curia

Introduction to hybridoma technologies for antibody discovery

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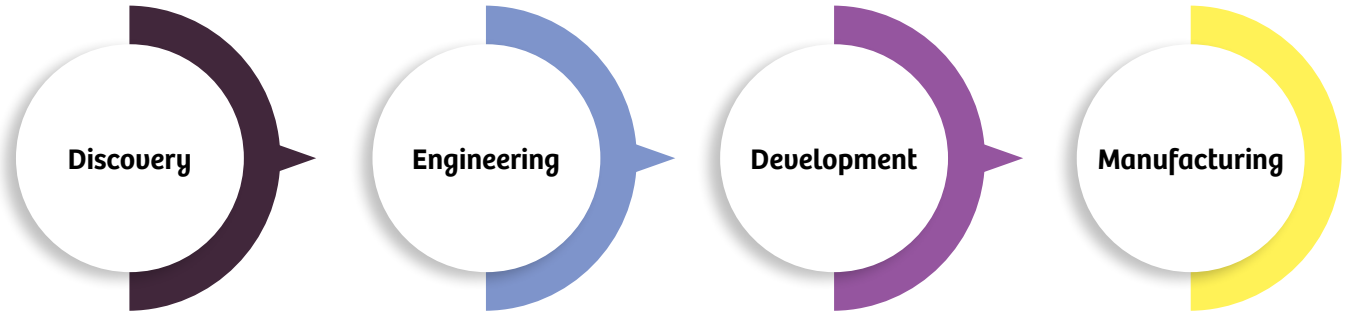
About Curia

Curia, formerly AMRI, is a leading contract research, development and manufacturing organization providing products and services from R&D through commercial manufacturing of large and small molecules to pharmaceutical and biopharmaceutical customers.



- Hybridoma platform for *in vivo* discovery
- *In vitro* phage and yeast display platforms
- Antibody functional characterization
- Epitope binning & mapping

- Humanization
- Affinity maturation & measurement
- Sequence liability identification
- Therapeutic developability assessment

- Transient antibody production using TunaCHO℠ platform
- Stable cell line development using proprietary CHO-GSN℠ platform
- Process development

- GMP manufacturing
- Tech transfer
- Biorepository

History

The origin of monoclonal antibodies

Köhler and Milstein, *Nature* Vol. 256 August 7, 1975 p495–7.

In the early 1970s, the field of antibody research was restricted by an inability to generate, isolate and purify single antibodies of a known specificity. On one hand, immortal myeloma cell lines were known to produce monoclonal antibodies or antibody fragments, though of unknown specificity. On the other hand, Norman Klinman and others had developed methods for cloning primary B cells that produced single antibodies of known specificity but were limited by low mAb yield and short cell lifespan. César Milstein's lab (Medical Research Council Laboratory of Molecular Biology, Cambridge, UK) had been studying the origin of antibody diversity for a number of years and at the time was using the technique of cell:cell fusion to study the potential role of allelic exclusion in antibody expression in myeloma cells.

Georges Köhler joined César Milstein's lab as a postdoctoral fellow in 1974. Together they developed the idea of a hybrid cell resulting from a cell:cell fusion between an immortal myeloma cell and a short-lived antibody-producing B cell with a designed target specificity. These "hybridomas" could theoretically make monoclonal antibodies against any specific antigen. The scientists immunized mice with sheep red blood cells, a target known to elicit a strong antibody response *in vivo,* and fused the splenocytes with myeloma cells, creating the world's first hybridomas. Each hybridoma possessed the immortal growth feature of the myeloma and the antibody-producing feature of the plasma cell. They screened for antibody target specificity by a plaque assay (lysis of sheep red blood cells) and identified a number of hybridomas producing target-specific antibodies. These hybridomas could be cloned, thus for the first time enabling production of large amounts of target-specific monoclonal antibodies.

Köhler and Milstein shared the 1984 Nobel Prize in Physiology or Medicine for their groundbreaking discovery.

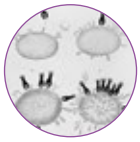
History

Evolution of antibody technologies

1796 British country doctor Edward Jenner uses cowpox to inoculate patients and protect them against smallpox. Today, Jenner is recognized as the "Father of Immunology" for his contributions to the field of vaccination.

1890 Emil von Behring and Shibasaburo Kitasato show that serum from infected animals can be used to treat as well as prevent infection in other animals. Eventually their idea is carried out in humans and used to treat pediatric cases of diphtheria.

1900 Paul Ehrlich introduces his side-chain theory: cells can express a variety of "side-chains" that can be released into the bloodstream and act as antitoxins or antikörpers (antibodies).



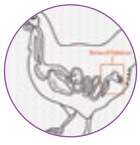
1914–1918 Horse serum therapy is expanded on a grand scale to combat tetanus during World War I; thousands of soldiers are presumed to have survived because of it.

1945 Following Karl Landsteiner's discovery of the ABO blood group system as well as rhesus factors, Robin Coombs develops the Coombs test which detects pre-existing antibodies to rhesus factors in the blood.

1947 During the completion of her doctorate, Astrid Fagraeus demonstrates that plasma cells (mature B cells) are responsible for the production of antibodies.

1957 Frank Macfarlane Burnet proposes his clonal selection theory, explaining how lymphocytes respond in the presence of antigens and how each lymphocyte produces antibodies with a single specificity.

1965 After working with children suffering from Wiskott-Aldrich syndrome, Max Cooper and his collaborators discover the bursa of Fabricius (an organ exclusively found in birds) as the organ generating antibody producing B cells.



1969 Gerald Edelman describes the structure of an antibody protein.

1975 While working at Cambridge University, Georges Köhler and César Milstein concretize the idea of fusing myeloma cells with B cells, resulting in the creation of synkaryon cells, later named hybridomas, that have the capabilities of secreting antibodies of a single specificity.

