ALCHEMAB THERAPEUTICS

Mining the immune system for protective antibodies

Genomics England

4 May 2022

OUR VISION: TO USE THE POWER OF THE HUMAN IMMUNE SYSTEM TO DISCOVER NEW MEDICINES AND DIAGNOSTICS

ALCHEMAB THERAPEUTICS

Discovering and developing protective, patient-originated therapeutic antibodies

- Focus on protective antibody responses
- Convergent in resilient individuals
- Target agnostic approach
- Advanced computational approaches

B CELL RECEPTORS ARE A KEY COMPONENT OF ADAPTIVE IMMUNITY

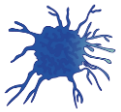
ALCHEMAB THERAPEUTICS

INNATE | NONSPECIFIC

Fast response (0-4 hours)

MONONUCLEAR PHAGOCYTE SYSTEM

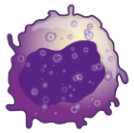
Macrophage



Dendritic Cell

Monocyte

Complement Protein



Natural Killer Cell

Mast Cell

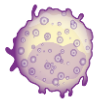
Basophill

Eosinophil

Neutrophil

granulocites

Yδ &T Cell



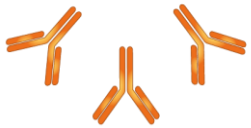
Natural Killer T Cell

ADAPTIVE | SPECIFIC

Slow response (4-14days)

HUMORAL

B cell



Antibodies

CELLULAR

T Lymphocyte

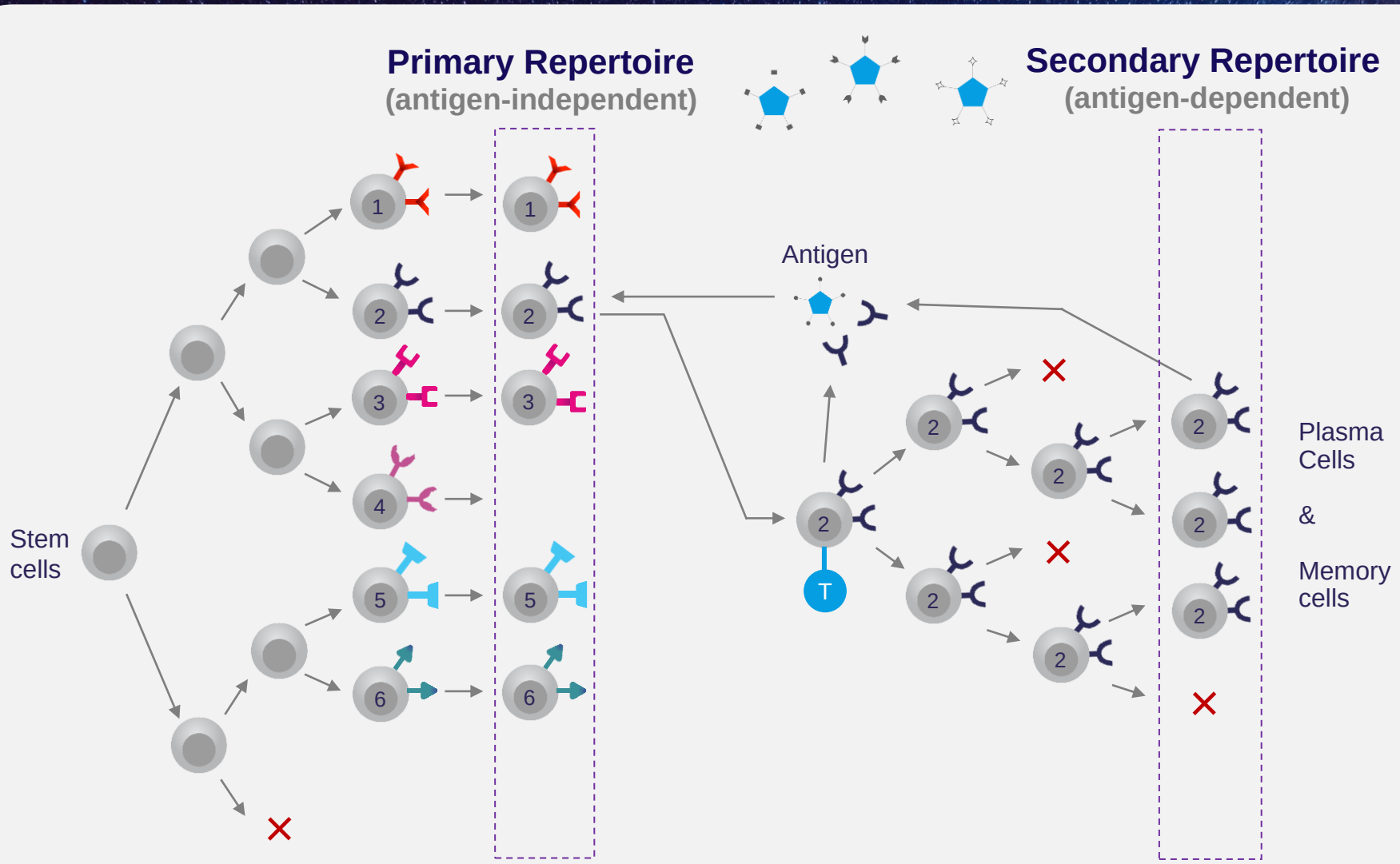
CD4+

CD8+

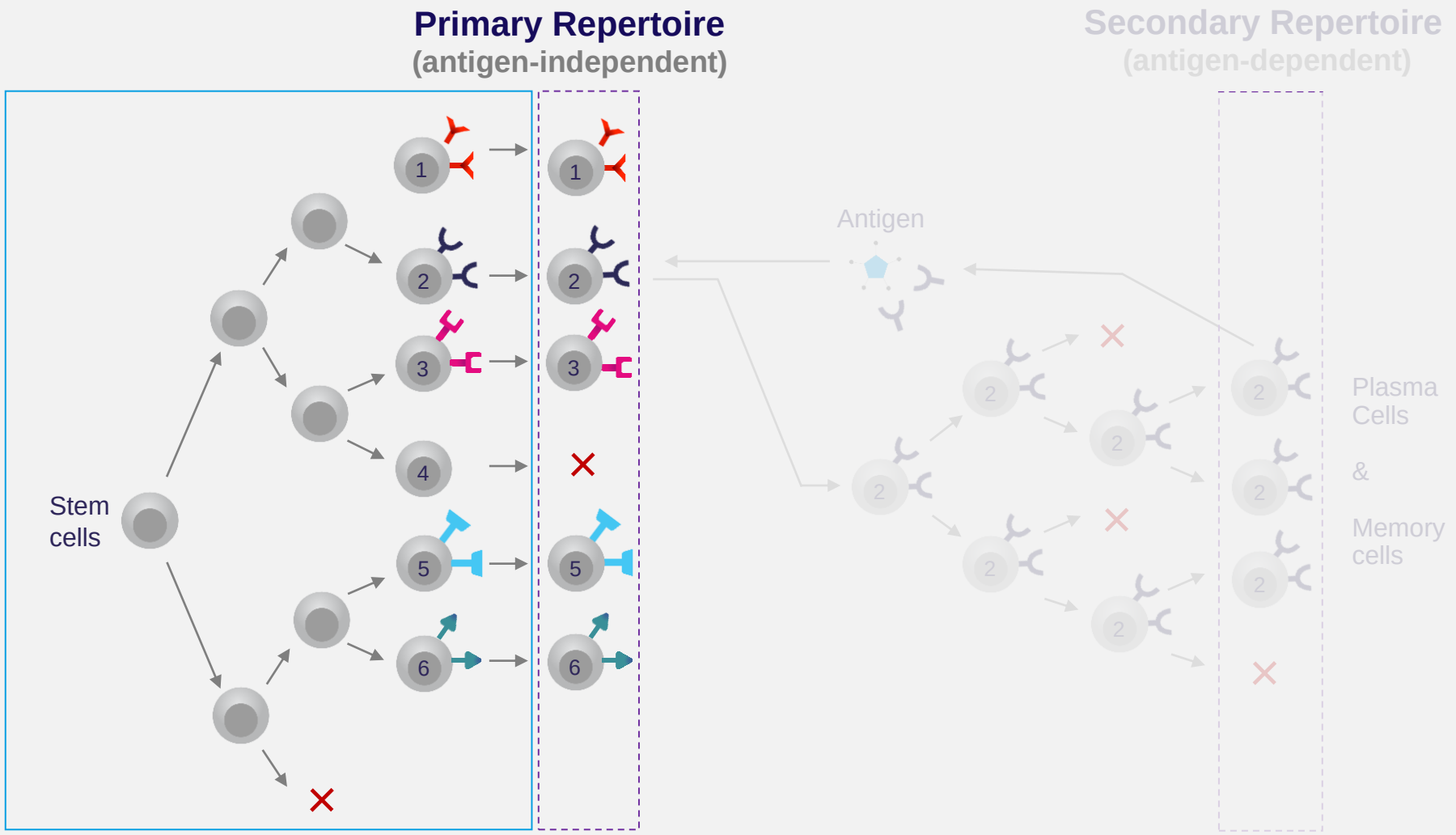
THE B CELL RECEPTOR (ANTIBODY) REPERTOIRE

"The sum of all B cells expressing distinct B cell receptors, necessary to bind diverse antigens and produce an effective humoral immune response"

