

From cells to therapeutics **Vivalis**[®]



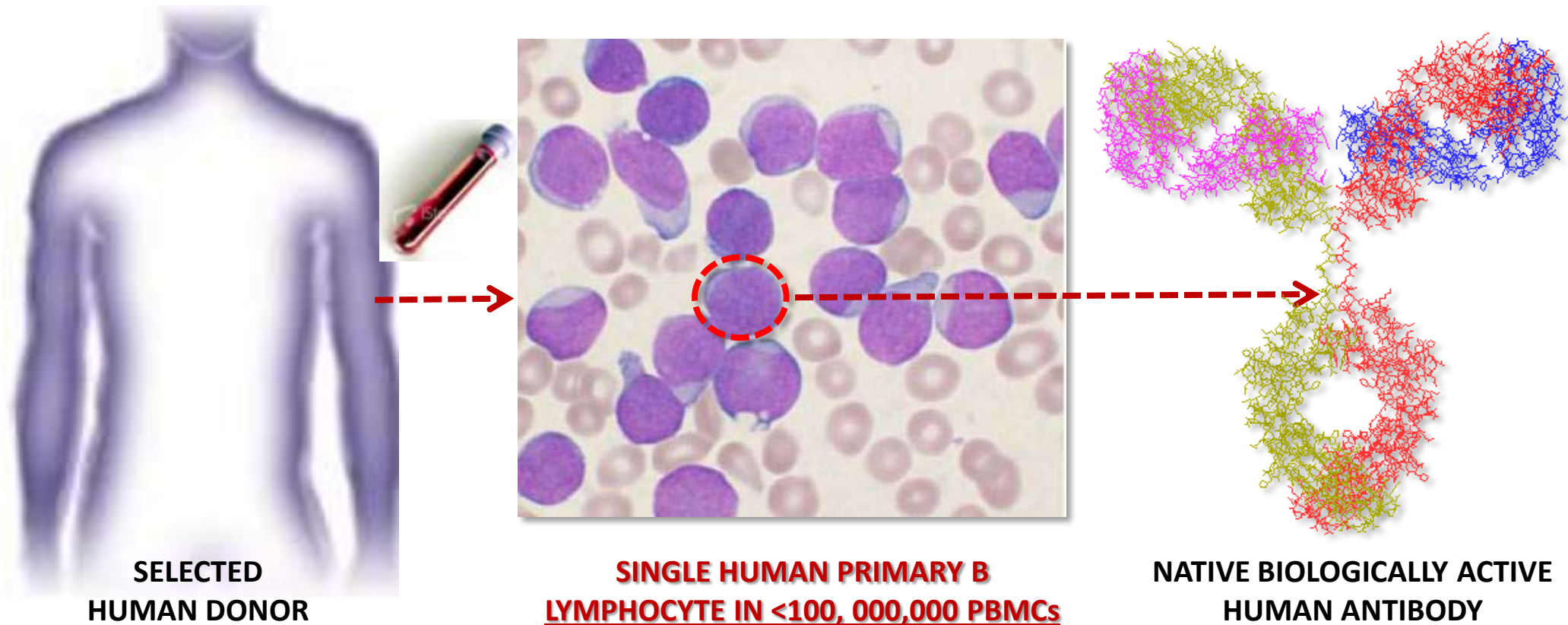
Screening & Discovery of Native Human Monoclonal Antibodies