1976 Susumu Tonegawa determines the rearrangement of immunoglobulin genes and demonstrates the genetic mechanism that results in antibody diversity.

1977 The Food and Drug Administration (FDA) approves first home pregnancy test which uses antibodies specific for the human chorionic gonadotropin (hCG) hormone.

1986 The FDA approves the first therapeutic monoclonal antibody: muromonab-CD3 for prevention of kidney transplant rejection.

1992 The FDA approves the first diagnostic monoclonal antibody: indium-111 satumomab pendetide, targeting the tumor-associated glycoprotein 72 (TAG-72), for the detection and imaging of colorectal and ovarian tumors.

1992–1995 James Allison and Tasuku Honjo independently discover the first cancer checkpoint inhibitors: programmed cell death-1 (PD-1) and cytotoxic T-lymphocyte-associated protein-4 (CTLA-4).

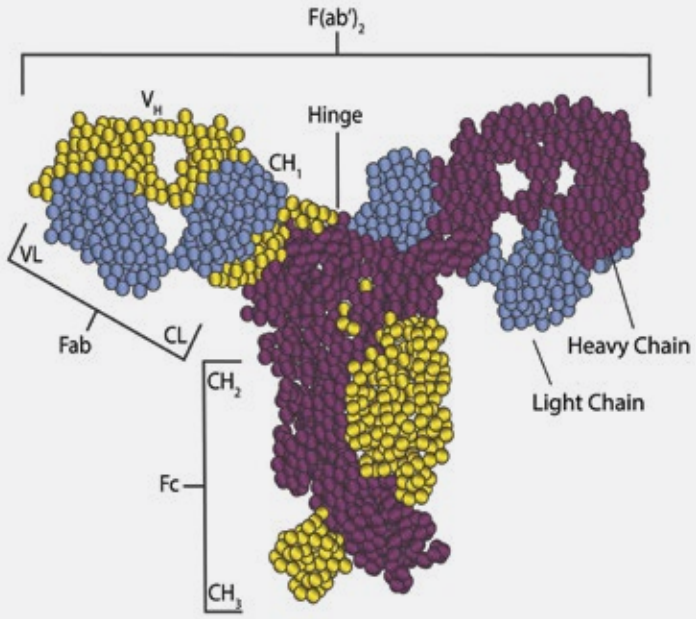
1998 The FDA approves the first cancer "immunotherapy": trastazumab for human epidermal growth factor receptor (HER2) overexpressing breast cancer.

2006 The FDA approves the first fully human monoclonal antibody derived from a transgenic mouse: panitumumab for the treatment of epidermal growth factor-receptor (EGFR) positive colorectal cancer.

2020 Combined worldwide sales of monoclonal antibody-based therapies is estimated to reach $150 billion.

Antibody structure

Along with the double helix of DNA, the distinctive Y-shape of an antibody is one of the most recognized structures in biology and perhaps all of science. There are five classes of antibodies in humans and rodents defined by their respective immunoglobulin (Ig) heavy chains: IgG, IgM, IgD, IgA, and IgE. Here we focus on IgG, as it is one of the most abundant proteins in human serum (10-20% of total plasma protein), comprises 70-85% of the total immunoglobulin pool, has the longest plasma half-life (20-24 days), and it is the most common format used in antibody-based therapeutics. IgG (~150 kD) comprises four peptide chains: two identical heavy chains and two identical light chains connected by disulfide (S-S) bonds. The heavy chain in IgG comprise ~450 residues. There are four IgG subclasses in human (IgG_1, IgG_2, IgG_3, and IgG_4), five in mice (IgG_1, IgG_{2a}, IgG_{2b}, IgG_{2c}, IgG_3), and four in rats (IgG_1, IgG_{2a}, IgG_{2b}, IgG_{2c}). The light chain in IgG comprise ~215 residues, and there are two light chain subclasses in humans, mice, and rat: kappa (κ) and lambda (λ).



ANTIBODY STRUCTURE	GENE SEGMENT LOCATION
Heavy Chain	Chromosome 14
Light Chain (κ)	Chromosome 2
Light Chain (λ)	Chromosome 22

The two identical complementarity-determining regions (CDRs) at the upper tips of the Y-structure make up the antigen recognition surface for the antibody. There are 3 CDRs in the heavy chain and light chain (CDR1, CDR2, and CDR3), and in most cases antigen binding interactions require contributions from both heavy and light chain CDRs. Furthermore, in most cases the CDR3 region in the heavy chain is the key determinant of antigen recognition specificity. The CDRs are contained within the Fragment variable (Fv) domain (~25 kD): the variable heavy (VH) and variable light (VL) chain regions make up the Fv domain. The antigen-binding fragment (Fab, ~50 kD) domain includes the entire light chain (VL and constant light (CL)), the VH and the first constant domain of the heavy chain (CH1). Monomeric Fabs can be generated by digesting an antibody with papain protease. A divalent F(ab')2 fragment (~100 kD) can be generated by digesting an antibody with Immunoglobulin-degrading enzyme from *Streptococcus pyrogenes* (IdeS) or by using the pepsin peptidase. The fragment crystallizable (Fc, ~50 kD) region forms the base of the Y-structure and comprise the CH2 and CH3 domains of the heavy chain (HC). The Fc region binds to Fc receptors and complement proteins, and is often the site of modifications for therapeutic purposes (e.g. coupling to cytotoxic molecules), such is the case with antibody-drug conjugates (ADCs).