THE B CELL RECEPTOR REPERTOIRE HAD TWO ELEMENTS



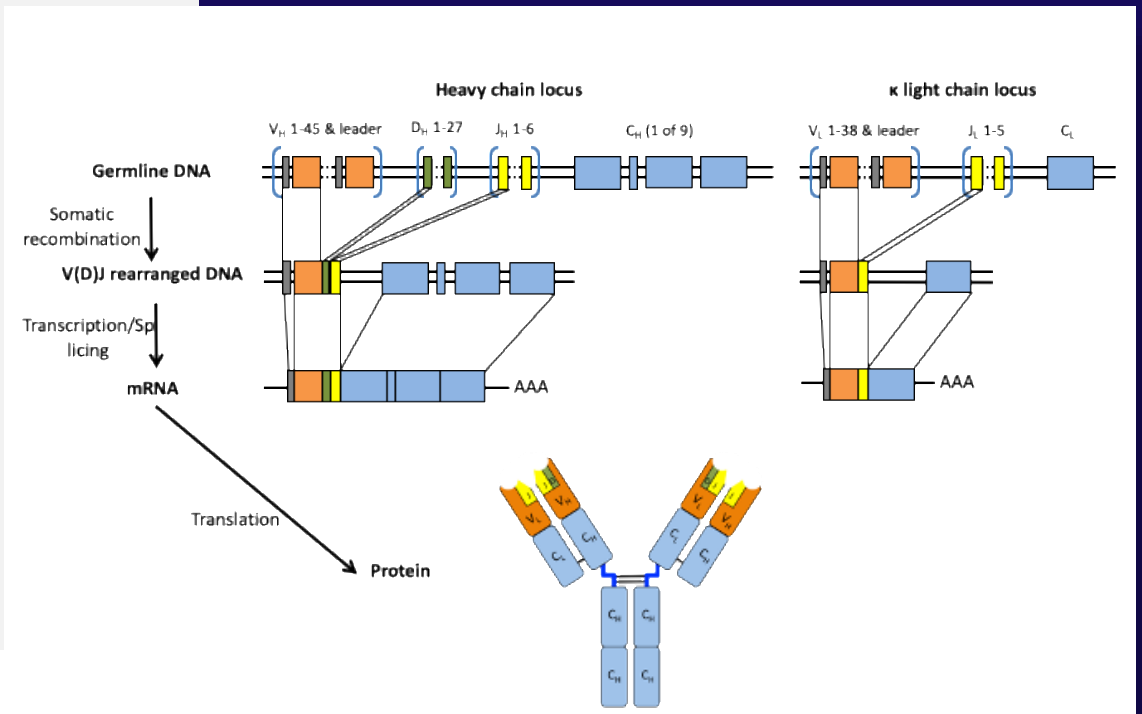
FORMING THE PRIMARY REPERTOIRE



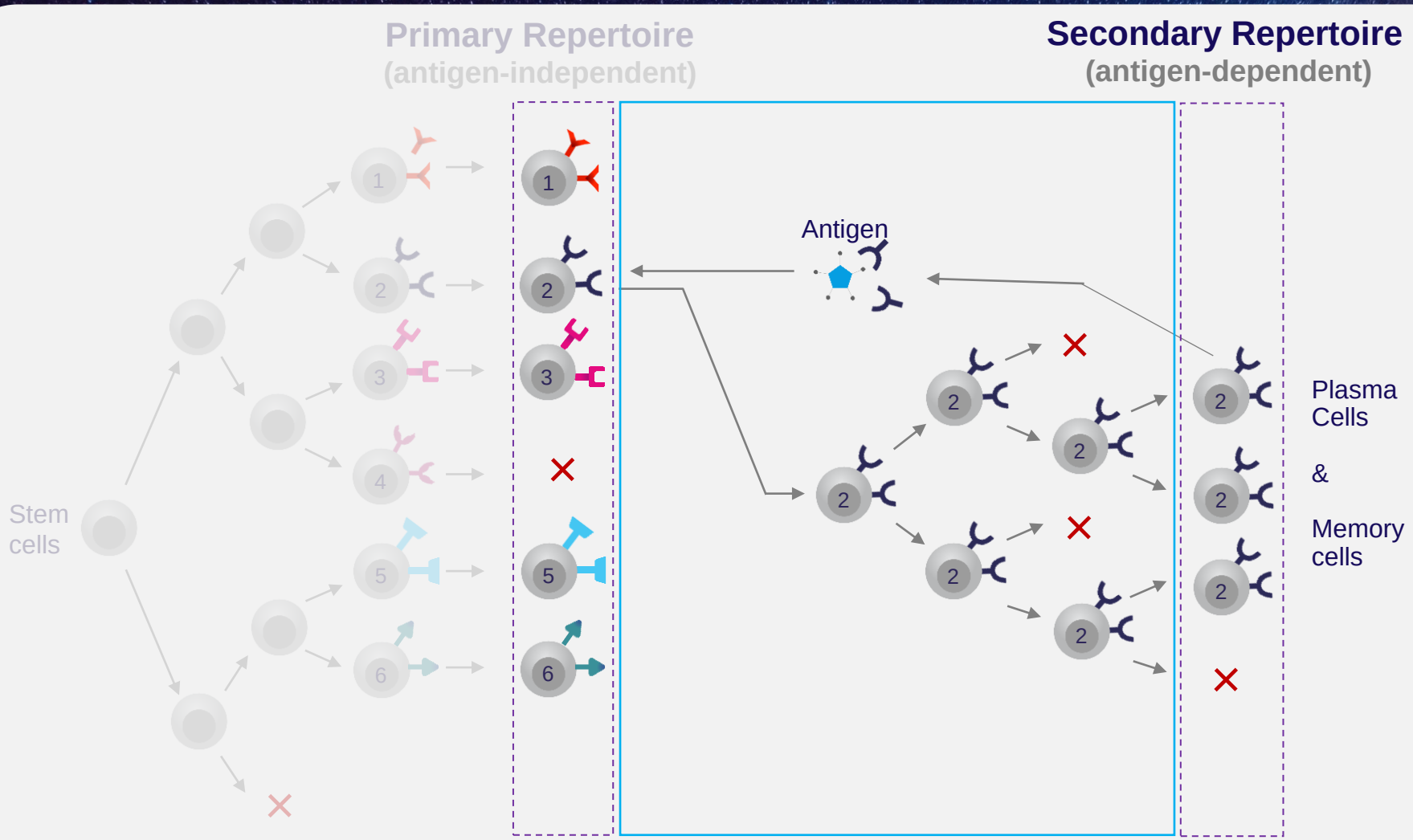
COMBINATORIAL DIVERSITY – VDJ RECOMBINATION

Somatic recombination of gene segments during B cell development

Element	Immunoglobulin	
	H	K+ λ
Variable segments (V)	~40	~70
Diversity segment (D)	23	0
D segments read in three frames	rarely	-
Joining segments (J)	6	5(κ) 4(λ)
Joints with N- and P-nucleotides	2	50% of joints
Number of V gene pairs	1.9×10^6	
Junctional diversity	$\sim 3 \times 10^7$	
Total diversity	$\sim 5 \times 10^{13}$	

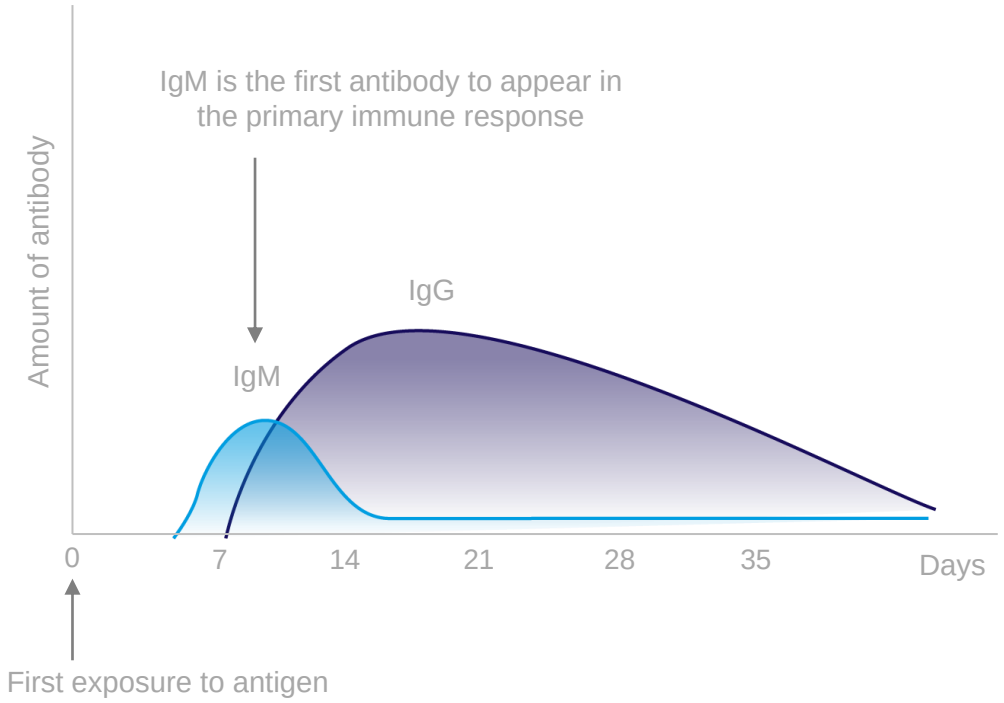


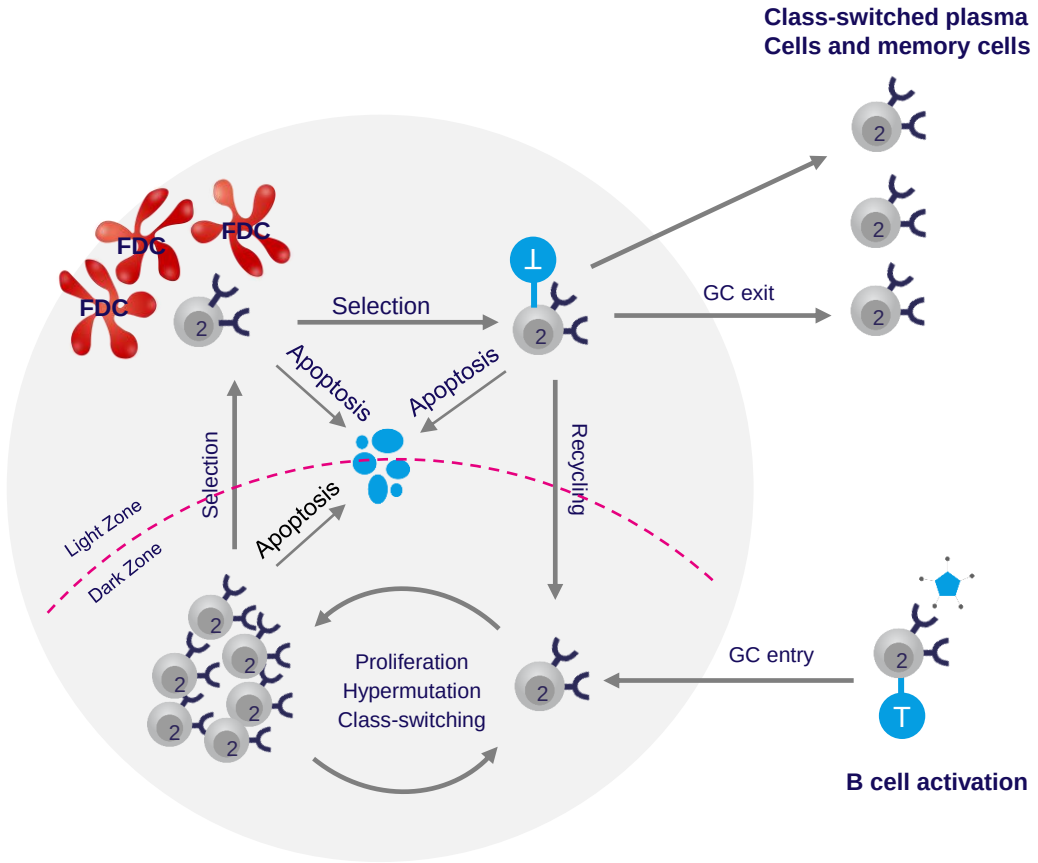
FORMING THE SECONDARY REPERTOIRE



AFFINITY MATURATION IN THE GERMINAL CENTRE

RAPID B CELL EVOLUTION OVER THE COURSE OF DAYS





BCR HIGH THROUGHPUT SEQUENCING: HAS BEEN EVOLVING SINCE 2009

High-Throughput Sequencing of the Zebrafish Antibody Repertoire

Joshua A. Weinstein,[1]* Ning Jiang,[2]* Richard A. White III,[3]
Daniel S. Fisher,[1,4,5] Stephen R. Quake[1,2,3,4]†

Despite tremendous progress in understanding the nature of the immune system, the full diversity of an organism's antibody repertoire is unknown. We used high-throughput sequencing of the variable domain of the antibody heavy chain from 14 zebrafish to analyze VDJ usage and antibody sequence. Zebrafish were found to use between 50 and 86% of all possible VDJ combinations and shared a similar frequency distribution, with some correlation of VDJ patterns between individuals. Zebrafish antibodies retained a few thousand unique heavy chains that also exhibited a shared frequency distribution. We found evidence of convergence, in which different individuals made the same antibody. This approach provides insight into the breadth of the expressed antibody repertoire and immunological diversity at the level of an individual organism.

The nature of the immune system's antibody repertoire has been a subject of fascination for more than a century. This repertoire is highly plastic and can be directed to create antibodies with broad chemical diversity and high selectivity (*1*, *2*). There is also a good understanding of the potential diversity available and the mechanistic aspects of how this diversity is generated. Antibodies are composed of two types of chains (heavy and light), each containing a highly diversified antigen-binding domain (variable). The V, D, and J gene segments of the antibody heavy-chain variable genes go through a series of recombination events to generate a new heavy-chain gene (Fig. 1). Antibodies are formed by a mixture of recombination among gene segments, sequence diversification at the junctions of these segments, and point mutations throughout the gene (*3*). Estimates of immune diversity for antibodies or the related T cell receptors either have attempted to extrapolate from small samples to entire systems or have been limited by coarse resolution of immune receptor genes (*4*). However, certain very elementary questions have remained open more than a half-century after being posed (*1*, *5*, *6*): It is still unclear what fraction of the potential repertoire is expressed in an individual at any point in time and how similar repertoires are between individuals who have lived in similar environments. Moreover, because each individual's immune system is an independent experiment in evolution by natural selection, these questions about repertoire similarity also inform our understanding of evolutionary diversity and convergence.

