WHITE PAPER

Leveraging the immunological diversity of the PentaMice® platform for COVID-19 antibody discovery

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Hybridoma technology is a popular method for antibody discovery, but the conventional approach of using a single inbred mouse strain for immunization fails to generate the diversity and antibody titers needed to maximize the discovery of high-quality leads. This white paper introduces an alternative immunization approach — the PentaMice platform, a collection of five wildtype mouse strains bred in-house for increased MHC class II diversity — and highlights how Curia is leveraging it for COVID-19 antibody discovery.

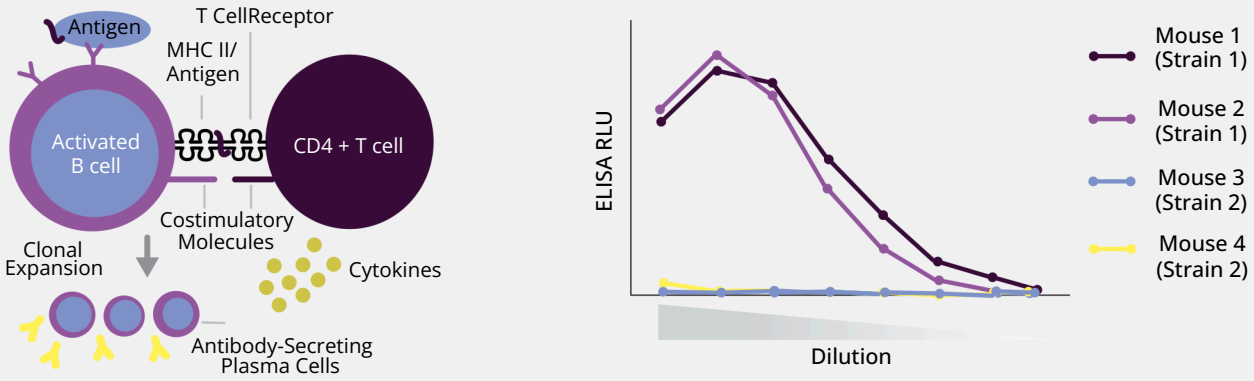
Most approved therapeutic antibodies on the market today were derived from hybridomas,[1] a technology that has remained largely unchanged since its invention by Köhler and Milstein 47 years ago. To create a hybridoma, animals are first immunized with a target antigen, after which their B cells are isolated and fused to immortal myelomas. Hybridoma clones are then screened and selected for target reactivity. After a target-specific clone has been identified, the originating hybridoma serves as an endless source for further production of the clonal antibody.

With hybridoma technology, antibody diversity and plasma titers, which are predictive of antibody discovery success, are generated by the B cells of immunized animals. These B cells use major histocompatibility complex (MHC) class II molecules to present the target antigen peptides to T cells, activating them and causing them to express co-stimulatory molecules and secrete cytokines (Figure 1a). These signals converge to stimulate clonal B cell amplification and high affinity antibody production. Maximizing this response requires CD4 T cell help, which is driven by T cell receptor recognition of peptides presented by MHC II.

MHC II molecules are highly polymorphic, which means there is substantial within-species variation among MHC class II genes, and highly polygenic, which means that each allele in the MHC class II locus

Figure 1. The concept behind the PentaMice platform

a.b.



c.

Peptide 1	—	—	—	—	—
Peptide 2	—				
MHC Class II Haplotype	IAk,IAg7, IEk	IAd, IAu, IEd, IEu	IAb, IAs	IAv, IAq, IEv	IAmixed,IE^{mixed}
PentaMice Strain	k x g7	d x u	b x s	q x v	mixed

Strong interactions between T and B cells during immunization (a) are essential to obtaining high antibody plasma titers. Because there are strain-dependent differences in plasma titers for most targets (b), using several mice representing different MHC II haplotypes (c) improves the chances for maximal engagement of T cell help and, consequently, high affinity antibody generation.

can harbor several different versions of the gene. These characteristics of the MHC class II locus likely contribute to differences in plasma titers observed between different strains of mice (Figure 1b), as different MHC class II alleles (called haplotypes) confer different peptide-binding profiles. For example, one peptide may be effectively presented by most MHC II molecules, whereas another may be effectively presented by only one (Figure 1c).