In vivo humoral immune response

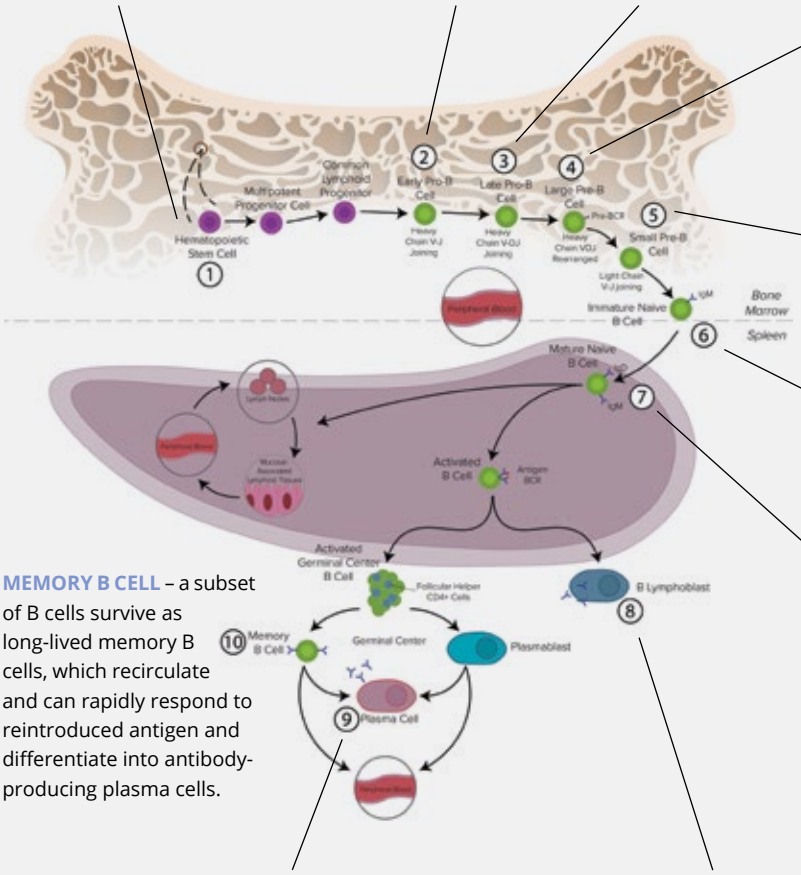
Terminally differentiated B cells capable of secreting high affinity antibodies (aka plasma cells) arise following an extensive process of cell differentiation and activation that begins with hematopoietic stem cells (HSC) in the bone marrow. Antigen-independent B cell development occurs in five discrete stages that are coupled with the sequential somatic recombination of the variable (V), diversity (D), and joining (J) gene segments. Antigen-dependent B cell development takes place initially in the bone marrow and then in the periphery.

B cell development pathway

HEMATOPOIETIC STEM CELL – heavy chain and light chain genes are in germline configuration.

EARLY PRO-B CELL – heavy chain undergoes D–J gene rearrangement.

LATE PRO-B CELL – heavy chain undergoes V–DJ rearrangement.



LARGE PRE-B CELL – transient surface expression of IgM heavy chain with invariant pseudo light chain (pre-B cell receptor). Successful cell surface expression of pre-B cell receptor triggers allelic exclusion to prevent rearrangement of the second allele and also initiates pre-B cell proliferation, which results in different light chains matched with the same heavy chain in different daughter cells. Dividing cells are larger than resting cells, hence the name large pre-B cell.

SMALL PRE-B CELL – light chain undergoes V–J rearrangement. Individual cells first attempt κ chain rearrangement. If rearrangement of both κ alleles is unsuccessful, cells attempt λ chain rearrangement.

IMMATURE B CELL – successfully rearranged heavy and light chains are expressed as IgM on the cell surface. At this stage the cells are highly sensitive to antigen binding: if they bind self-antigen in the bone marrow they die (negative selection).

MATURE NAÏVE B CELL – if the immature B cell survives, it matures in the bone marrow or spleen and expresses surface IgD and IgM, made from alternatively spliced transcripts. Mature naïve B cells recirculate between blood, spleen, peripheral lymph nodes and other secondary lymphoid tissues (e.g. Peyer's Patches in the gut) in search of cognate antigen.

MEMORY B CELL – a subset of B cells survive as long-lived memory B cells, which recirculate and can rapidly respond to reintroduced antigen and differentiate into antibody-producing plasma cells.

PLASMA CELL – continuous antibody secretion, estimated at 2,000 IgM or 15,000 IgG molecules per second per cell. Alternative splicing yields some membrane-bound Ig, but most is secreted. Most plasma cells are incapable of proliferation, with their protein-synthesizing machinery dedicated entirely to making antibody. Although many die after several days, some survive in the bone marrow for months or years and continue to secrete antibodies into the blood.

B LYMPHOBLAST – antigen binding leads to alternative splicing to secrete Ig. The location of antigen encounter triggers isotype switching (gut microenvironmental signals lead to IgA, whereas lymph node signals lead to IgG). Somatic hypermutation is triggered in germinal centers in the follicles of secondary lymphoid tissues. CD4+ T cell help in germinal centers drives *in vivo* affinity maturation.

Approved monoclonal antibodies

Whether it's a pioneering antibody like OKT3, or an effective and financially attainable biosimilar, each approved antibody plays an important role in the quest to improve patient health. By our estimation, as of January 2020, 100 originator monoclonal antibodies have been approved in the US (FDA) and/or Europe (EMA) since OKT3 in 1986. These antibodies cover a wide range of indications and worldwide antibody sales are expected to reach nearly $150 billion by 2020*. The current rate of approval (last 5 years) by the FDA is 7.6 antibodies per year. It's important to note that of all of the monoclonal antibodies that have been approved by the FDA, the vast majority (roughly 88%) have been discovered via hybridoma-based technologies (with a small but growing portion from transgenic human antibody-producing animals). That's quite a track record for what many consider to be an "ageing" technology!