Zebrafish are an ideal model system for studying the adaptive immune system because in evolutionary terms they have the earliest recognizable adaptive immune system whose features match the essential human elements (*7*, *8*). Like humans, zebrafish have a recombination activating gene (RAG) and a combinatorial rearrangement of V, D, and J gene segments to create antibodies. They also have junctional diversity during recombination and somatic hypermutation of antibodies to improve specificity, and the organization of their immunoglobulin (Ig) gene loci approximates that of human (*9*). In addition, the zebrafish immune system has only ~300,000 antibody-producing B cells, making it three orders of magnitude simpler than mouse and five orders simpler than human in this regard.

We developed an approach to characterize the antibody repertoire of zebrafish by analyzing complimentarity-determining region 3 (CDR3) of the heavy chain, which contains the vast majority of immunoglobulin diversity (*10*, *11*) and can be captured in a single sequencing read (Fig. 1). Using the 454 GS FLX high-throughput pyrosequencing technology allowed sequencing of 640 million bases of zebrafish antibody cDNA from 14 zebrafish in four families (Fig. 1B). Zebrafish were raised in separate aquaria for each family and were allowed to have normal interactions with the environment, including the development of natural internal flora. We chose to investigate the quiescent state of the immune system, a state where the zebrafish had sampled a complex but fairly innocuous environment and had established an equilibrium of normal immune function. mRNA was prepared from whole fish, and we synthesized cDNA using primers designed to capture the entire variable region.

Between 28,000 and 112,000 useful sequencing reads were obtained per fish, and we focused our analysis on CDR3 sequences. Each read was assigned V and J by alignment to a reference with a 99.6% success rate (table S3); failures were due to similarity in some of the V gene segments. D was determined for each read by applying a clustering algorithm to all of the reads within a given

[1]Biophysics Program, Stanford University, Stanford, CA 94305, USA. [2]Department of Bioengineering, Stanford University, Stanford, CA 94305, USA. [3]Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305, USA. [4]Department of Applied Physics, Stanford University, Stanford, CA 94305, USA. [5]Department of Biology, Stanford University, Stanford, CA 94305, USA.

*These authors contributed equally to this work.
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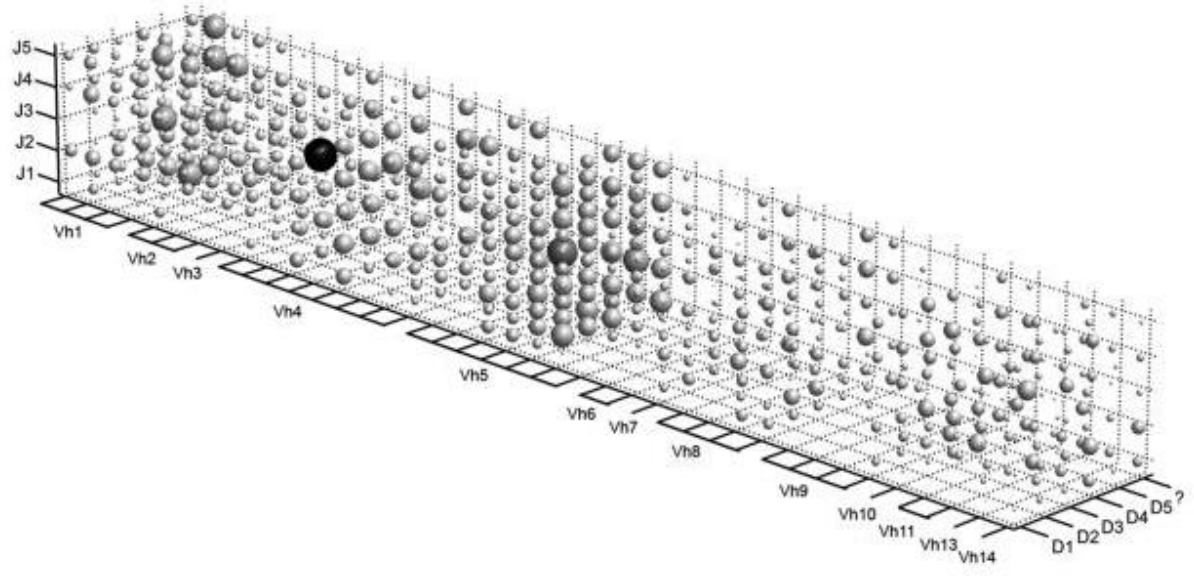


2009 | Enabled by 454 sequencing

HOW RARE IS CONVERGENCE?

DIVERSITY OF THE HUMAN REPERTOIRE IS THEORETICALLY 1 X 10^{13}

- Number of peripheral B cells in a healthy individual is approx. $1x10^9$
- Circulating B Cell Repertoire is therefore a fraction of the total diversity
- The amount of information encoded in “genome” of the adaptive immune system exceeds the human genome by 4 orders of magnitude

10 INDIVIDUALS VH SEQUENCED

3 BILLION HEAVY CHAINs

Briney *et al Nature 2019*

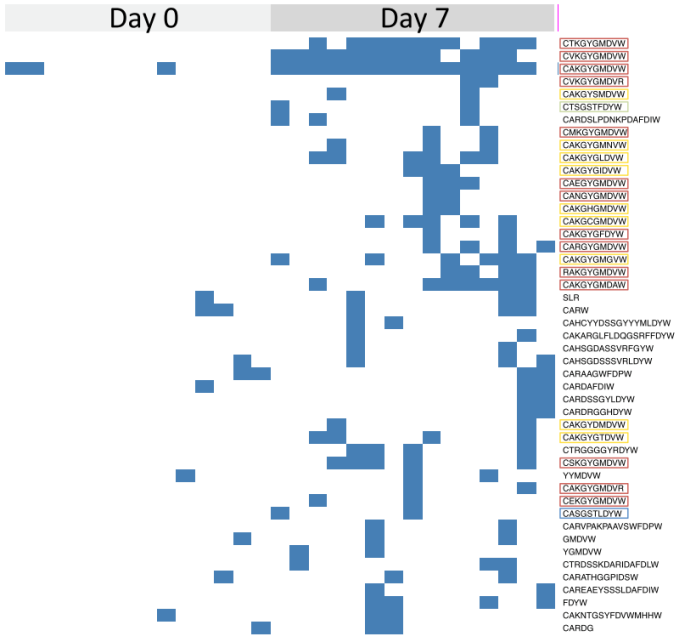
- Largely unique repertoires for each individual
- Between 2 individuals 0.95% of the repertoire was shared. Shared clonotypes were skewed towards short CDR3s
- Only 0.022% of clones were shared between all 10 individuals
- Commonality is driven by early BCR development rather than common antigen-specific selection, although there is some convergence due to vaccination and common infections

CONVERGENCE INCREASES AFTER COMMON ANTIGEN EXPOSURE

- Convergence increases after a **common** antigen stimulus and can be used to identify rare antigen-specific sequences
- Vaccination used as an example of a strong, specific stimulus

Identification of Antigen-Specific B Cell Receptor Sequences Using Public Repertoire Analysis

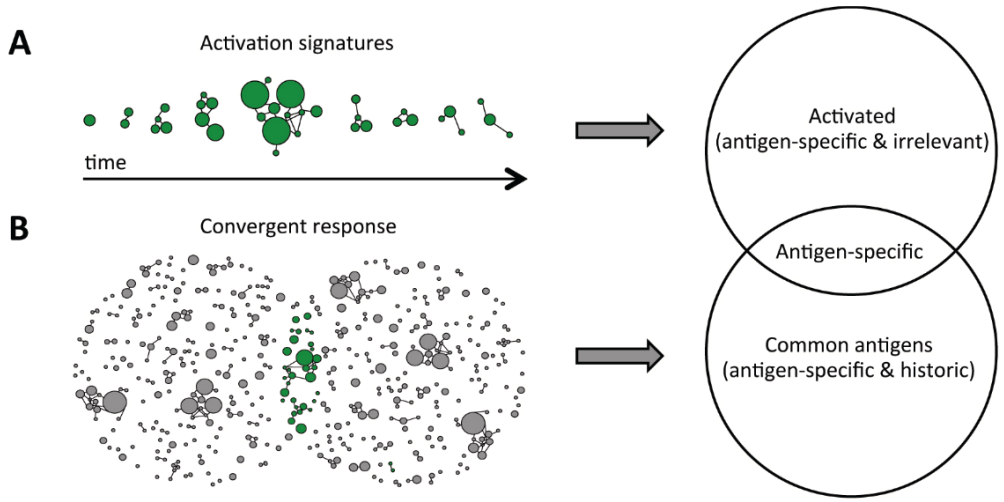
Johannes Trück,*,[1] Maheshi N. Ramasamy,*,[1] Jacob D. Galson,* Richard Rance,[†] Julian Parkhill,[†] Gerton Lunter,[‡] Andrew J. Pollard,* and Dominic F. Kelly*



Critical Reviews™ in Immunology, 35(6): 463-478 (2015)

Identification of Antigen-Specific B-Cell Receptor Sequences from the Total B-Cell Repertoire

Jacob D. Galson[a,*], Dominic F. Kelly[a], & Johannes Trück[a,b]



AUTOANTIBODIES ARE GENERALLY VIEWED AS ‘BAD GUYS”

MANY AUTOIMMUNE DISEASES ARE THE RESULT OF PATHOGENIC AUTOANTIBODIES:

- SLE (Polyclonal autoreactive B cells)
- Myasthenia Gravis (anti-AchR)
- Neurological disease (anti-NMDA Receptor encephalitis
- Pemphigus (anti- desmosomal proteins)

TREATMENTS:

- Ablate B cells (anti-CD20)
- Plasmapheresis
- Anti-FcRn
- High dose IVIG to block
- Anti-IDs (e.g. anti-anti-Dsg3 autoantibodies)

IF THERE ARE BAD GUYS THERE ARE ALWAYS GOOD GUYS

Published: 19 March 2013

Protective autoantibodies in the rheumatic diseases: lessons for therapy

Gregg J. Silverman ✉, Jaya Vas & Caroline Grönwall

Nature Reviews Rheumatology **9**, 291–300 (2013) | Cite this article

ALDH4A1 is an atherosclerosis auto-antigen targeted by protective antibodies

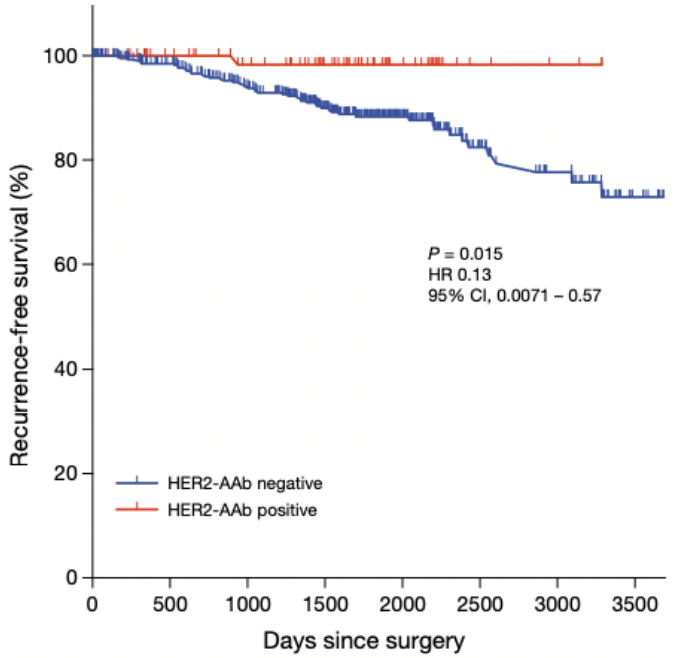
https://doi.org/10.1038/s41586-020-2993-2
Received: 3 December 2019
Accepted: 5 October 2020
Published online: 2 December 2020

Cristina Lorenzo[1], Pilar Delgado[1,6], Christian E. Busse[2,7], Alejandro Sanz-Bravo[1,7], Inmaculada Martos-Folgado[1,7], Elena Bonzon-Kulichenko[3,4,7], Alessia Ferrarini[3], Ileana B. Gonzalez-Valdes[3], Sonia M. Mur[1], Raquel Roldán-Montero[5], Diego Martinez-Lopez[5], Jose L. Martin-Ventura[4,5], Jesús Vázquez[3,4], Hedda Wardemann[2] & Almudena R. Ramiro[1]✉