M. Mehtali

Mykonos, June 26th 2012

Native Human Antibody Discovery Platform

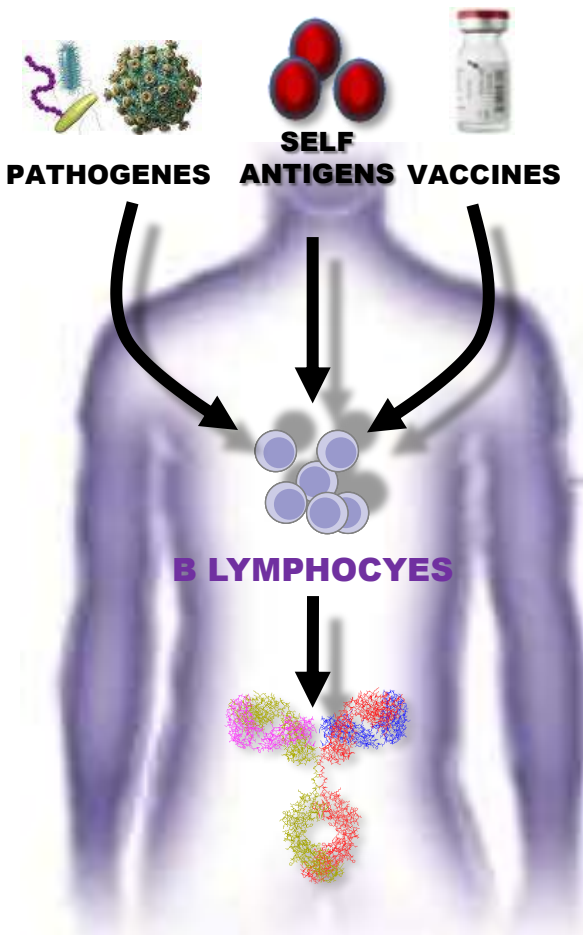
PRINCIPLE



DISCOVERY OF RARE NATIVE ANTIGEN SPECIFIC & FUNCTIONAL ANTIBODIES

Native Human Monoclonal Antibodies

From Primary Human B Lymphocytes to Human Monoclonal Antibodies



AN OPTIMAL PHARMACEUTICAL FORMAT

1. Humans naturally develop highly active antibodies against disease-related exogenous or self antigens
2. Antigens in their native conformation processed naturally by the human immune system
3. Native antibodies with no foreign sequences, naturally selected from full human repertoire (*minimal toxicity, limited off-target cross-reactivities*)
4. “Ready to Use”: High affinity (pM to nM) functional antibodies, with no need for further engineering
5. *However, technologies to access rare B lymphocytes secreting such antibodies of high therapeutic value have long been lacking*

Discovery of Native Human Monoclonal Antibodies

From Human Lymphocytes to Human Monoclonal Antibodies



- **Healthy donor**
- **Vaccinated donor**
- **Patient**

A TECHNOLOGY PLATFORMS THAT ALLOWS

1. Access to a Large Pool of Human Donors
(healthy & diseased)
2. High-Throughput Screening of Large Populations of Primary B Cells for antibody Function & Binding
3. Rapid Single Cell Screening of Primary B Cells & single cell gene cloning
4. Selection of rare B cells (<1/10 million) secreting antigen-specific & biologically active antibodies

VIVA | SCREEN™ TECHNOLOGY PLATFORM

Human Donors

Rapid access to a large pool of donors



STRATEGIC COLLABORATION WITH BLOOD TRANSFUSION CENTERS & HOSPITALS

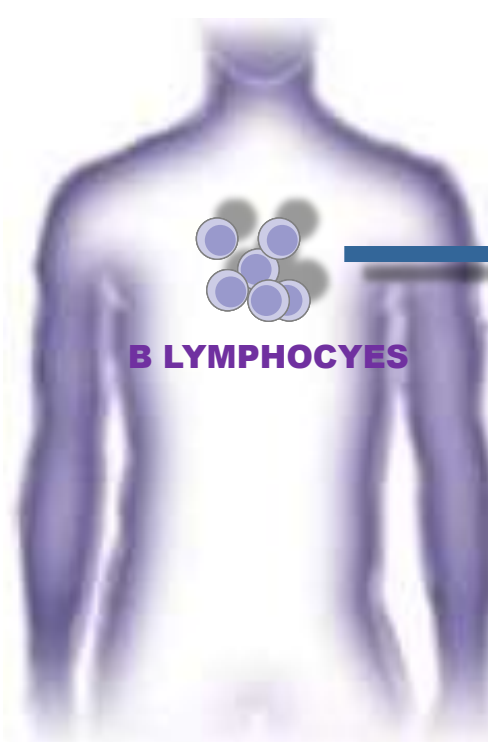
- Blind screening of thousands of healthy donors in a week
- Access to patients through specific agreements with hospitals
- Consolidated legal framework, including for future commercial applications (*IRB, ministry of health, agreement with blood transfusion center, Informed consent from blood donors*)
- Examples of donors already selected in past studies
 - Healthy blood donors: >8000 tested in various projects
 - Patients infected by viral or bacterial pathogens
 - Patients with specific pathologies (inflammatory diseases, cancer)
 - Parents of infants with specific viral infections
 - Medical staff exposed to patients with specific viral infections
 - Mother developing an allogenic response towards her infant platelets (single AA mutation)