The table below provides a list of all of the hybridoma-derived monoclonal antibodies that have been granted FDA approval in the last 10 years.

Trade Name	International Non-proprietary Name (INN)	Company	Target	Therapeutic Indication(s)	Year of first FDA approval
Padcev®	Enfortumab vedotin-ejfv	Astellas Pharma and Seattle Genetics	Nectin-4	Locally advanced or metastatic urothelial cancer (mUC)	2019
Adakveo®	Crizanlizumab-tmca	Novartis	P-selectin	Sickle cell disease	2019
Beovu®	Brolucizumab-dbll	Novartis	VEGF-A	Macular degeneration	2019
Polivy®	Polatuzumab vedotin-piiq	Genentech (Roche)	CD79b	Diffuse large B-cell lymphoma (DLBCL)	2019
Skyrizi®	Risankizumab-rzaa	Abbvie	IL-23	Plaque psoriasis	2019
Evenity®	Romosozumab-aqqg	Amgen and UCB Pharma	Sclerostin	Osteoporosis	2019
Ultomiris®	Ravulizumab-cwvz	Alexion Pharmaceuticals	Complement protein C5	Paroxysmal nocturnal hemoglobinuria (PNH)	2018
Ilumya®	Tildrakizumab-asmn	Sun Pharma	IL23 (p19)	Plaque psoriasis	2018
Trogarzo®	Ibalizumab-uiyk	Theratechnologies Inc	CD4	Human immunodeficiency virus-1 (HIV-1)	2018
Libtayo®	Cemiplimab-rwlc	Sanofi	PD-1	Advanced/metastatic cutaneous squamous cell carcinoma (CSCC)	2018
Poteligeo®	Mogamulizumab-kpkc	Kyowa Hakko Kirin	CC chemokine receptor 4 (CCR4)	Mycosis fungoides (MF) or Sézary syndrome (SS)	2018
Crysvita®	Burosumab-twza	Ultragenyx Pharmaceuticals and Kyowa Kirin	FGF-23	X-linked hypophosphatemia (XLH)	2018
Emgality®	Galcanezumab-gnlm	Eli Lilly	Calcitonin gene-related peptide (CGRP) receptor	Migraine	2018

* Lu et al. (2020). Development of Therapeutic Antibodies for the Treatment of Diseases. *J Biomed Sci.* 2020 Jan2:27(1):1 doi: 10.1186/s12929-019-0592-z

Trade Name	International Non-proprietary Name (INN)	Company	Target	Therapeutic Indication(s)	Year of first FDA approval
Ajovy®	Fremanezumab-vfrm	Teva Pharmaceuticals	Calcitonin gene-related peptide (CGRP) receptor	Migraine	2018
Aimovig®	Erenumab-aooe	Amgen	Calcitonin gene-related peptide (CGRP) receptor	Migraine	2018
Hemlibra®	Emicizumab-kxwh	Chugai and Genentech (Roche)	Factor IXa and X	Hemophelia A	2017
Fasenra®	Benralizumab	MedImmune (AstraZeneca)	IL-5R	Asthma	2017
Besponsa®	Inotuzumab ozogamicin	Pfizer	CD22	B-cell precursor acute lymphoblastic leukemia (ALL)	2017
Kevzara®	Sarilumab	Sanofi and Regeneron Pharmaceuticals	IL-6R	Rheumatoid arthritis	2017
Dupixent®	Dupilumab	Regeneron Pharmaceuticals	IL-4Rα	Asthma; dermatitis	2017
Imfinzi®	Durvalumab	AstraZeneca	PD-L1	Metastatic urothelial carcinoma	2017
Ocrevus®	Ocrelizumab	Genentech (Roche)	CD20	Multiple sclerosis (primary-progressive)	2017
Siliq®	Brodalumab	Amgen and AstraZeneca	IL-17RA	Plaque psoriasis	2017
Zinplava®	Bezlotoxumab	Merck Sharp & Dohme Limited	C. difficile toxin B	Enterocolitis; pseudomembranous	2016
Taltz®	Ixekizumab	Eli Lilly	IL-17A	Plaque psoriasis	2016
Cinqair®	Reslizumab	Teva Pharmaceuticals	IL-5	Asthma	2016
Lartruvo®	Olaratumab	Eli Lilly	PDGFR-α	Sarcoma	2016
Anthim®	Obiltoxaximab	Elusys Therapeutics INC	PA component of B. anthracis toxin	Anthrax infection	2016
Tecentriq®	Atezolizumab	Genentech (Roche)	PD-L1	Metastatic non-small cell lung cancer	2016
Darzalex®	Daratumumab	Janssen-Cilag	CD38	Multiple myeloma	2015
Empliciti®	Elotuzumab	Bristol-Myers Squibb	SLAMF7	Multiple myeloma	2015
Cosentyx®	Secukinumab	Novartis Europharm	IL-17A	Arthritis; psoriatic psoriasis; spondylitis; ankylosing	2015
Nucala®	Mepolizumab	GlaxoSmithKline	IL-5	Asthma	2015
Opdivo®	Nivolumab	Bristol-Myers Squibb	PD-1	Carcinoma; non-small-cell lung carcinoma; renal cell Hodgkin disease melanoma	2015
Praluent®	Alirocumab	Sanofi-Aventis Group	PCSK9	Dyslipidemias	2015