Breast Cancer Res Treat (2016) 157:55–63
DOI 10.1007/s10549-016-3801-4

Protective effect of naturally occurring anti-HER2 autoantibodies on breast cancer

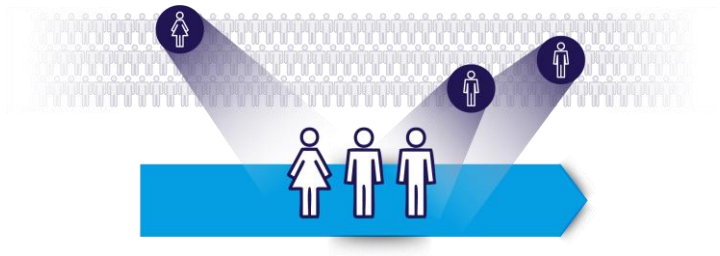
Yukiko Tabuchi[1] · Masafumi Shimoda[1] · Naofumi Kagara[1] · Yasuto Naoi[1] · Tomonori Tanei[1] · Atsushi Shimomura[1] · Kenzo Shimazu[1] · Seung Jin Kim[1] · Shinzaburo Noguchi[1]



ALCHEMAB CONCEPT

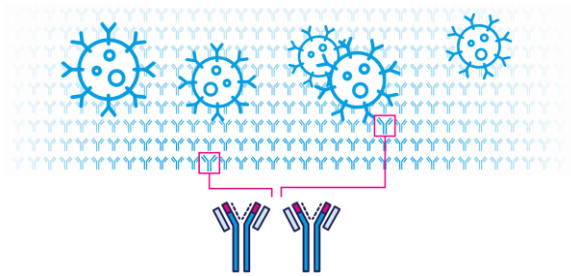
WE IDENTIFY

We identify especially resilient individuals – and learn how they overcome or resist disease



WE SEQUENCE

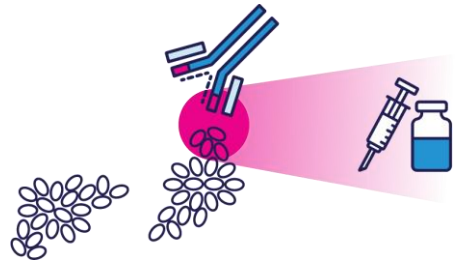
We sequence B cells from the resilient individuals and identify antibodies with similar properties



Antibodies

WE DISCOVER

We discover the binding targets of the antibodies, understand their protective properties and develop candidates that replicate the protective effect



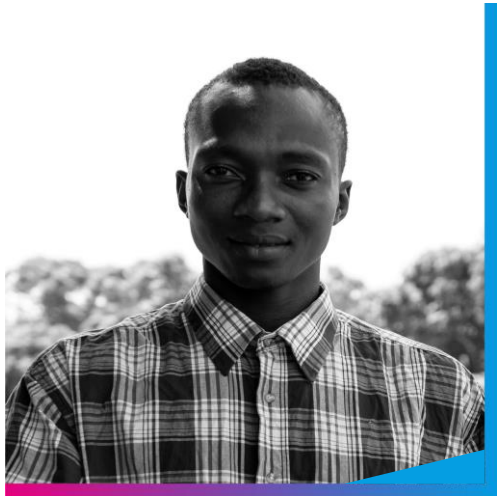
Novel Antibody Therapeutic

Unbiased platform to identify novel therapeutics

DEFINING “RESILIENCE”

Patients who survive advanced cancer

Patients progressing unusually slowly with neuro-degenerative disorders



People who survive grievous, deadly infectious disease

Long-lived, healthy individuals

WE COLLABORATE WITH A BROAD AND GROWING NETWORK OF INSTITUTIONS

ALCHEMAB THERAPEUTICS

NEURODEGENERATION

CHDI FOUNDATION
Accelerating therapeutic development for Huntington's disease

Queen Mary University of London

lumind idsc DOWN SYNDROME FOUNDATION

EPAD
European Prevention of Alzheimer's Dementia Consortium

UCL

ONCOLOGY

CY-Biobank
Research and Innovation Center of Excellence

HCL HOSPICES CIVILS DE LYON

St MARK'S THE NATIONAL BOWEL HOSPITAL

CENTRE DE LUTTE CONTRE LE CANCER LEON BERARD

CANCER RESEARCH UK

GUSTAVE ROUSSY CANCER CAMPUS GRAND PARIS

UNIVERSITY OF OXFORD

AstraZeneca

PLATFORM TECHNOLOGY

JOHNS HOPKINS UNIVERSITY

illumina

UNIVERSITY OF OXFORD

OTHER AREAS

Amsterdam UMC
Universitair Medische Centra

NHS Barts Health NHS Trust

NVIDIA

CONVERGENT AUTOANTIBODY SEQUENCES PROVIDE THE STARTING POINT FOR DISCOVERY

POPULATION B CELL REPERTOIRE ANALYSIS

Sequences of disease-free population

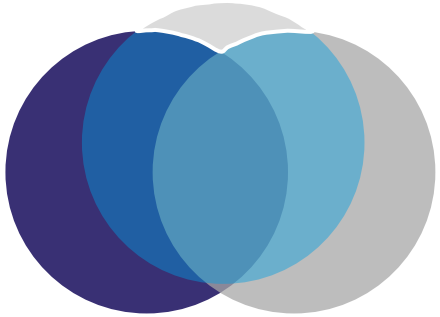
Sequences of resilient patients

Sequences of disease progressors

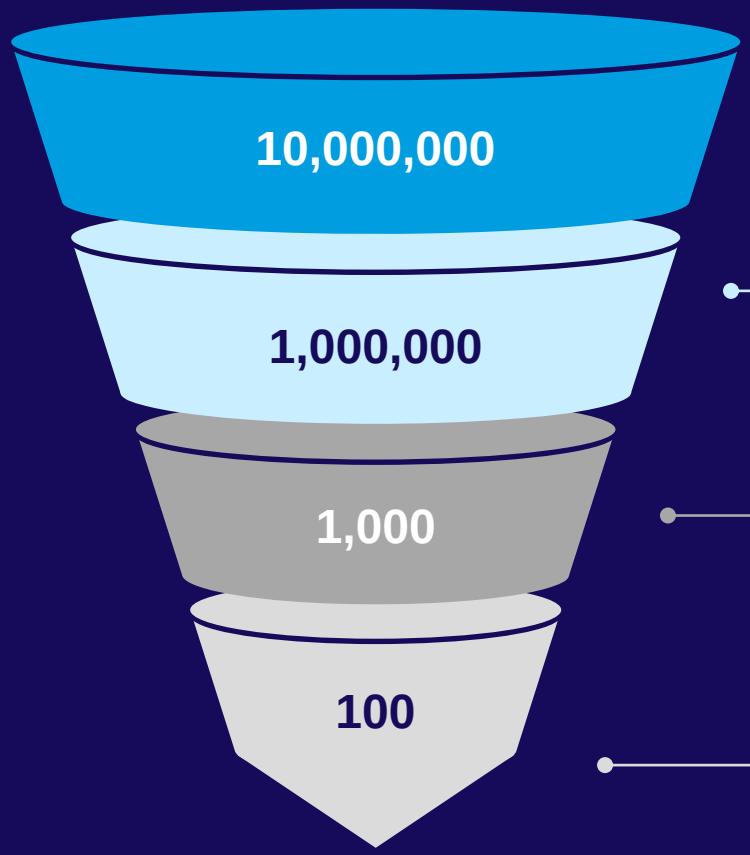
10’s-100’s of Individuals;
Millions of BCR sequences

CONVERGENCE

Shared antibodies that do not occur in controls or progressors



REDUCING THE VAST B CELL REPERTOIRE DOWN TO CANDIDATE ANTIBODIES



CLUSTERING

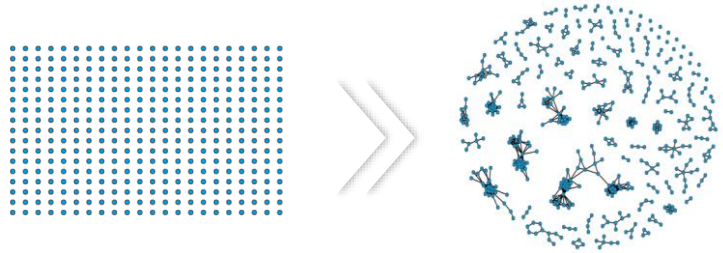
Grouping together antibodies based on sequence relatedness

CONVERGENCE

Searching for clusters containing sequences derived from multiple resilient individuals – indicates selection for similar specificities