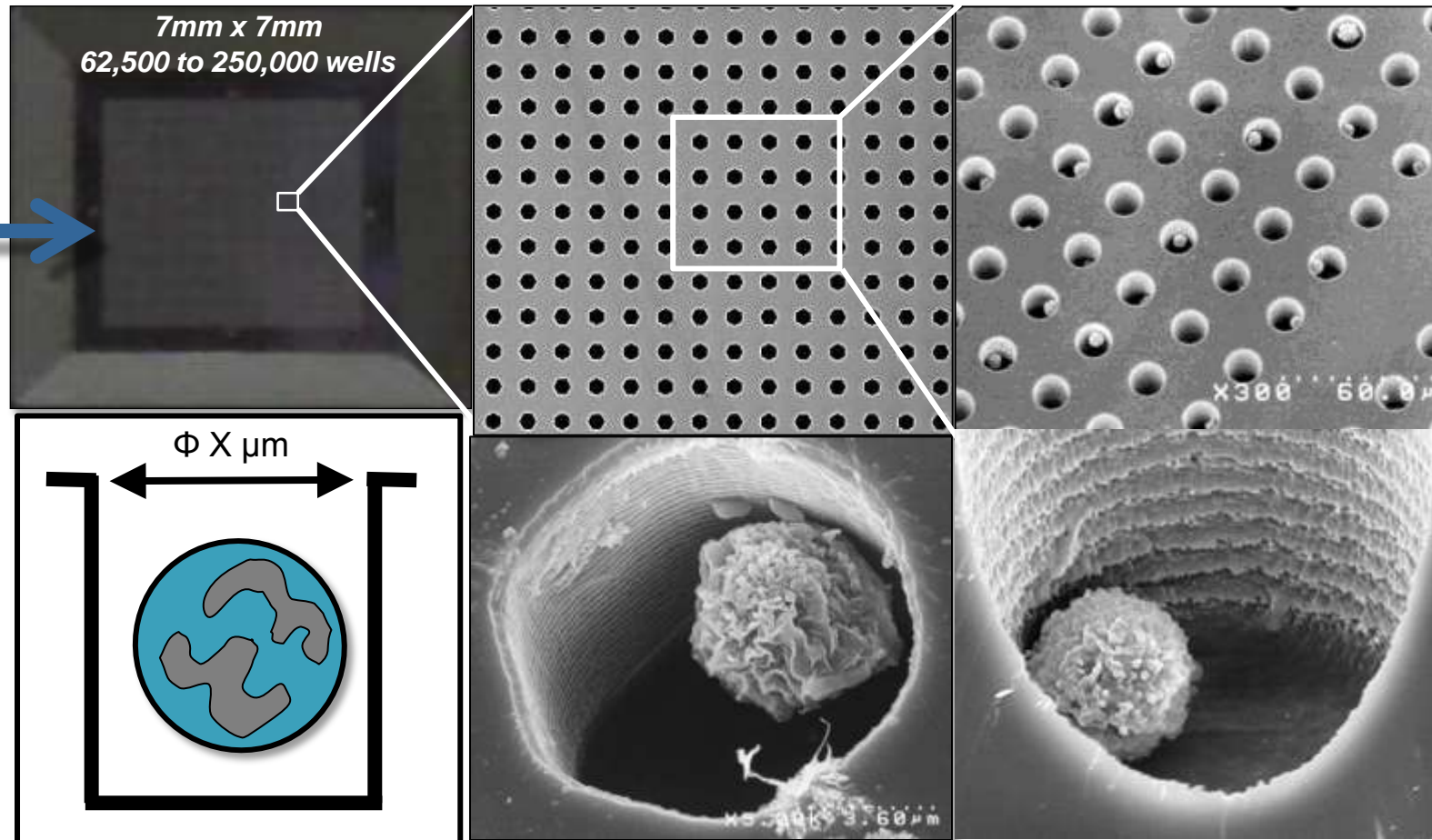
ISAAC™ Antibody Discovery Platform

Direct Single Cell Screening of B lymphocytes on microarray chips

Kishi et al. (Toyama, JPN) **PATENTED TECHNOLOGY (WO2009/ 017226)**



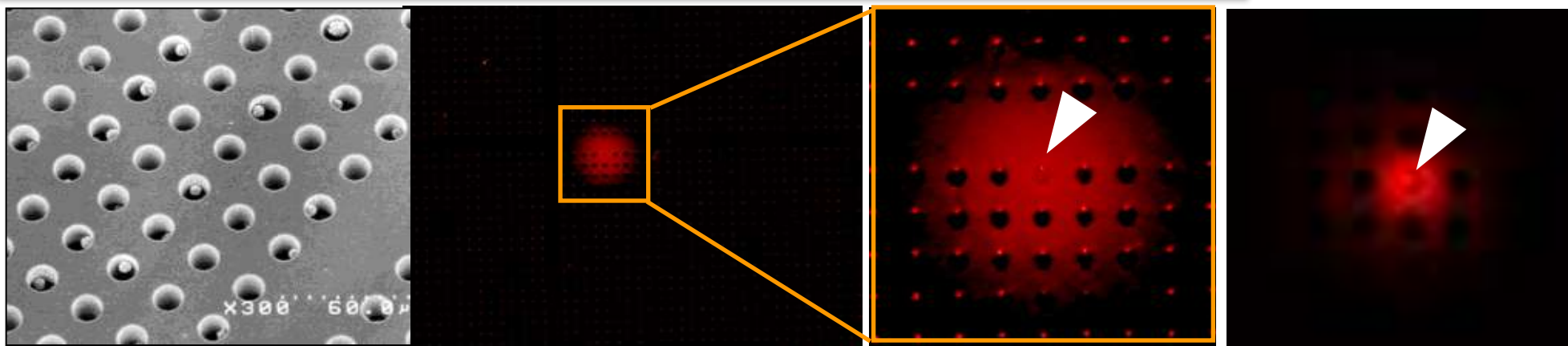
- Healthy donor
- Vaccinated donor
- Patient