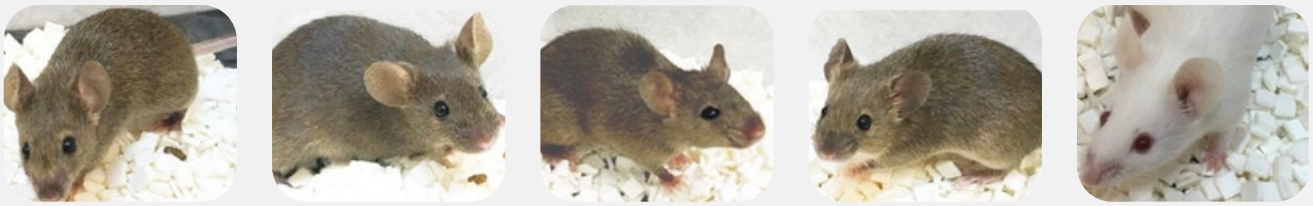
Conventional immunization strategies generate limited antibody diversity and titers because they typically use a single inbred mouse strain (e.g., C57Bl/6) of a single homozygous MHC II haplotype. To vastly improve antibody diversity and titers in hybridoma-based antibody discovery, Curia developed the PentaMice platform, a set of five wildtype mouse strains representing nine MHC II haplotypes. This white paper highlights the PentaMice platform and its application to the discovery of COVID-19 therapeutics including neutralizing antibodies.

The PentaMice platform

The PentaMice platform comprises five wildtype mouse strains — b x s, d x u, k x g7, q x v and mixed — generated by crossing strains of different MHC II haplotypes. Unlike most mouse strains used for antibody discovery, each PentaMice strain possesses heterozygous, or two unique, MHC II haplotypes.

In mice, MHC II molecules are encoded by IA and IE loci. Whereas a single MHC II molecule is possible with inbred strains like the C57Bl/6 strain, 42 MHC II molecules are possible with the PentaMice platform:

Figure 2. The five strains of the PentaMice platform



b x s | d x u | k x g7 | q x v | Mixed

Two mice from each strain, totaling 10 mice, are used in hybridoma campaigns.

- b x s: $IA\alpha^{b}A\beta^{b}$, $IA\alpha^{s}A\beta^{s}$, $IA\alpha^{b}A\beta^{s}$, $IA\alpha^{s}A\beta^{b}$
- d x u: $IA\alpha^{d}A\beta^{d}$, $IE\alpha^{d}E\beta^{d}$, $IA\alpha^{d}E\beta^{d}$, $IE\alpha^{d}A\beta^{d}$, $IA\alpha^{u}A\beta^{u}$, $IE\alpha^{u}E\beta^{u}$, $IA\alpha^{u}E\beta^{u}$, $IE\alpha^{u}A\beta^{u}$, $IA\alpha^{d}A\beta^{u}$, $IA\alpha^{d}E\beta^{u}$, $IE\alpha^{d}A\beta^{u}$, $IE\alpha^{d}E\beta^{u}$, $IA\alpha^{u}A\beta^{d}$, $IA\alpha^{u}E\beta^{d}$, $IE\alpha^{u}A\beta^{d}$, $IE\alpha^{u}E\beta^{d}$
- k x g7: $IA\alpha^{k}A\beta^{k}$, $IE\alpha^{k}E\beta^{k}$, $IA\alpha^{k}E\beta^{k}$, $IE\alpha^{k}A\beta^{k}$, $IA\alpha^{g7}A\beta^{g7}$, $IA\alpha^{k}A\beta^{g7}$, $IA\alpha^{g7}A\beta^{k}$, $IE\alpha^{k}A\beta^{g7}$, $IA\alpha^{g7}E\beta^{k}$
- q x v: $IA\alpha^{v}A\beta^{v}$, $IE\alpha^{v}E\beta^{v}$, $IA\alpha^{v}E\beta^{v}$, $IE\alpha^{v}A\beta^{v}$, $IA\alpha^{q}A\beta^{q}$, $IA\alpha^{q}A\beta^{v}$, $IA\alpha^{v}A\beta^{k}$, $IE\alpha^{v}A\beta^{q}$, $IA\alpha^{q}E\beta^{v}$
- Mixed: $IA\alpha^{mixed}A\beta^{mixed}$, $IE\alpha^{mixed}E\beta^{mixed}$, $IA\alpha^{mixed}E\beta^{mixed}$, $IE\alpha^{mixed}A\beta^{mixed}$

This 42-fold increase in MHC II diversity enables a greater diversity of peptides to be presented by B cells to T cells during immunization, increasing the probability of a robust antibody response and high-quality lead generation.