VALIDATE & TRIAGE

Validating convergent signals, and understanding features of the best antibodies



B cell activation

Structure

Liabilities

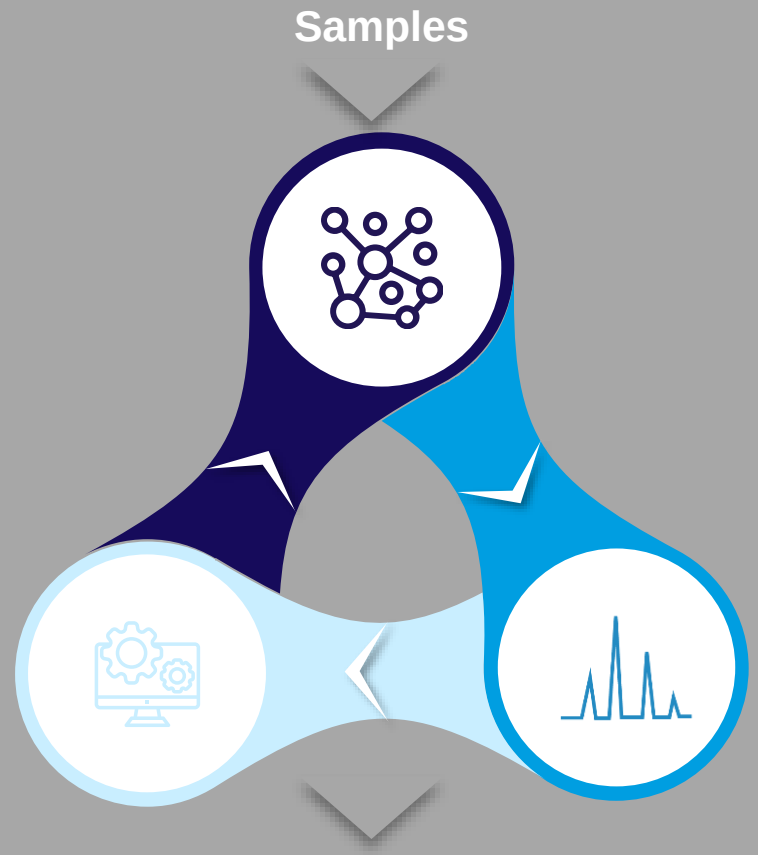
DISCOVERY PROCESS POWERED BY ADVANCED SEQUENCING, BIG DATA, AND DEEP LEARNING

ALCHEMAB THERAPEUTICS

CORE TECHNOLOGIES

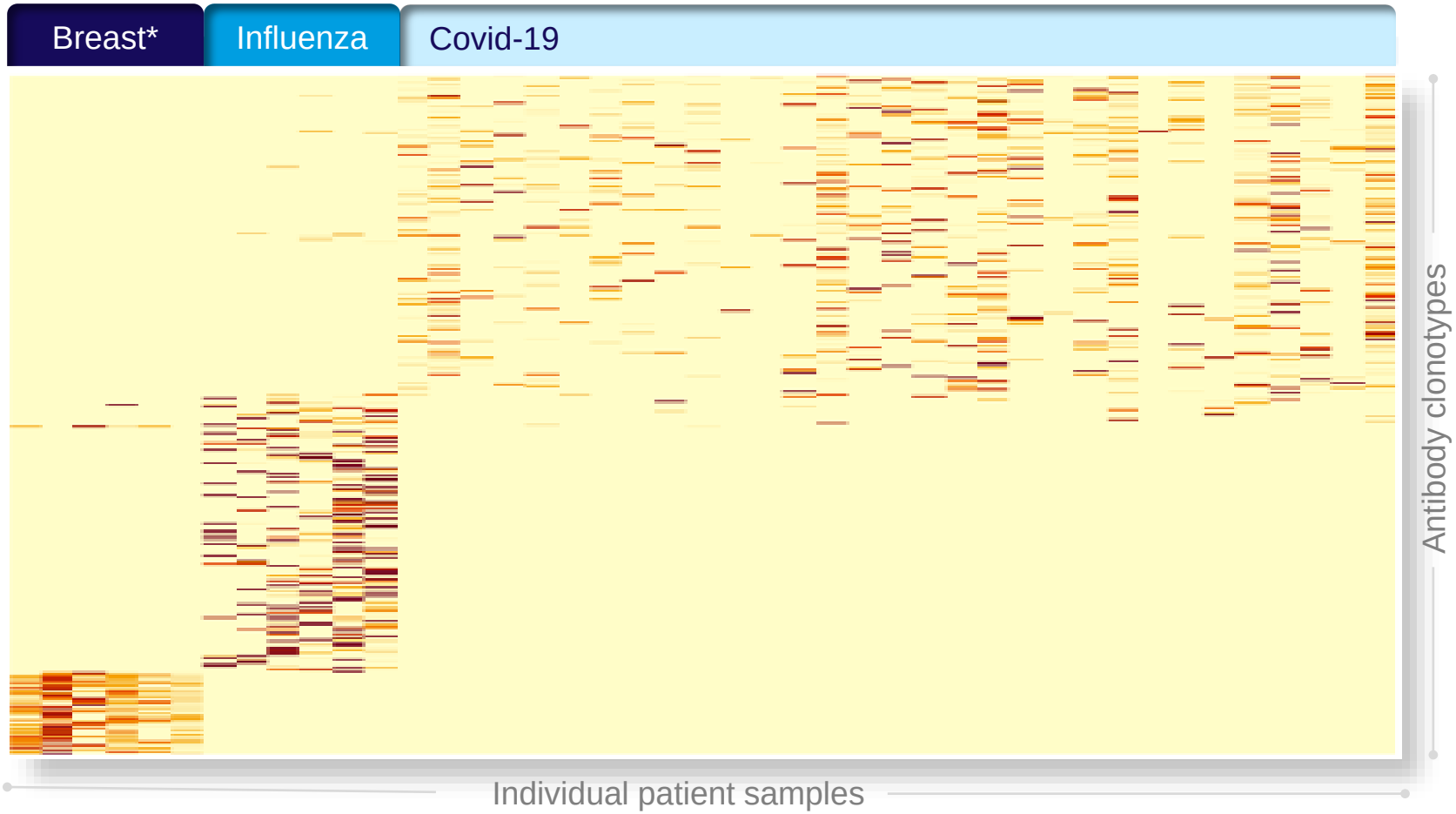
- **Repertoire analysis** — Leading expertise & capabilities in BCR repertoire analysis
- **Antigen proteomics** — Serum analysis and target deconvolution tools
- **Deep learning** — Proprietary technologies

PROCESS



DISEASE-ASSOCIATED ANTIBODY SIGNATURES

Shared by individuals within a disease group and distinct from healthy controls



Heatmap of 1,337 COVID-19 clonotypes, 1,180 Influenza clonotypes and 351 breast cancer clonotypes, demonstrating that the convergent signatures are unique to each disease cohort. Healthy controls subtracted.

*Breast cancer

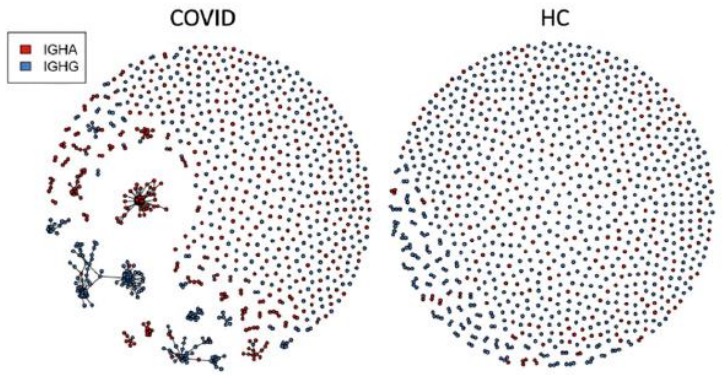
Infectious Disease CASE STUDY

COVID-19 STUDY

frontiers in Immunology

Deep Sequencing of B Cell Receptor Repertoires From COVID-19 Patients Reveals Strong Convergent Immune Signatures

Jacob D. Galson[1], Sebastian Schaetzle[1], Rachael J. M. Bashford-Rogers[1,2], Matthew I. J. Raybould[3], Aleksandr Kovaltsuk[3], Gavin J. Kilpatrick[1], Ralph Minter[1], Donna K. Finch[1], Jorge Dias[1], Louisa K. James[4], Gavin Thomas[4], Wing-Yiu Jason Lee[4], Jason Betley[5], Olivia Cavlan[1], Alex Leech[1], Charlotte M. Deane[3], Joan Seoane[6], Carlos Caldas[7], Daniel J. Pennington[4], Paul Pfeffer[4] and Jane Osbourn[1]*

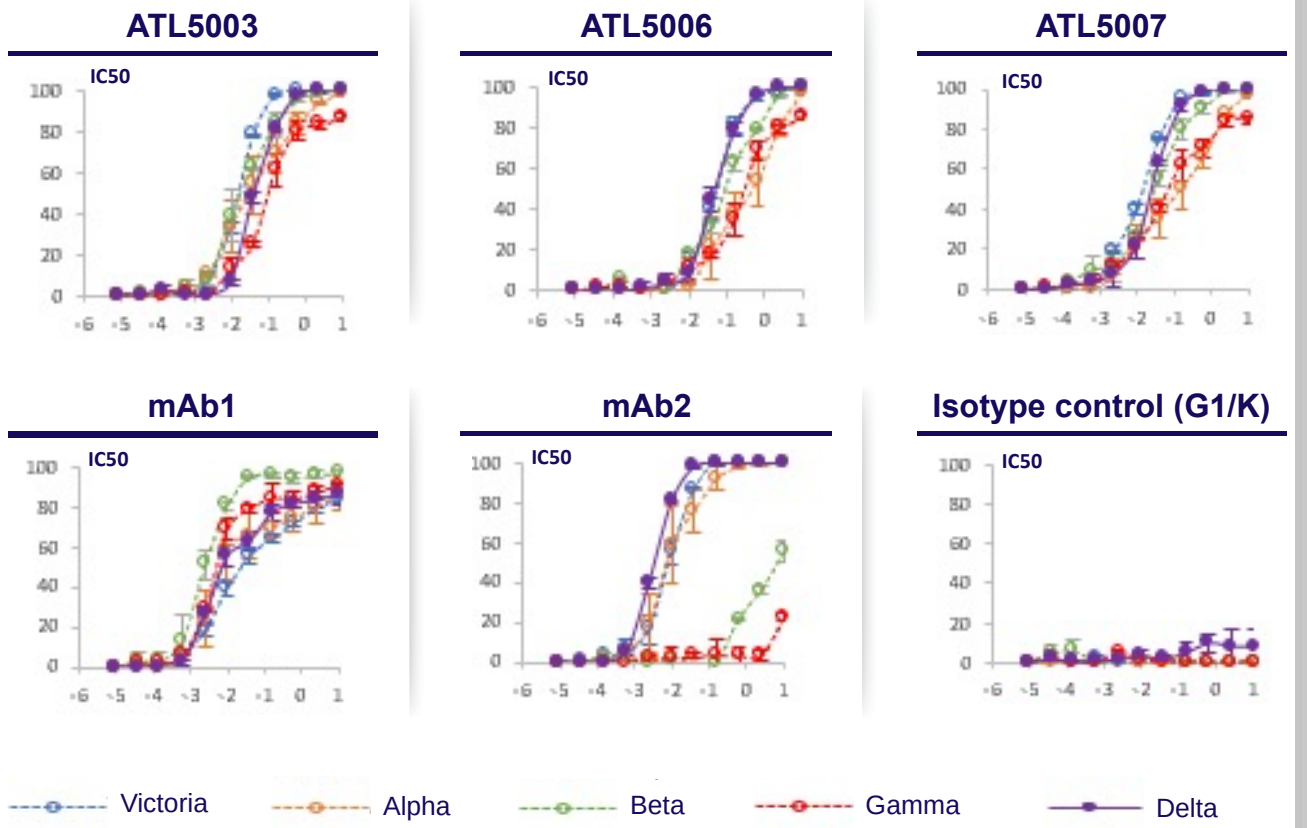


Galson *et al.*, https://doi.org/10.3389/fimmu.2020.605170

Potent multi-strain covid neutralizing antibodies identified

Alchemab

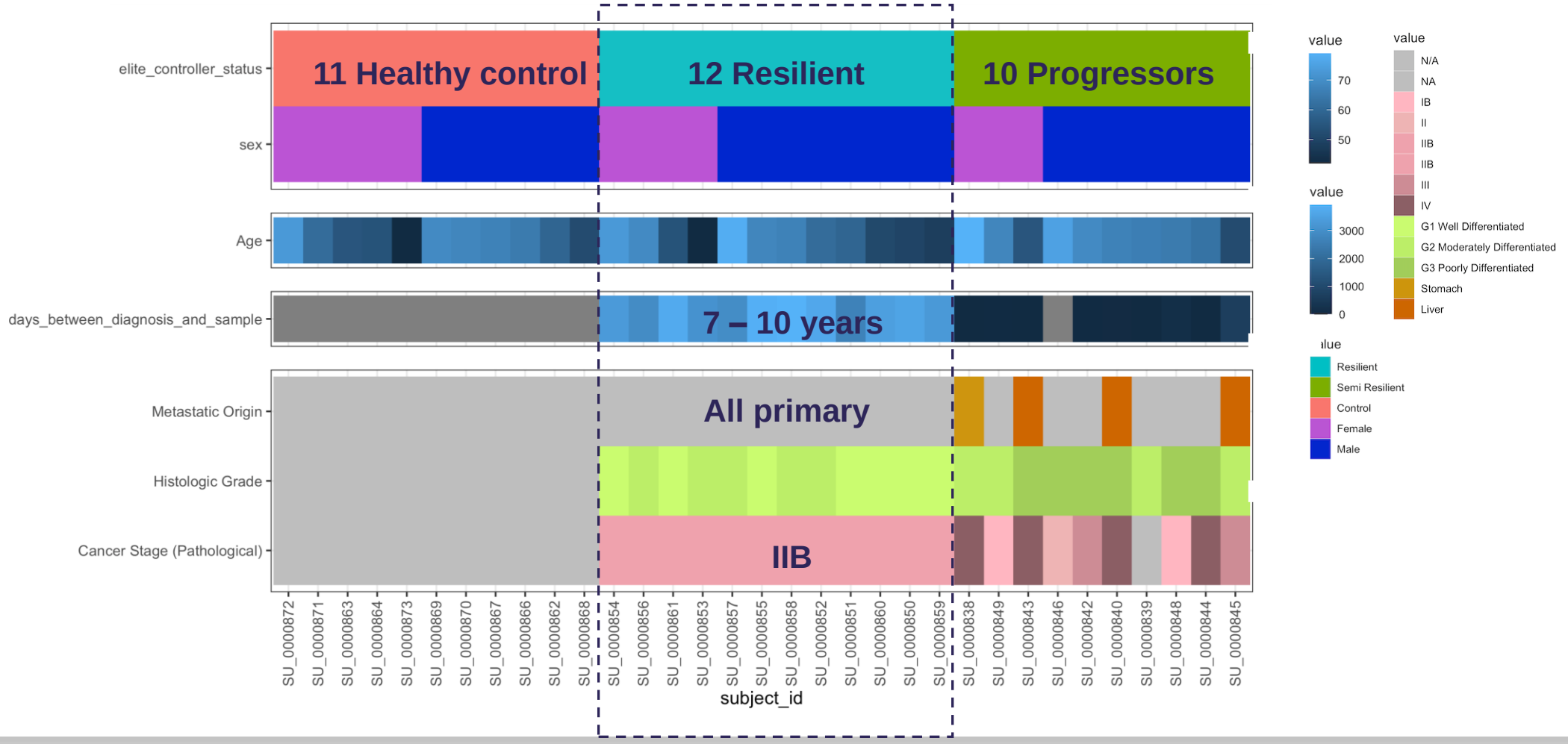
Commercial cocktail & control



Oncology
CASE STUDY

PANCREATIC CANCER COHORT

ALL PDAC PATIENTS HAVE UNDERGONE RESECTION



HIGHLY CONVERGENT RESILIENT CLONOTYPES FOUND IN MULTIPLE INDIVIDUALS

ALCHEMAB THERAPEUTICS

10 clonotypes found with exceptional convergence among resilient individuals **which were not found in progressors:**