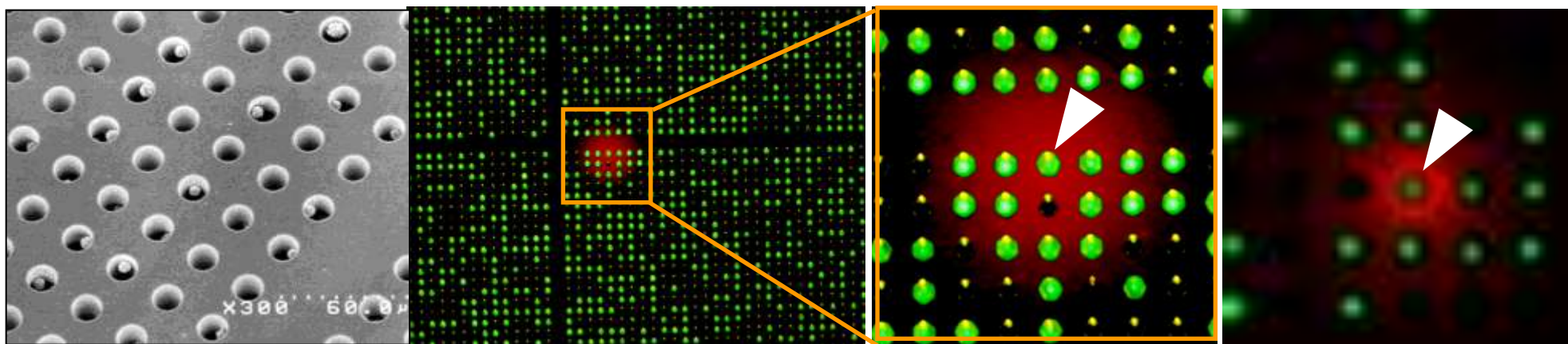
ISAAC™ Antibody Discovery Platform

Direct Single Cell Screening of B lymphocytes on microarray chips

Exemple for the discovery of human antibodies targeting antigen X