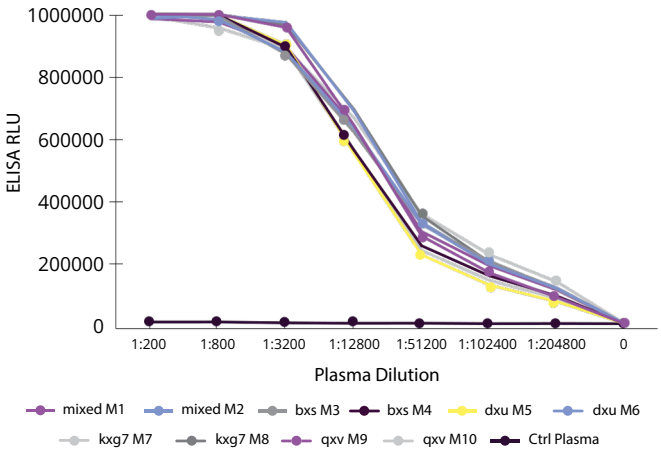
Discovering SARS-CoV-2 variant-specific antibodies

Since the COVID-19 pandemic began in January 2020, the discovery of therapeutic and diagnostic antibodies against SARS-CoV-2 has been complicated by the virus's unyielding evolution. SARS-CoV-2 Beta (B.1.351) was the first variant to demonstrate significant escape from natural and acquired (i.e., vaccine-induced) immunity.[2]

To discover antibodies that could be used to research, diagnose and treat SARS-CoV-2 Beta, Curia combined its PentaMice platform with a cascading screening workflow aimed at isolating Beta-specific antibodies. Immunization with the SARS-CoV-2 Beta spike protein elicited robust antibody responses in all PentaMice strains by day 17 post-immunization (Figure 3).

After B cell isolation, hybridomas were generated and screened for Beta selectivity. The primary screen identified 74 Beta-specific hybridomas, 80% of which were confirmed to be specific for the variant in the secondary screen. The tertiary screen, which

Figure 3. The PentaMice platform produces high antibody titers after immunization with the SARS-CoV-2 Beta (B.1.351) spike protein



All mice rapidly achieved goal plasma titer of 100,000 relative light units (RLU) at 1:50,000 dilution by day 17. Plate was coated with B.1.351 spike protein.

incorporated spike proteins beyond the wildtype and Beta variants, further narrowed the set of Beta-specific clones to 49. Thus, the PentaMice platform can be used to discover variant-specific antibodies against spike proteins that share at least as much as 99.3% identity with other spike proteins.

Discovering diverse, high-quality antibodies against SARS-CoV-2

In an example of the quality and diversity of antibodies that can be obtained with the PentaMice platform, a broader campaign targeting the wildtype SARS-CoV-2 spike (isolate WIV02) uncovered a diverse array of ~350 antibodies, 30% of which were found to be SARS-CoV-2 neutralizers. A total of 42 leads were sequenced and purified for further characterization. Although several of these candidates specifically bound the receptor-binding domain (RBD) of SARS-CoV-2 S1, others showed alternative specificities, including:

- SARS-CoV-2 S1-specific, non-RBD-targeting antibodies
- SARS-CoV-2 S2-specific antibodies
- SARS-CoV and SARS-CoV-2 cross-reactive antibodies
- Pan-coronavirus antibodies capable of binding SARS-CoV-2 S2, SARS-CoV S, MERS S, HKU1 S, HCoV-NL63 S, HCoV-229E S and HCoV-OC43 S

Two leads — B13 and O24 — were compared to clinical-stage antibodies in a series of binding and neutralization assays (Table 1).3 In the binding assays, B13 cross-reacted with the spike proteins of SARS-CoV-2 and SARS-CoV like VIR-7831, whereas O24 was selective for the SARS-CoV-2 spike (specifically, the RBD). Both B13 and O24 neutralized pseudoviruses