- Length-independent super-convergence
- Low probability of generation & rare in healthy controls
- Predominantly IgG1

CLUSTER SIZE	CONVERGENCE LEVEL (OUT OF 12)	CDR3 LENGTH	MUTATIONS	GENERATION PROBABILITY	PROP. IN HEALTHY CONTROL	PREVALENT ISOTYPE
151	8	20	1.58	4.35E-12	0.060	IGHG1
40	8	20*	1.65	9.34E-12	0.050	IGHG1
43	8	19*	1.35	4.63E-20	0.186	IGHG1
73	7	16	1.51	2.13E-13	0.055	IGHG1
42	5	16	2.07	1.29E-12	0	IGHG1
73	5	15*	1.41	7.42E-11	0.082	IGHG1
33	5	14*	1.52	7.69E-11	0	IGHG1
17	5	13	1.18	1.14E-11	0.059	IGHG1
16	6	13	**17.63**	1.01E-11	0	IGHM
22	5	11	**25.59**	5.20E-10	0.091	IGHA1

HOMOLOGY TO APPROVED KDR MAB TRANSLATES TO TARGET BINDING

ALCHEMAB THERAPEUTICS

Ramucirumab is a VEGFR2 (KDR) antibody, approved for treatment of solid tumors

ARTICLES | VOLUME 383, ISSUE 9911, P31-39, JANUARY 04, 2014

Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial

Dr Prof Charles S Fuchs, MD • Jiri Tomasek, MD • Prof Cho Jae Yong, MD • Filip Dumitru, MD • Rodolfo Passalacqua, MD • Prof Chanchal Goswami, MD • et al. Show all authors • Show footnotes

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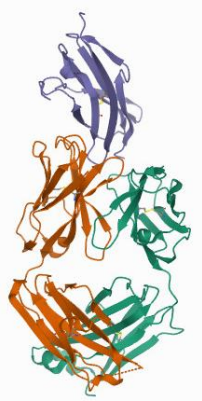
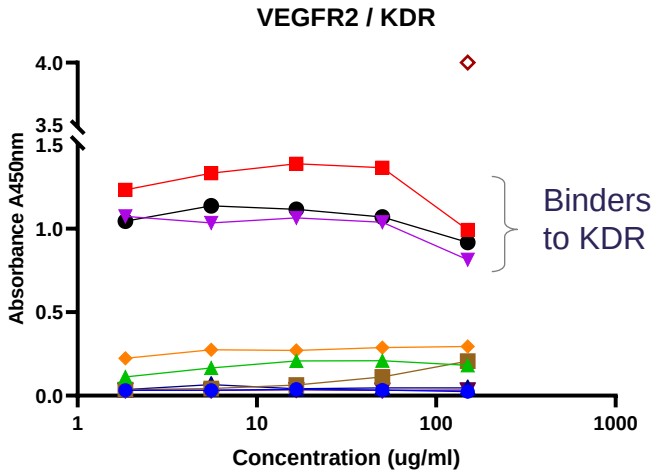


One of Alchemab's convergent clusters is highly homologous to ramucirumab

- Assay shows binding for multiple antibodies from clonotype 41671 to KDR



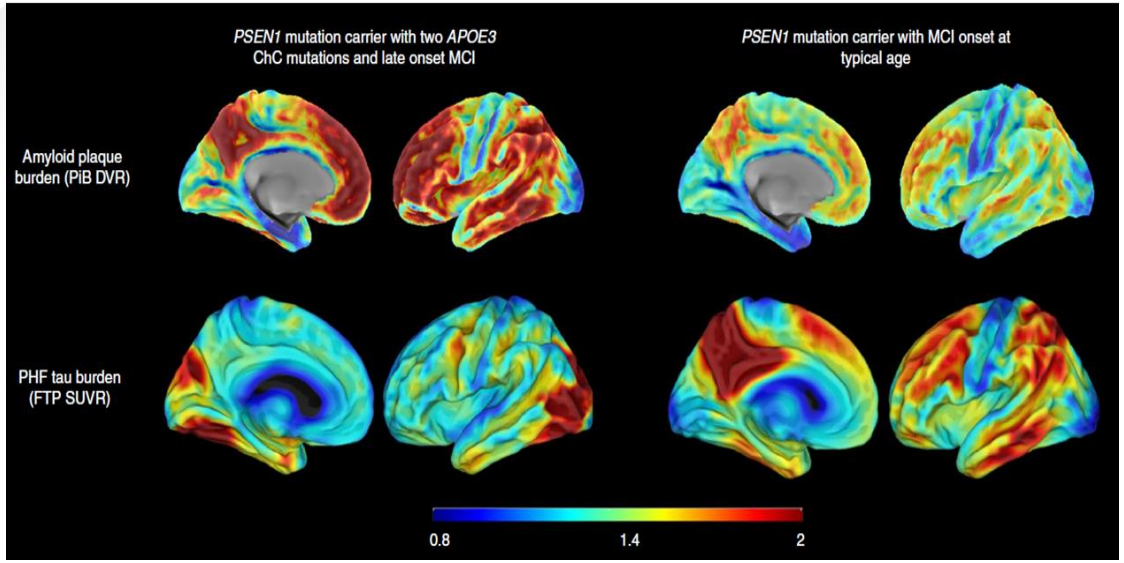
Neuro degeneration
CASE STUDY

DEFINING RESILIENCE IN AD

MEASURE OF RESILIENCE	RESILIENT SAMPLE COHORTS
Mini Mental State exam Cognitive function	Commercial study **100+ cohort** EPAD*
ApoE status and other genetic mutations	EPAD **100+ cohort**
B-amyloid (CSF/ plaques) Tau (CSF/plaques) Additional biomarkers (NfL)	EPAD

Report of Extraordinary individual with Presenilin (PSEN1) mutation but considerable delay of cognitive decline, with high b-amyloid plaque load but low Tau

Arboleda-Valasquez et al (2019) Nature Medicine



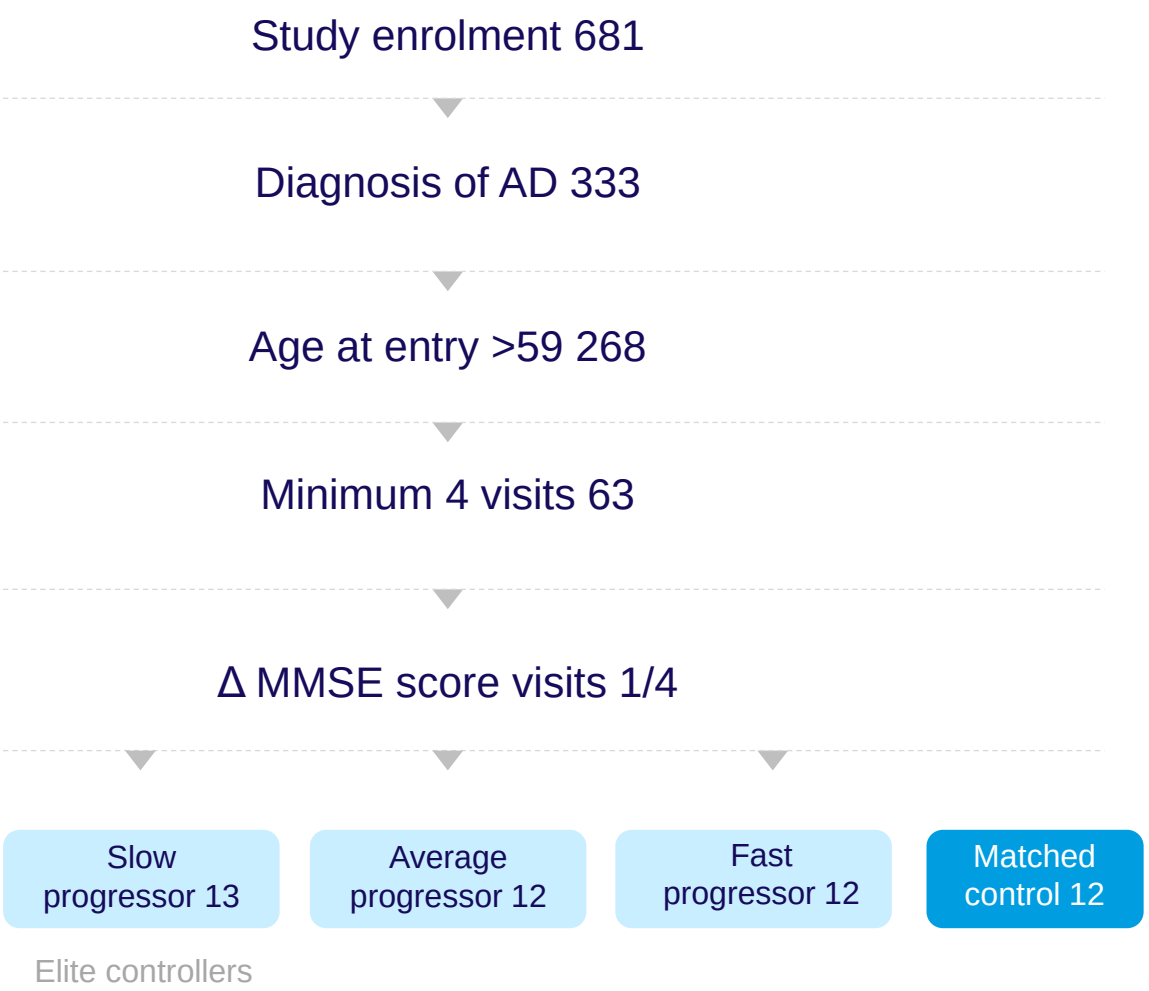
* European Prevention of Alzheimer's Dementia

ALZHEIMER'S DISEASE PILOT STUDY

Longitudinal study over 2 years (visits every 6 months)