Trade Name	International Non-proprietary Name (INN)	Company	Target	Therapeutic Indication(s)	Year of first FDA approval
Praxbind®	Idarucizumab	Boehringer Ingelheim International GmbH	Dabigatran	Hemorrhage (inactivating oral anticoagulant dabigatran; dabigatran etexilate prodrug)	2015
Repatha®	Evolocumab	Amgen	LDL-C / PCSK9	Dyslipidemias; hypercholesterolemia	2015
Unituxin®	Dinutuximab	United Therapeutics Europe	GD2	Neuroblastoma	2015
Entyvio®	Vedolizumab	Takeda Pharma	Integrin-α4β7	Colitis; ulcerative Crohn's disease	2014
Blincyto®	Blinatumomab	Amgen Europe	CD19	Precursor cell lymphoblastic leukemia-lymphoma	2014
Keytruda®	Pembrolizumab	Merck Sharp & Dohme Limited	PD-1	Melanoma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), head and neck squamous cell cancer (HNSCC), Hodgkin lymphoma, urothelial carcinoma, GI tract cancers	2014
Sylvant®	Siltuximab	Janssen-Cilag International	cCLB8	Giant lymph node hyperplasia, Castleman's disease	2014
Lemtrada®	Alemtuzumab	Sanofi	CD52	Multiple sclerosis (relapsing-remitting)	2014
Kadcyla®	Trastuzumab emtansine	Genentech (Roche)	HER2	Breast Cancer	2013
Gazyva®	Obinutuzumab	Genentech (Roche)	CD20	Chronic Lymphocytic leukemia (CLL)	2013
Perjeta®	Pertuzumab	Genentech (Roche)	HER2	Breast Cancer	2012
Adcetris®	Brentuximab vendotin MMAE	Seattle Genetics	CD30	Hodgkin lymphoma (HL), systemic anaplastic large cell lymphoma (ALCL)	2011
Yervoy®	Ipilimumab	Bristol-Myers Squibb	CTLA-4	Melanoma	2011
Prolia®/ Xgeva®	Denosumab	Amgen	RANKL	Osteoporosis/ Prevention of skeletal-related events (SREs) in patients with bone metastases from solid tumors	2010/ 2011
Actemra®	Tocilizumab	Chugai (Roche)	IL-6 receptor	Rheumatoid arthritis	2010

Marketing Approval Agencies:

Approval by the US Food and Drug Administration (FDA) grants marketing authorization in all 50 US states as well as other US territories and possessions.

Approval by the European Medicines Agency (EMA) grants marketing authorization in all Eurpean Union (EU) and European Economic Area (EEA)-European Free Trade Association (EFTA) states (which includes Iceland, Liechtenstein, and Norway).

What will you target next?

Hybridoma technology

Overview

Hybridoma technology utilizes a wide variety of experimental procedures to yield antigen-specific monoclonal antibody (mAb)-producing immortal hybridoma clones.

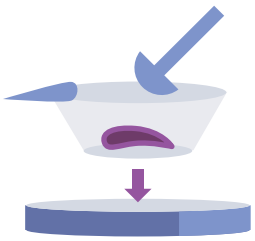
Conventional hybridoma technology utilizes *in vivo* immunizations, cell:cell fusion, specialized cell culture conditions, and various screening techniques to yield antigen-specific monoclonal antibody (mAb)-producing immortal hybridoma clones. The concept is essentially unchanged from Köhler and Milstein's original approach reported in 1975. Animals (typically rodents) are immunized with the target antigen, splenocytes are fused with myeloma partners and grown in HAT media to select for hybridomas, and the hybridoma supernatants are screened for target reactivity. Single cell cloning is performed to yield monoclonal antibody-producing hybridomas.

1

Mouse immunization

Rodents are typically immunized with antigen and adjuvant to generate a strong *in vivo* B cell antibody response.

2



Spleen harvest

The spleen is harvested and single cell suspensions of splenocytes are generated.

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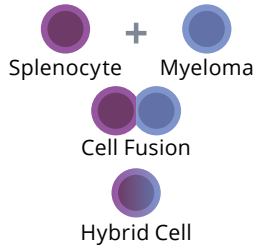

Cell fusion

Splenocytes are fused with myeloma partners to generate hybridomas.

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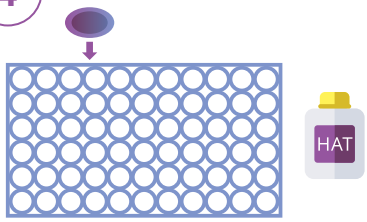

Planning and selection with HAT media

The fusion mixture is plated into 96-well plates where HAT culture media drives selection of hybridomas vs. unfused myelomas or B cells.

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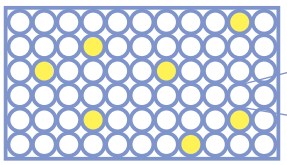
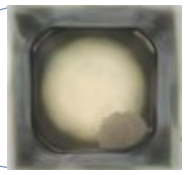


Antibody screening and cell cloning

The plates are screened for antigen-specific antibodies. Positive wells are expanded, and single cell hybridoma clones are generated from the parental hybridomas.

6



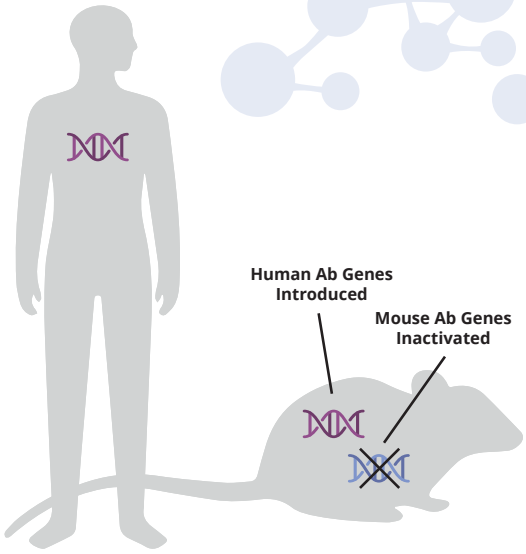
Hybridoma expansion and monoclonal antibody production

The clonal hybridoma is expanded for production of antigen-specific mAbs.