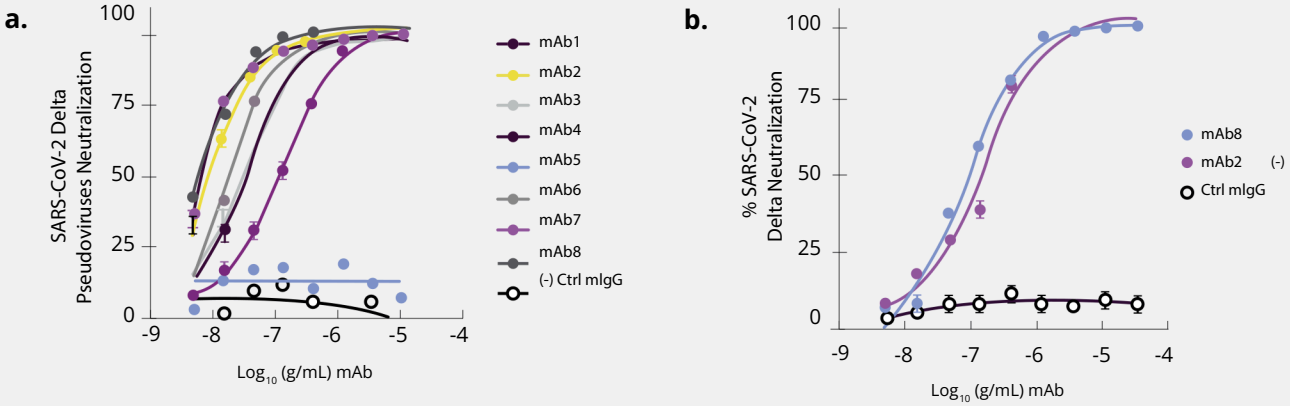
Table 1. Comparison of selectivity and potency of SARS-CoV-2 monoclonal antibodies[3]

							Neutralization IC50 (nM)		
			Binding			SARS-CoV-2 Live Virus	SARS-CoV-2 Pseudovirus		
	Antibody	Isotype	SARS-CoV-2 Spike	SARS-CoV-2 RBD	SARS-CoV-1 Spike	D614G	D614G	B.1.351	
	B13	IgG1 κ	+	+	+	2.48	0.052	1.53	
	O24	IgG1 κ	+	+	–	No data	0.024	0.012	
EUA for combination treatment in areas with <5% certain variants	LY-CoV555	IgG1 κ	+	+	–	No data	0.028	> 10	Bamlanivimab
	LY-CoV016	IgG1 κ	+	+	–	No data	0.168	> 10	Etesevimab
EUA requested	AZD1061	IgG1 κ	+	+	–	No data	0.035	0.045	Cilgavimab
EUA requested	AZD8895	IgG1 κ	+	+	–	No data	0.016	0.088	Tixagevimab
EUA	VIR-7831	IgG1 κ	+	+	+	No data	0.318	0.466	Sotrovimab
EUA requested	CT-P59	IgG1 λ	+	+	–	No data	0.002	0.286	Regdanvimab
EUA	REGN10987	IgG1 λ	+	+	–	No data	0.051	0.035	Imdevimab
EUA	REGN10933	IgG1 κ	+	+	–	No data	0.035	> 10	Casirivimab

encoding the D614G and Beta spike proteins with potencies rivaling clinical-stage antibodies such as AZD1061 (Cilgavimab), CT-P59 (Regdanvimab) and REGN10987 (Imdevimab), among others.

SARS-CoV-2 continues to evade COVID-19 public health measures, as demonstrated by the twin public health crises caused by SARS-CoV-2 variants Delta and Omicron. Indeed, most clinically relevant monoclonal antibodies fail to neutralize the recent Omicron BA.2 subvariant.[4] From our discovery campaign, we identified several antibodies that neutralize SARS-CoV-2 Delta (B.617.2) (Figure 4) and Omicron (BA.1 and BA.2) (Figure 5). Notably, clones mAb8 and mAb2 potently neutralize Delta pseudovirus (Figure 4a) and authentic Delta virus (Figure 4b), while mAb8 and mAb1 potently neutralize Omicron BA.1 (Figure 5a) and Omicron BA.2 (Figure 5b). These data show that the PentaMice

Figure 4. Neutralization of SARS-CoV-2 Delta by PentaMice antibodies



(a) A total of seven candidates potently neutralized Delta pseudoviruses. (b) In authentic virus assays, mAb8 and mAb2 neutralized Delta with IC50s of 0.56 and 1.10 nM, respectively.