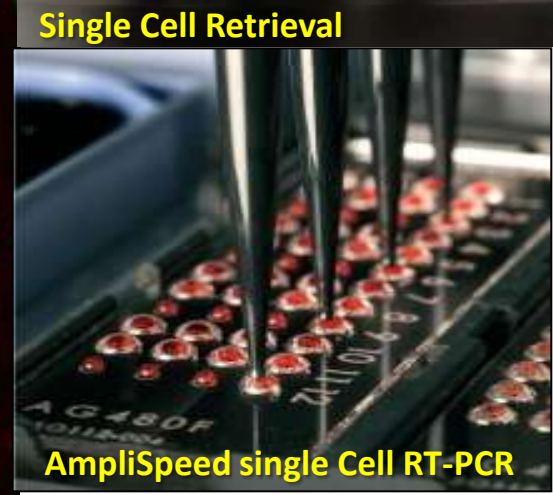
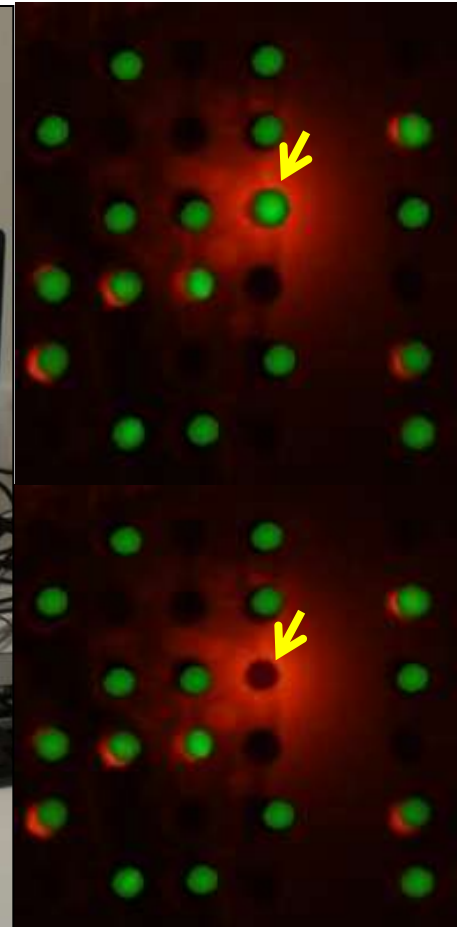
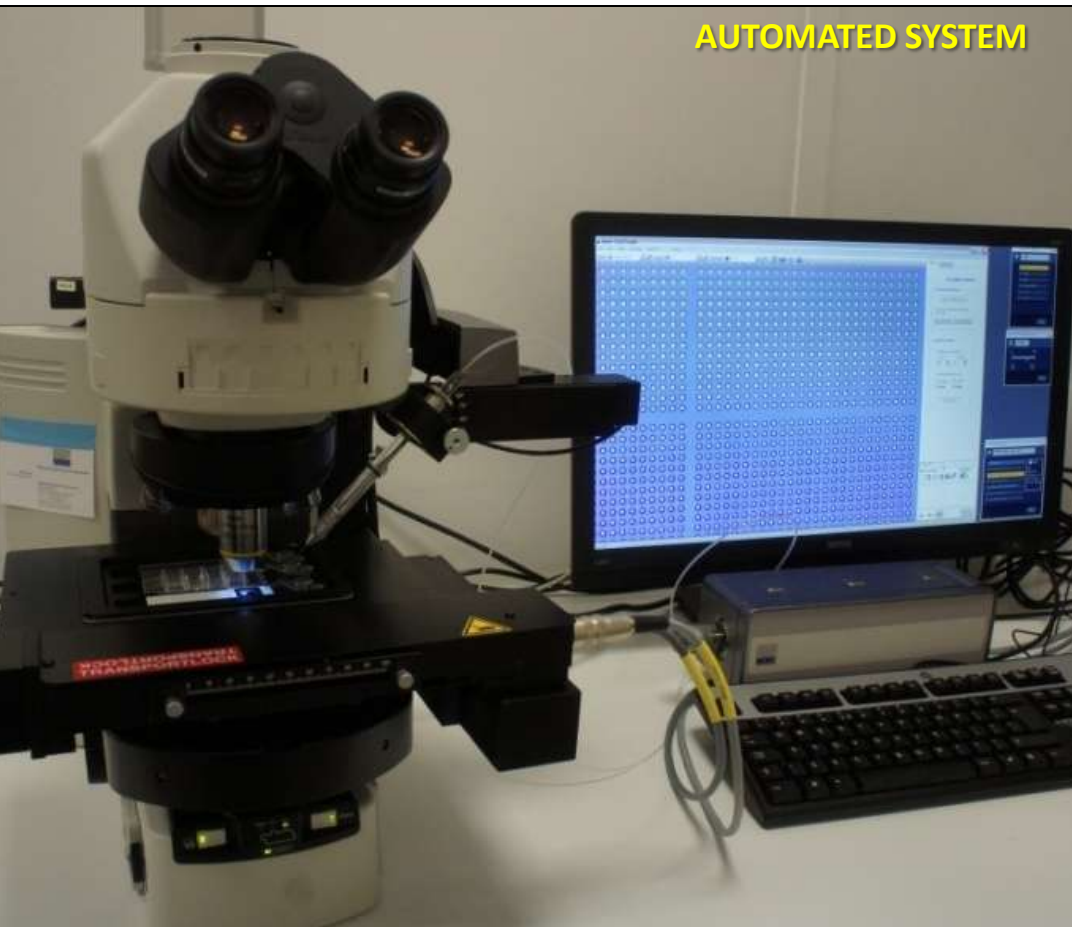
Labelling of secreted antibodies targeting antigen X