Hybridoma technology

Human antibody-producing rodents

Multiple innovative platforms are currently available for antibody discovery using genetically modified mice that express fully-human heavy and light chain variable regions. These animals can generate diverse repertoires of *in vivo* affinity-matured antibodies with intrinsic drug-like properties necessary for successful development, including high potency, specificity, solubility, and manufacturability. A key advantage is that in having fully human variable regions, the antibodies have low risk of immunogenicity, thus mitigating efficacy-killing anti-drug responses when reformatted as therapeutics.



	Ligand OmniMouse®	Harbour H2L2 Mouse	Trianni Mouse®	Alloy ATX-GK Mouse	Ablexis AlivaMab® Mouse
Light Chain	Hu κ Hu λ	Hu κ	Hu κ Hu λ	Hu κ	Hu κ Hu κ Hu λ Hu λ
Heavy Chain Fc	Rat IgG1 IgG2b IgG2c	Rat IgG1 IgG2b IgG2c	Mouse IgG1 IgG2b IgG2c IgG3	Not disclosed	Mouse IgG
Heavy Chain Repertoire (no. V Gene Segments)	44	18	44	50+	Not disclosed
Light Chain Repertoire (no. V Gene Segments)	20 κ 15 λ	11 κ	39 κ 38 λ	22+ λ	Not disclosed
Parental Strain(s)	C57Bl/6 SJL	C57Bl/6 FVB 129	C57Bl/6	C57Bl/6	Not disclosed
MHC Haplotype(s)	H-2b H-2s	H-2b H-2q	H-2b	H-2b	Not disclosed

References

Lowitz J, Lin G, Somera J, Santibanez-Vargas L, Vo C, Rodriguez E, Nguyen B, Trang M, Nichols J, Kenney J. Optimization of Therapeutic Discovery Strategies for Human Antibody Transgenic Animal Platforms. Poster presented at PEGS; 2019 Apr. 8-12; Boston, MA.

Maximizing the AlivaMab Mouse Advantage. Retrieved from https://alivamab.com/alivamab-mouse.

Hybridoma technology

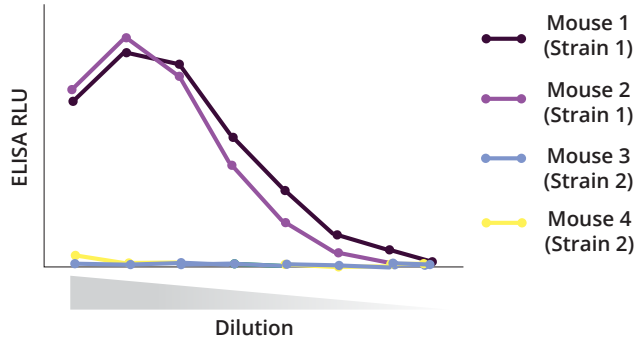
Curia's PentaMice® platform

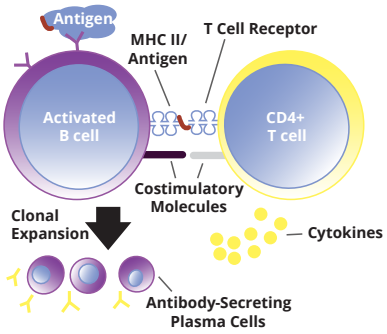
A proprietary set of wildtype mice designed to achieve maximum plasma titers in hybridoma campaigns

Conventional immunization approaches utilized in hybridoma-based antibody discovery campaigns typically use one or two common wildtype (WT) mouse strains (e.g. Balb/c or C57Bl/6). Curia's scientists have noted through a course of over 100 campaigns that this approach likely limits the identification of high-quality antibodies to just those target antigens that are efficiently processed and presented by a restricted major histocompatibility complex (MHC) repertoire that is distinct for each WT strain.

Curia's PentaMice platform is a royalty-free set of mice comprising 5 WT strains that cover 9 distinct MHC haplotypes. A total of 10 mice (2 mice of each strain) are included in each set to achieve maximum plasma titers, thus boosting the opportunity to generate high-quality antibodies *in vivo.*

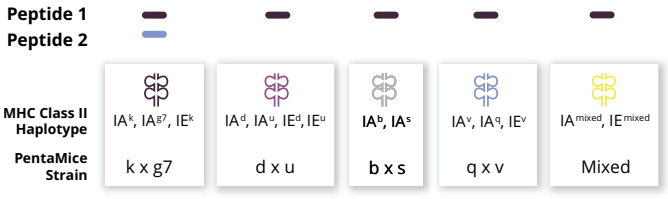
The concept behind the PentaMice platform





1

Plasma titers are highly predictive of antibody discovery success. Based on Curia's experience, there is often a strong strain-dependent difference in plasma titers for most targets.



Two different peptide binding profiles are shown as examples. Peptide 1 is efficiently presented by most MHC II. Peptide 2 is only efficiently presented by IA^{g7}.

2

High plasma titers require T cell help, and one of the requirements for effective T cell activation is recognition of cognate antigens presented by the MHC. Only certain peptides are effectively presented by certain MHC.

3

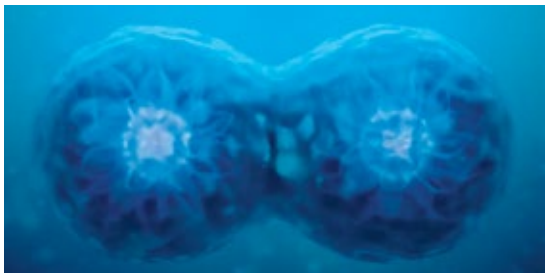
MHCs are highly polymorphic. Curia's scientists hypothesize that this polymorphism drives strain-dependent differences in plasma titers. Hence, the PentaMice platform is designed to cover a wide range of MHC haplotypes to enable effective T cell help.