Figure 5. Neutralization of SARS-CoV-2 Omicron by PentaMice antibodies

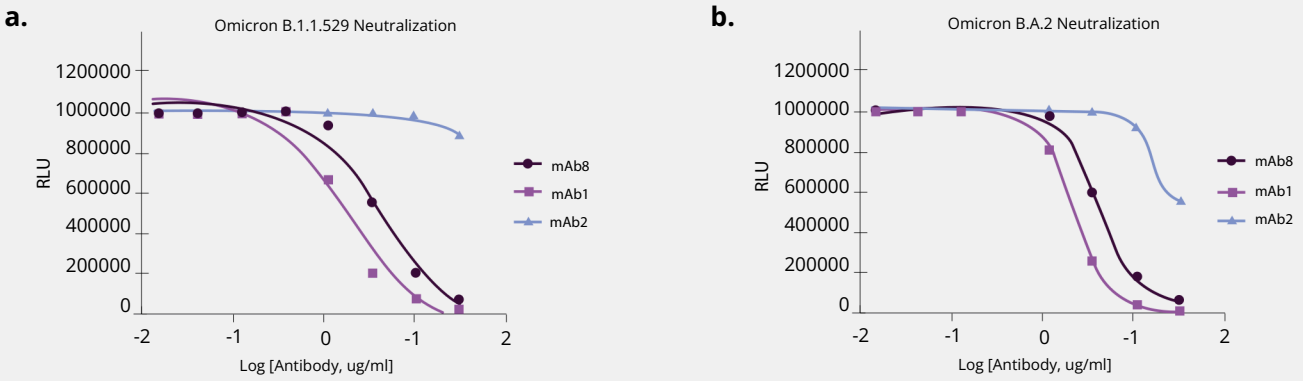


Table 2. Discovery of potent Omicron-spike binding and neutralizing mAbs

	Omicron B.1.1.529		Omicron BA.2	
mAb clone ID	EC50 (nM)	IC50 (nM)	EC50 (nM)	IC50 (nM)
mAb8	0.5	23.4	0.8	25.8
mAb1	0.5	10.1	1.1	13.8
mAb2	20.6	NI	61.9	partial

Clones mAb8 and mAb1 neutralized Omicron and B.1.1.529 (Figure 5a) and BA.2 (Figure 5b) binding to ACE2 by ELISA with nanomolar potencies (Table 2). NI: no inhibition.

platform yields high-quality antibodies capable of neutralizing SARS-CoV-2 variants, including Omicron (Table 2).

In addition to generating broadly neutralizing antibodies, the PentaMice platform also produced antibodies with different spike binding selectivities that could be potentially utilized in diagnostic applications. For example, clone mAb9 selectively binds to the WT and Gamma spikes; mAb11 to the Beta spike; mAb13 to the Beta and Omicron B.1.1.529 spikes and mAb14 to all spikes (Table 3). These varied binding selectivities highlight the diversity the PentaMice platform is capable of producing.

Table 3. Variant binding specificities of selected PentaMice antibodies

mAb	WT	Alpha	Beta	Gamma	Delta	Omicron B.1.1.529	Omicron BA.2
mAb9	+	–	–	+	–	–	–
mAb10	+	+	–	–	+	–	–
mAb11	–	–	+	–	–	–	–
mAb12	+	+	–	+	–	–	–
mAb13	–	–	+	–	–	+	–
mAb14	+	+	+	+	+	+	+

Conclusion

No matter the target, high-titer antibody production in immunized animals increases the quality and diversity of antibody leads during hybridoma campaigns. Curia's PentaMice platform leverages immunological diversity in the MHC II locus to maximize *in vivo* antibody production.

Let Curia help with your next hybridoma campaign. To learn more about the PentaMice platform, visit hybridoma.com.

Reference

1 Lu RM, Hwang YC, Liu IJ, et al. Development of therapeutic antibodies for the treatment of diseases. *J Biomed Sci.* 2020;27(1):1.

2. Harvey WT, Carabelli AM, Jackson B, et al. SARS-CoV-2 variants, spike mutations and immune escape. *Nat Rev Microbiol.* 2021;19(7):409-424.

3. Troitskaya L, Chan NLS, Frank B, et al. Mouse Antibodies with Activity Against the SARS-CoV-2 D614G and B.1.351 Variants. Preprint. *bioRxiv.* 2021;2021.07.05.451203.

4. Iketani S, Liu L, Guo Y, et al. Antibody evasion properties of SARS-CoV-2 Omicron sublineages. *Nature.* 2022;604(7906):553-556.

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