Merging of signals for cells and for secreted antibodies targeting antigen X

ISAAC™ Antibody Discovery Platform

Retrieval of single B lymphocytes secreting the antibody of interest

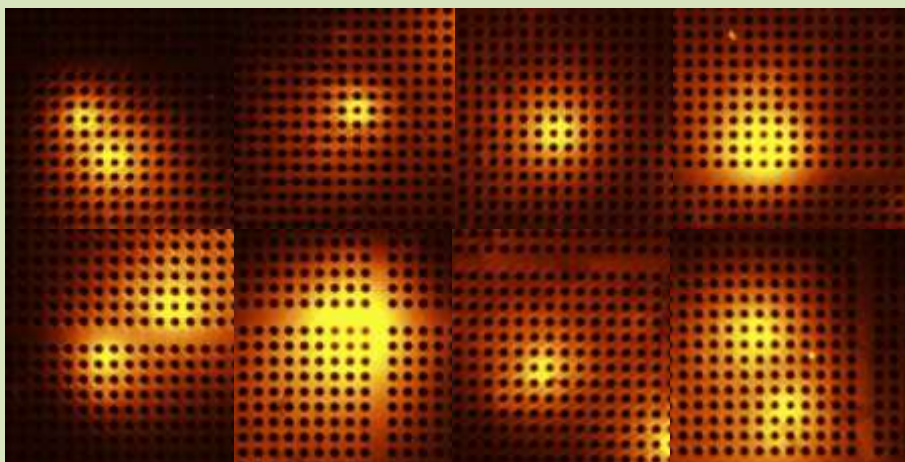


➔ Cloning of VH & VL coding genes & production of recombinant antibodies

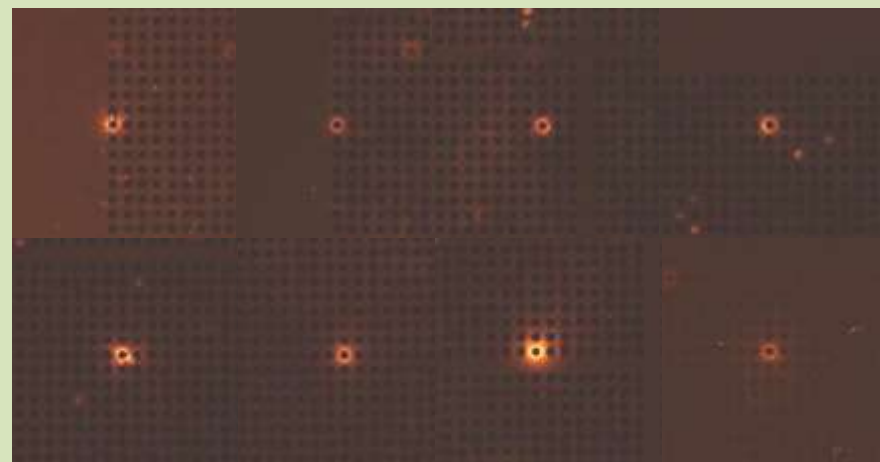
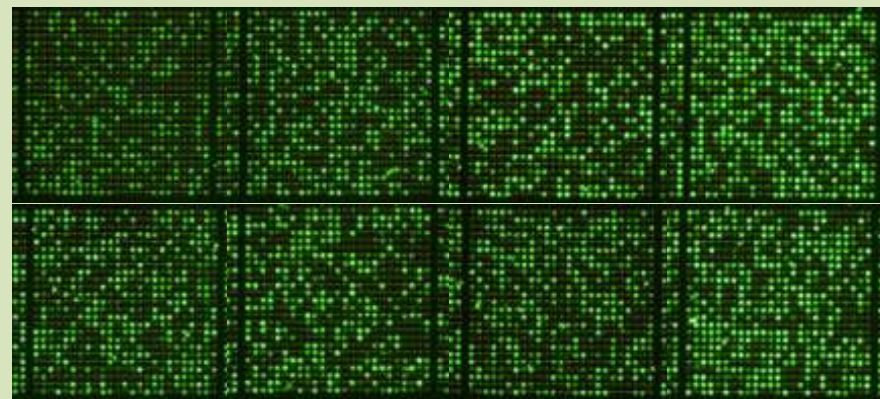
ISAAC™ Antibody Discovery Platform