Hybridoma technology

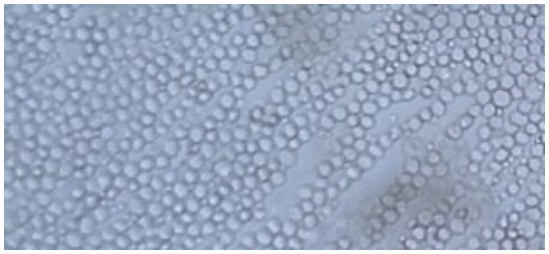
B cell: Myeloma cell fusion

Although cell fusion may seem a little odd at first, it is important to remember that it is quite common in the natural world. Such is the case with gamete fusion in the formation of a one-cell embryo, as well as later in development with the fusion of myocytes resulting in a myotube or skeletal muscle cell syncytium. Nevertheless, when it comes to hybridomas, B cells and myeloma cells make for an unlikely fusion pair, hence the need for an external catalyst. Köhler and Milstein harnessed the power of the Sendai virus, a murine respiratory tract virus. Once infected, a cell would be more prone to fuse with an adjacent cell via fusogens or fusion proteins. We have come a long way since the inception of the hybridoma cell, and nowadays the two most common techniques include polyethylene glycol (PEG) – mediated fusion and electrofusion.

	PEG-Mediated Fusion	Electrofusion
Procedure Specifics	PEG is a one-step process that consists of placing a mixture of the cells to be fused into a flask containing a PEG solution and shaking vigorously. Over time, PEG dehydrates the cells by making water molecules between them thermodynamically unfavorable, hopefully resulting in membrane fusion between adjacent cells.	Electrofusion is achieved by applying carefully controlled bursts of electricity to a suspended cell mixture. Cells are first brought into contact using the magnetic properties of dielectrophoresis (a form of alternating current – AC – field). Cells are then exposed to direct current – DC – pulses, stimulating electroporation, bringing the cell membranes into a permeable and fusogenic state.
Duration	Short (minutes)	Short (minutes); DC pulses range from µs to ns.
Fusion Efficiency	Very low (1:10,000)	Low (1:1,000)
Complexity	Low (PEG remains the simplest hybridoma fusion technique currently in use)	Medium (the procedure requires the use of high-grade fusion chambers)
Cost	**$**	**$ $**



Hybridomas are formed when a B cell is fused with a myeloma cell.



An AC field polarizes the cells and induces their linear "pearl chain" alignment. A DC pulse then triggers cell fusion.

Both methods rely extensively on cell-to-cell contact as well as the quality of the cells used. Although PEG fusions are more economically advantageous, results vary. Head-to-head comparisons have demonstrated that electrofusions not only had a greater number of fusion events but the resulting hybridomas grew more vigorously. Within minutes following the fusion event, intermingling of surface proteins and restructuring of the two separate cytoskeletons into one occurs, with nuclei fusion ensuing shortly after. When it comes to electrofusion, the number, duration, and frequency of pulses is optimized so as to preserve cell viability and increase fusion efficiency, rendering it the favored method for hybridoma-based antibody discovery!

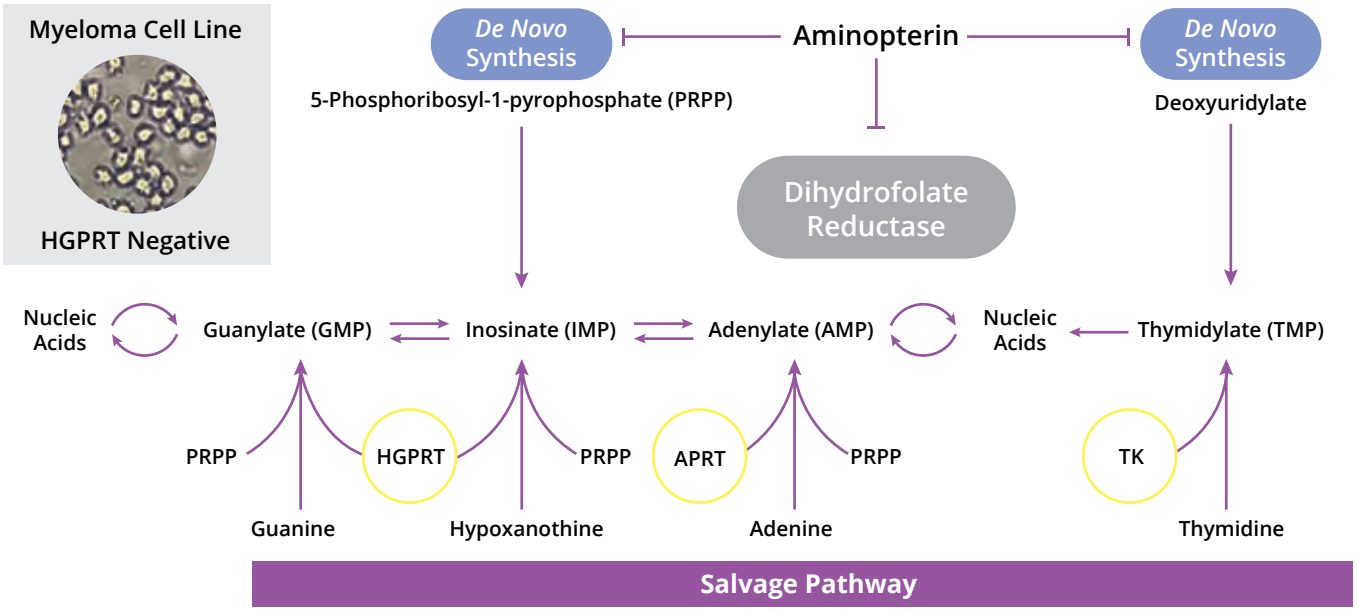
Hybridoma technology

Hybridoma selection

Cell division requires an adequate supply of free nucleotides for deoxyribonucleic acid (DNA) replication. Nucleic acids are generated either via:

