **IGHV3-25** IGKV6-21 IGHV3-29 - TC-mAb mice : Closely similar to hPBMC repertoire. - TC-mAb mice : Closely related with hPBMC repertoire. **hPMBC** - Immunized mice: Frequency of germline usage in VH region - Immunized mice : Frequency of germline usage in Vκ was conserved. region was also conserved.

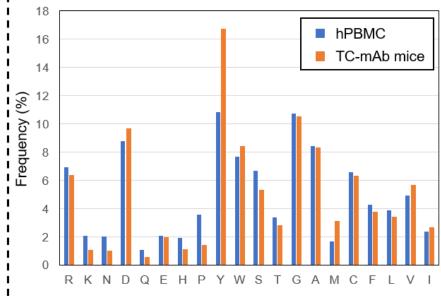


Normal Representation of Human Ab Repertoire by TC mAb™mice

CDR3H Length



CDR3H Amino acids usage



Both amino acid length and composition in CDR3 region of the antibody heavy chain variable region (VH) were almost the same with human PBMC.

It was speculated that antibodies similar to human individuals can be produced from TC-mAb mice.