Example of targets in infected and healthy donors

B CELLS FROM INFECTED DONOR/INFECTIOUS TARGET

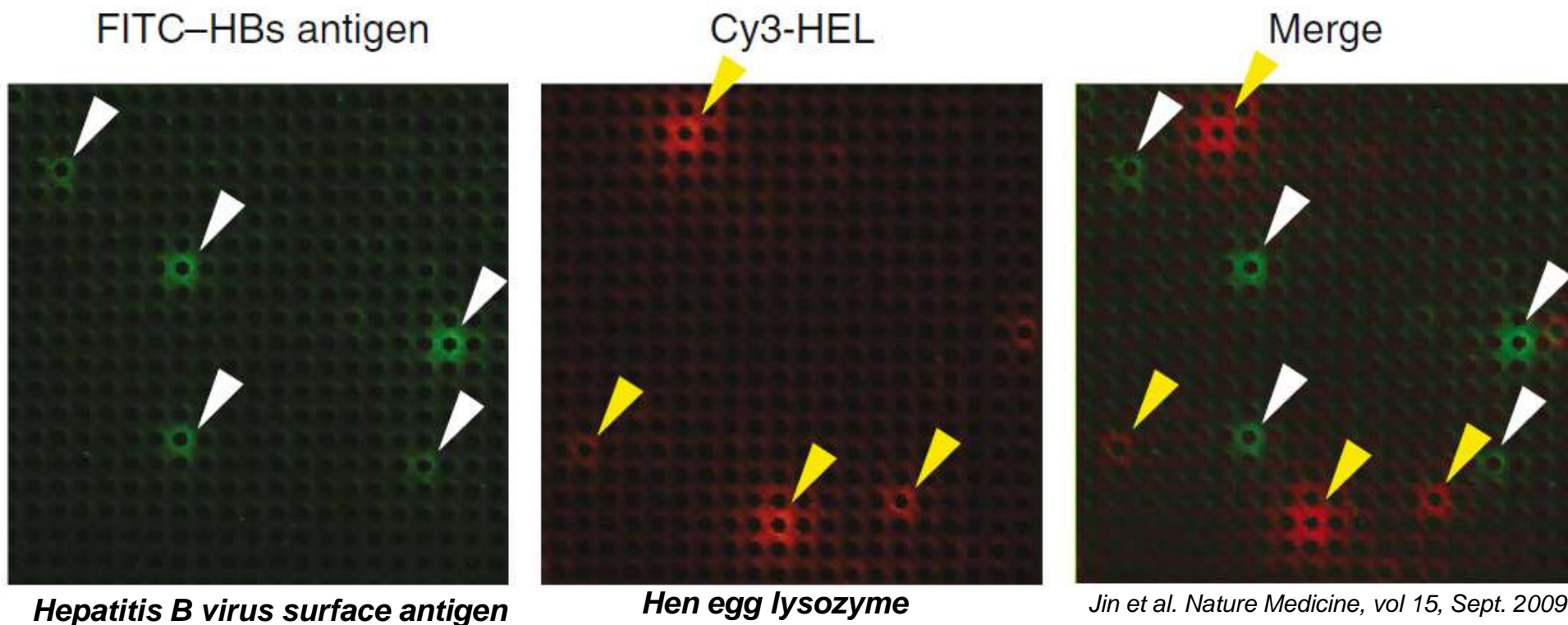


B CELLS FROM HEALTHY DONOR/TARGET X



ISAAC™ Antibody Discovery Platform

Simultaneous detection of B lymphocytes secreting mAbs specific to multiple Antigens



■ ISAAC™ Antibody Discovery Platform

Simultaneous detection of B lymphocytes secreting mAbs specific to multiple Antigens