- *De novo* DNA synthesis – which is dependent on the activity of dihydrofolate reductase to generate purine nucleotides (GMP, IMP, AMP) and thymidylate;
- Salvage pathway synthesis – which requires exogenous hypoxanthine and thymidine and the enzymes hypoxanthine-guanine phosphoibosyltransferase (HGPRT) and thymidine kinase (TK).

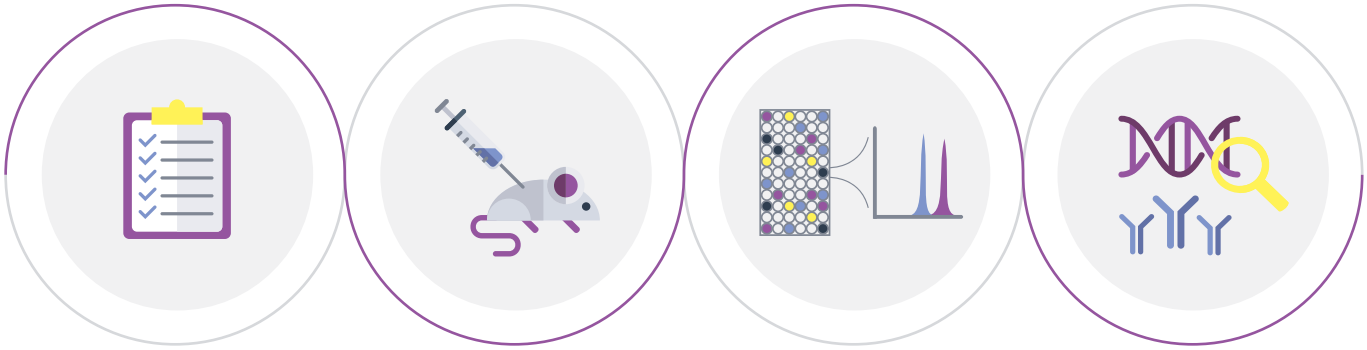
Aminopterin is a dihydrofolate reductase inhibitor: treatment of cells with aminopterin prevents *de novo* DNA synthesis, and in the absence of exogenous hypoxanthine and thymidine to supply the salvage pathway, the cells will die. A mutation in HGPRT disables the salvage pathway, which is lethal in aminopterin-treated cells even if they are cultured with exogenous hypoxanthine and thymidine. The identification of myeloma cells with mutations in HGPRT is straightforward. The HGPRT gene is on the X-chromosome, and due to X-linked inactivation a single mutation is all that is needed to result in the loss of HGPRT. Thus, specific cell culture conditions (HAT media: Hypoxanthine, Aminopterin, Thymidine) + a myeloma partner with a HGPRT mutation enables the selection and survival of hybridomas (B cell:myeloma hybrids) vs. unfused myeloma cells or B cells. The key concept is that the hybridoma receives a functional HGPRT gene from the primary B cell, thus enabling the survival of hybridomas vs. myeloma cells. While primary B cells can survive for a time in culture and produce antibody, they will eventually die without any additional selection, leaving hybridomas as the only living cell in the culture wells. A subset of hybridomas express antibody genes derived from their parental B cell contributor, and single cell cloning yields clonal hybridomas that express monoclonal antibodies.



Target the future today!

With a success rate of 98%, Curia's hybridoma platform has a proven track record in effectively identifying novel therapeutic or diagnostic monoclonal antibodies. The hybridoma team is ready to partner with you on your antibody quest.

Hybridoma discovery immunology – Chain of Discovery℠ approach



UPFRONT DUE DILIGENCE

- Target analysis and antigen design
- Goal-oriented proposal development
- Antigen biofunction QC

IMMUNIZATIONS

- Rapid immunization protocols
- DNA, protein, peptide, and cell-based immunizations
- In-life titer checks enable real-time optimization of immunizations, and selection of animals with optimal titers

HYBRIDOMA SCREENS

- Hybridoma generation by electrofusion
- 384-well plate high throughput screens (ELISA or multiplex FACS)
- Data Master Files for candidate selection

mAB SCREENS

- Hybridoma single cell cloning and variable region sequencing
- mAb characterization affinity, binding EC_{50} (ELISA or FACS), biofunction IC_{50} or EC_{50} (customized functional assays such as ligand blocking & receptor internalization

Complete campaigns incorporate Curia's unique suite of integrated services including:

- Target analysis and immunogen production
- Immunization, hybridoma generation, and single cell cloning
- Variable region sequencing and purified mAb production/characterization

Discovery Immunology mAbs can seamlessly transition to downstream GXP production, enabling a one-stop shop from discovery to development.

ABOUT CURIA

Curia is a Contract Development and Manufacturing Organization with over 30 years of experience, an integrated network of 29 global sites and over 3,500 employees partnering with customers to make treatments broadly accessible to patients. Our biologics and small molecule offering spans discovery through commercialization, with integrated regulatory and analytical capabilities. Our scientific and process experts and state-of-the-art facilities deliver best-in-class experience across drug substance and drug product manufacturing. From curiosity to cure, we deliver every step to accelerate and sustain life-changing therapeutics. ***Learn more at curiaglobal.com***

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