Mini Mental State Exam score used for assessment of disease progression

Starting range 15-24 out of a maximum score of 30

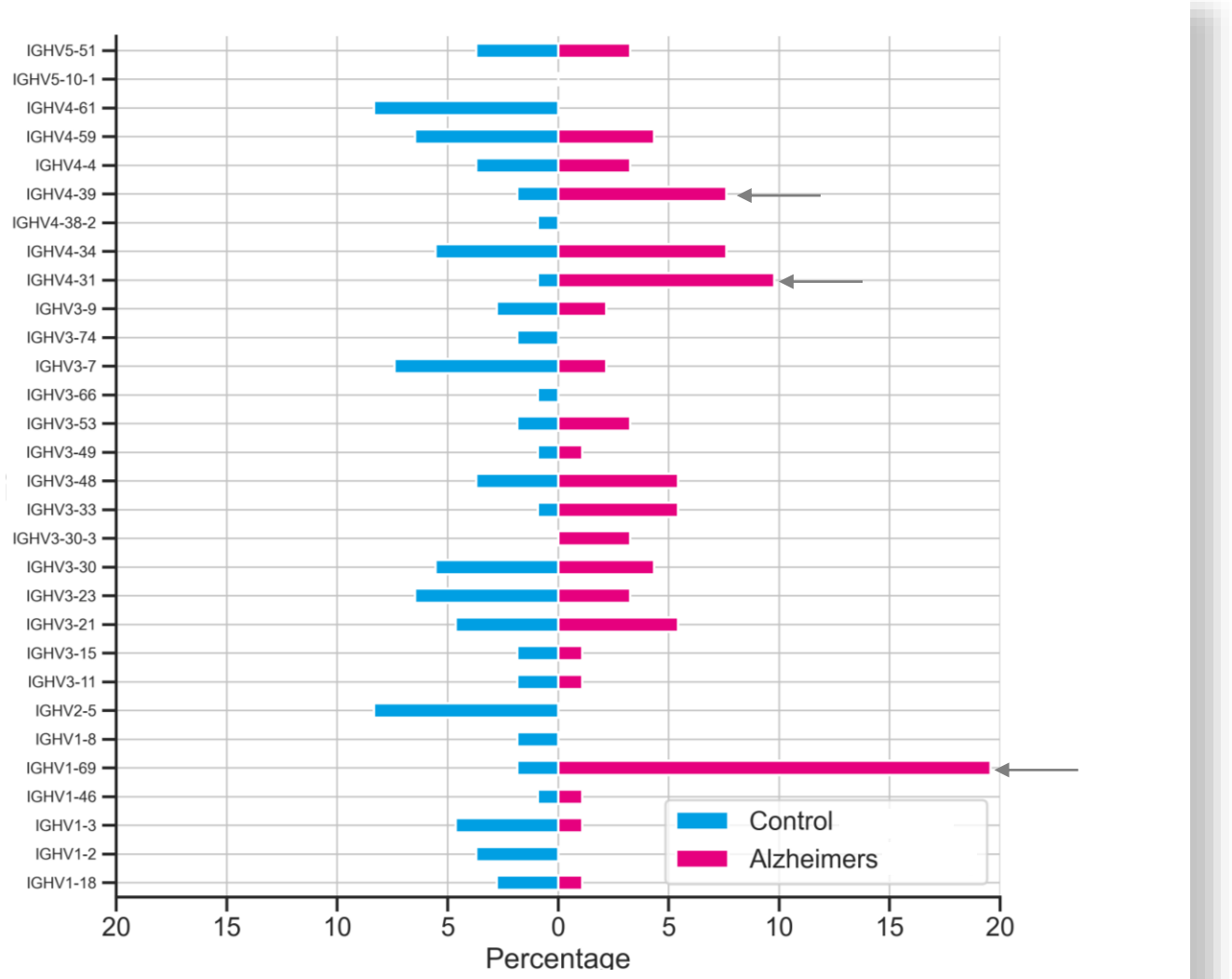


IDENTIFICATION OF AD CONVERGENT CLONES

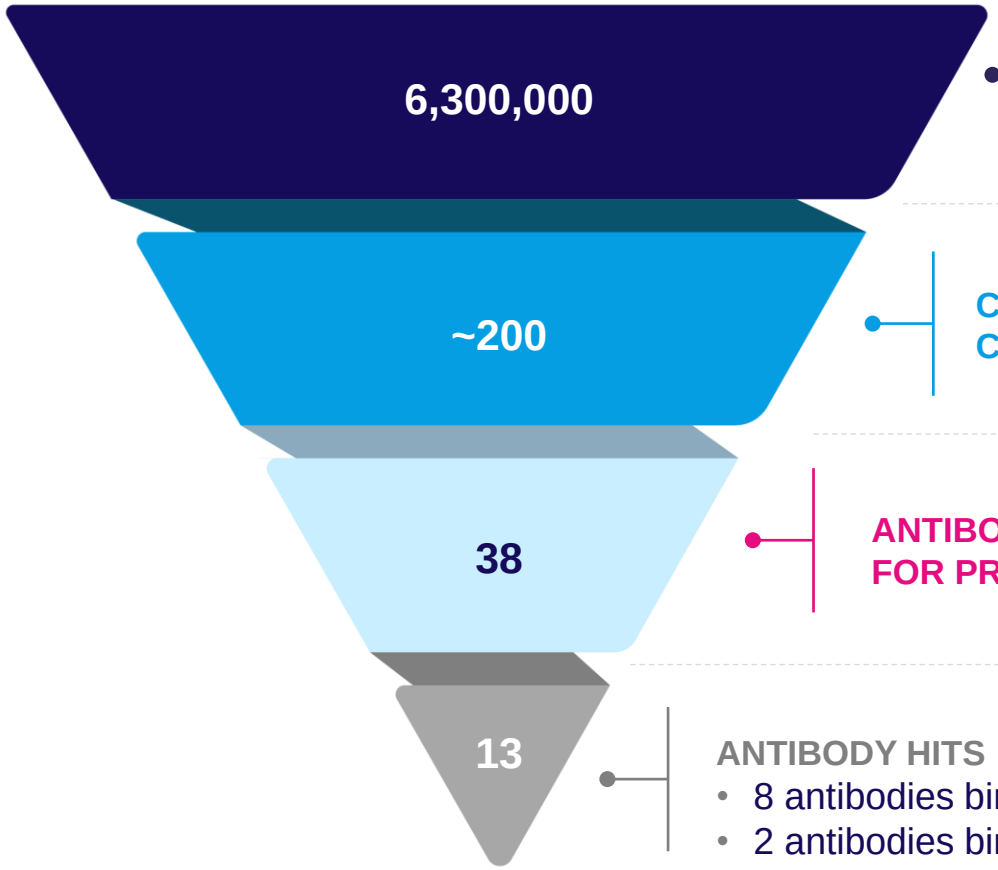
Sequence data from 30 Alzheimer's patients was triaged to select 38 antibodies according to the following workflow:

- **CLONOTYPING**:
  sequences with near identical V, J, and CDR3 length are clustered
- **EXCLUSIVITY:**
  Clonotypes chosen which are sare exclusive to resilient group and not seen in healthy controls
- **EXPRESSION FILTER:**
  Sequence seen more than once per sample
- **MUTATION FILTER:**
  Filter for clonotypes undergoing active selection via somatic hypermutation

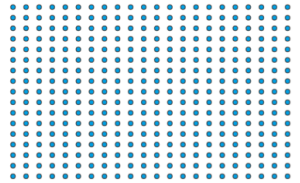
Exclusively convergent clonotypes have a highly skewed VH germline gene distribution which indicates disease specific activation



ALZHEIMER'S DISEASE ANTIBODY SELECTION



SEQUENCES



CONVERGENT CLUSTERS

ANTIBODIES TRIAGED FOR PRODUCTION

ANTIBODY HITS

- 8 antibodies bind immune cells
- 2 antibodies bind neuronal cells
- 3 antibodies bind astrocytes

Further analysis ongoing

Immunophenotyping

Neuronal cell lines

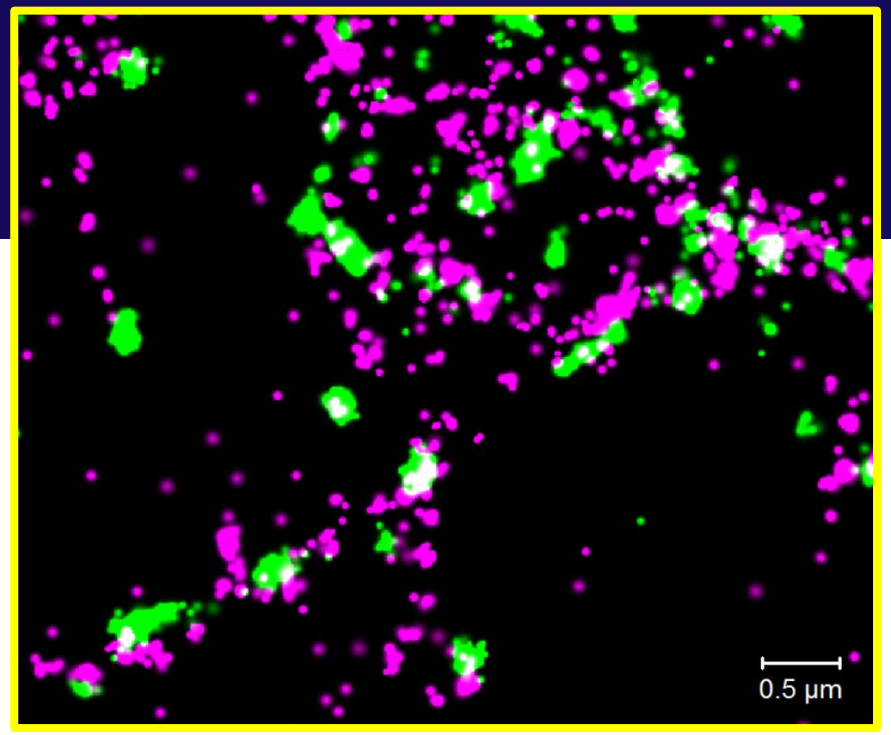
Human iPSC

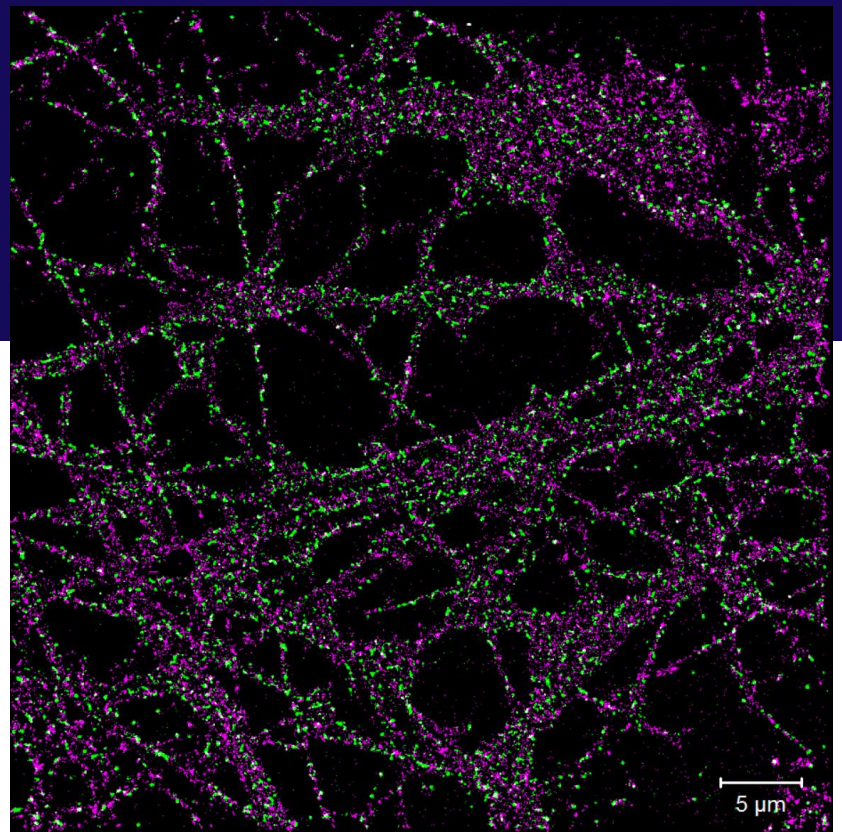
AD CONVERGENT ANTIBODY SHOWING NEURONAL BINDING (TARGET UNKNOWN)

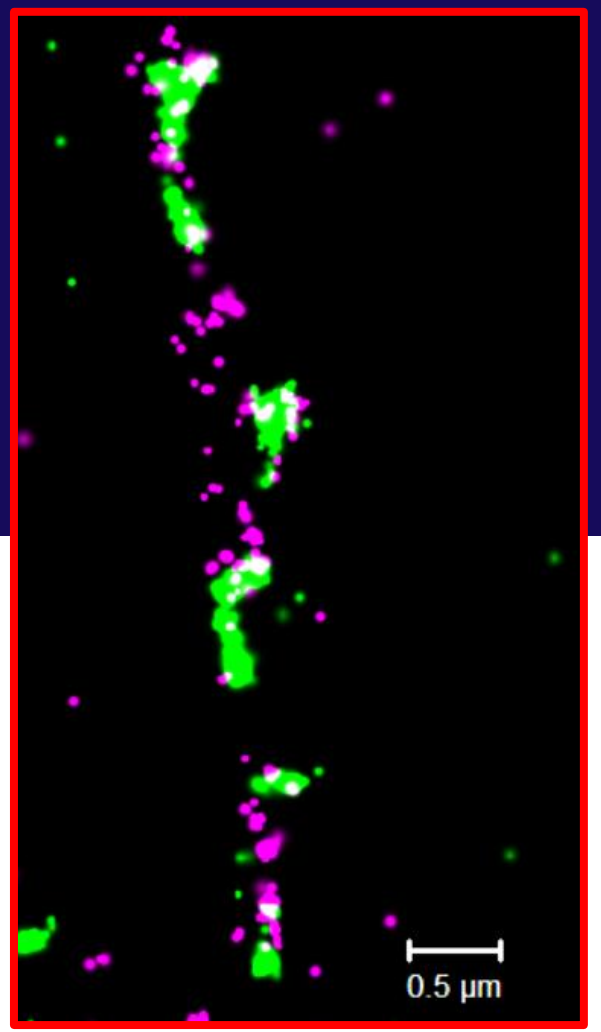
ALCHEMAB THERAPEUTICS

Alchemab Ab ATL 4527

VGLUT1 (pre-synaptic)



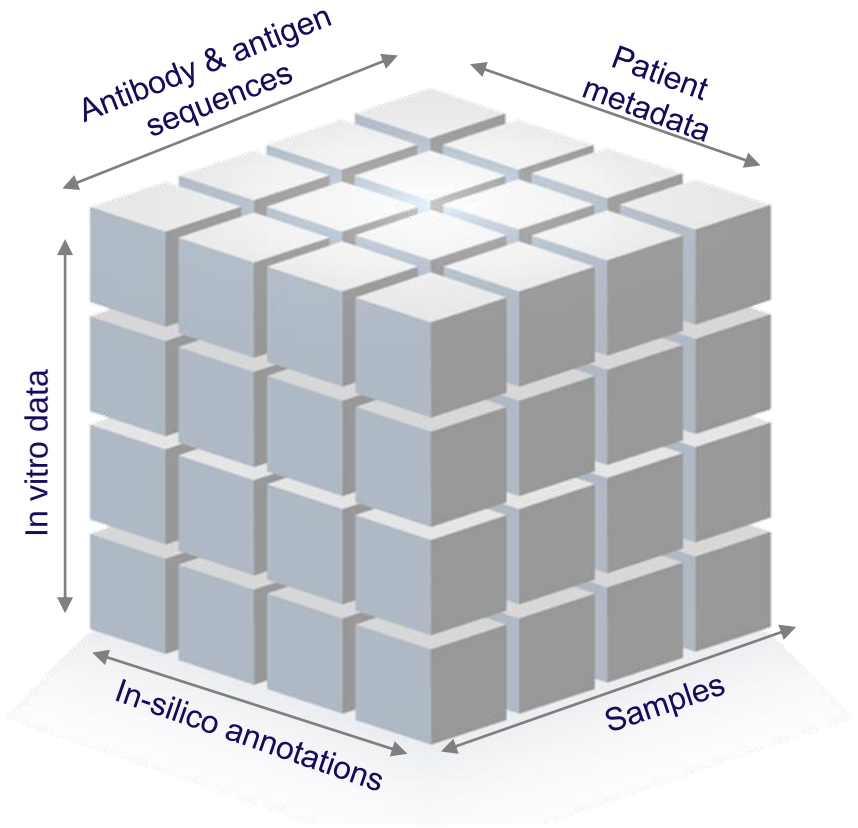




SUMMARY

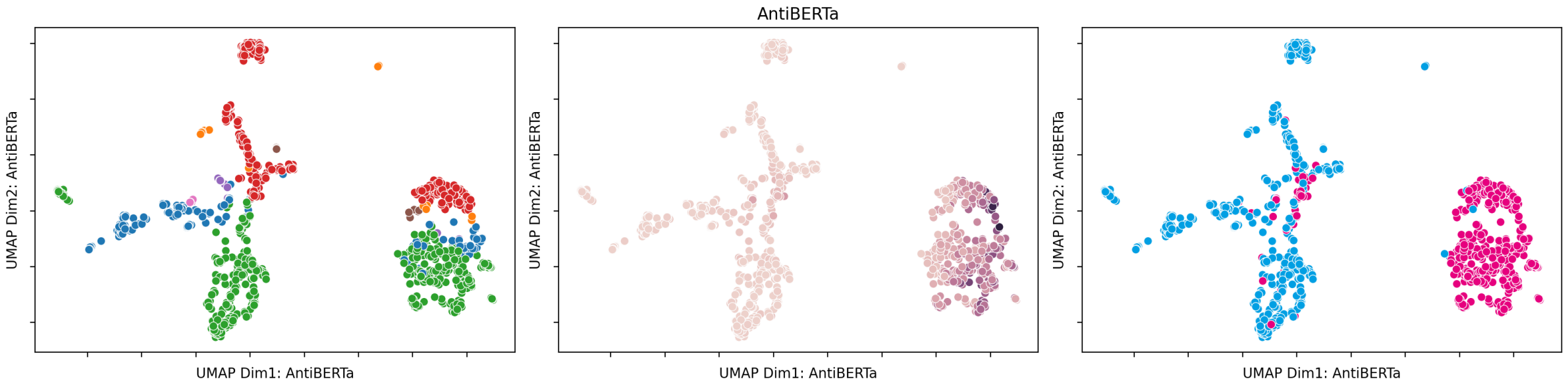
ALCHEMAB DATA CUBE: EXCEPTIONAL INSIGHT INTO BCR REPERTOIRES AND THEIR ASSOCIATION WITH DISEASE

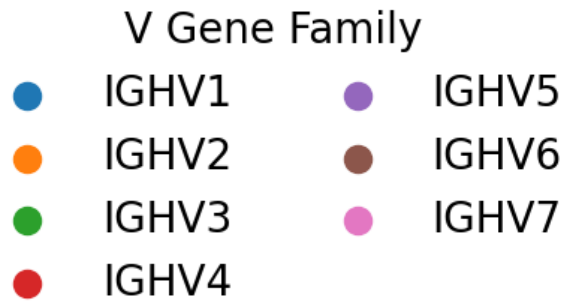
- FULL STACK AND ADVANCED ANALYTICS PIPELINE
- DEEP LEARNING TOOLKIT:
  - Quality assessments
  - Clustering
  - Cross disease analysis
  - In silico light chain pairing
  - Patient stratification

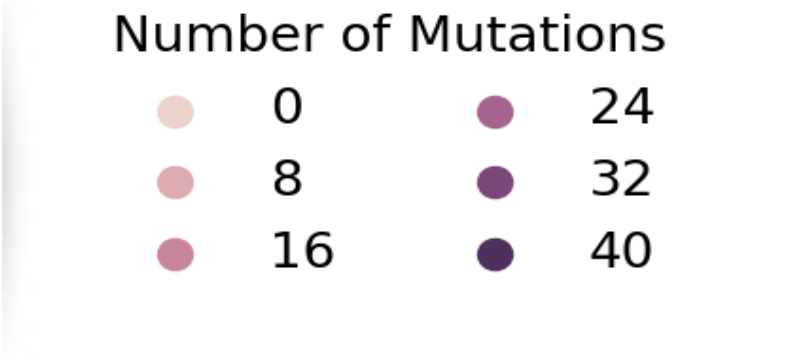


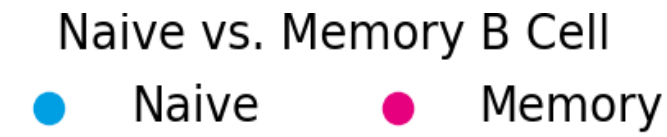
…enabling a powerful next generation drug discovery engine

ANTIBERTA: ALCHEMABS'S ANTIBODY-SPECIFIC LANGUAGE MODEL



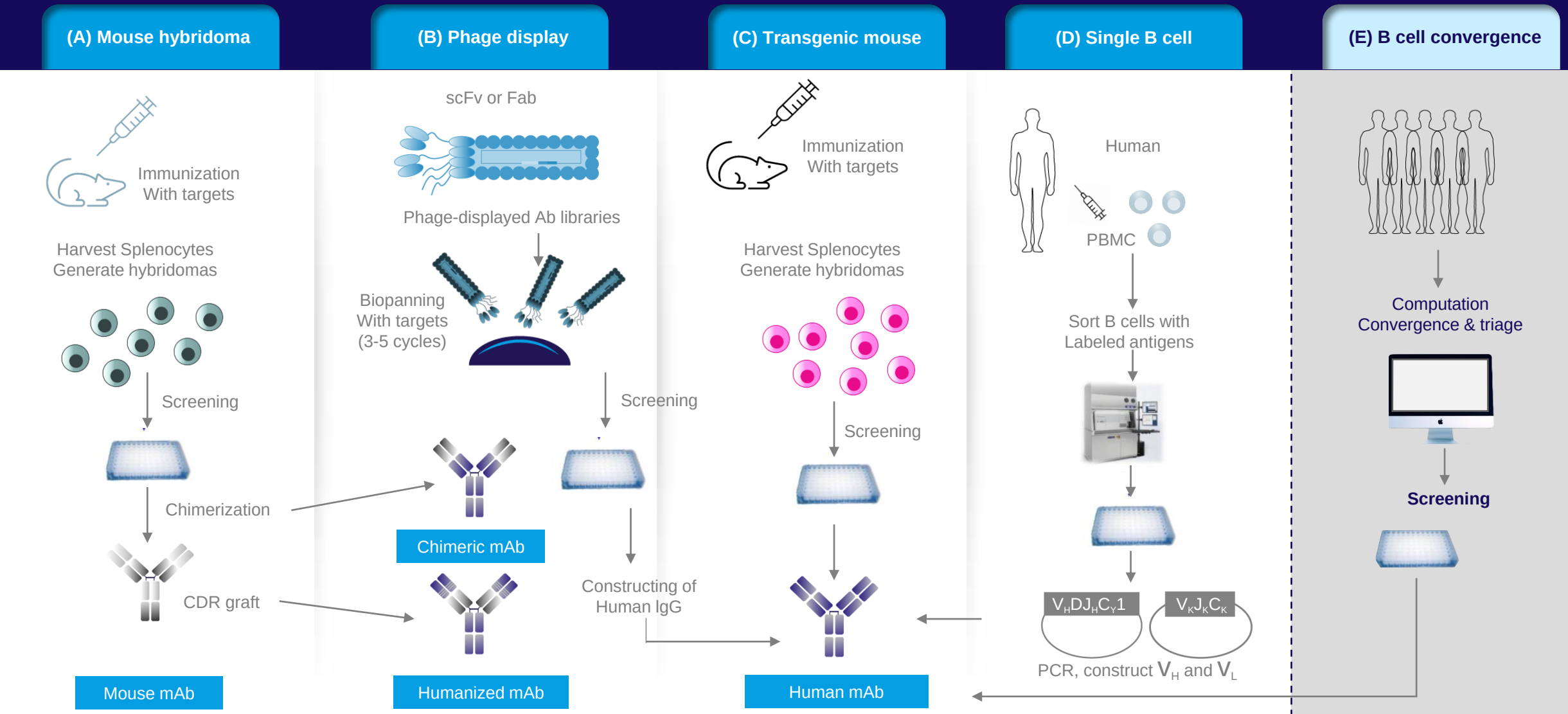






CONVERGENCE ANALYSIS COULD BE PART OF THE NEXT WAVE OF ANTIBODY GENERATION

ALCHEMAB THERAPEUTICS



Source: Journal of Biomedical Science, 21:1 (2020) - Adapted

OUR VISION: TO USE THE POWER OF THE HUMAN IMMUNE SYSTEM TO DISCOVER NEW MEDICINES AND DIAGNOSTICS

ALCHEMAB THERAPEUTICS

Discovering and developing protective, patient-originated therapeutic antibodies

- Focus on protective antibody responses
- Convergent in resilient individuals
- Target agnostic approach
- Advanced computational approaches