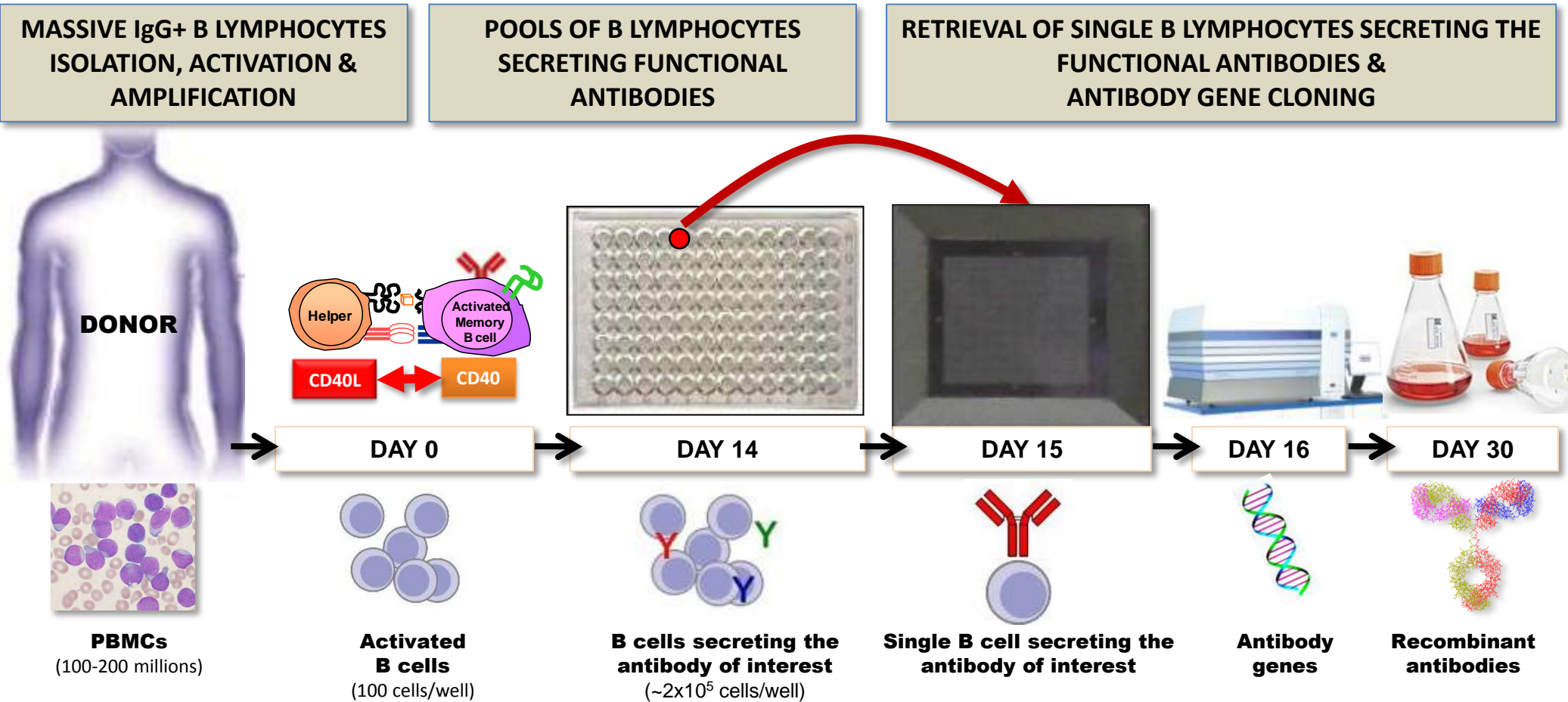
HOWEVER:

- Isolation of antigen-specific mAbs only
- Functional mAbs account for 1-5% of antigen-specific mAbs
- Evolve technology to zoom-in on functional antibodies only



VIVA | SCREEN™ Antibody Discovery Platform

High-Throughput massive single cell screening of B lymphocytes

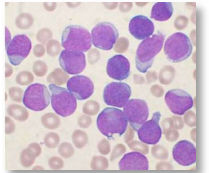
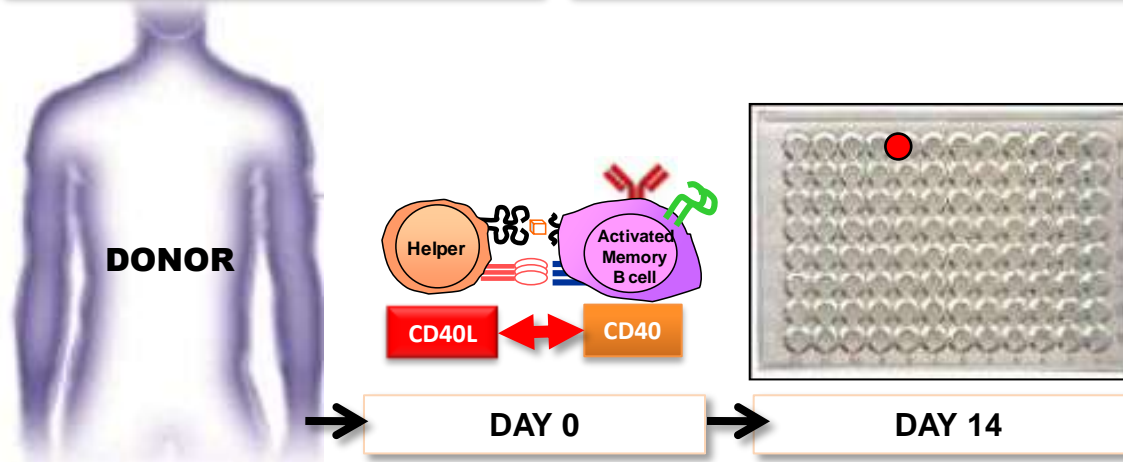


VIVA | SCREEN™ Antibody Discovery Platform

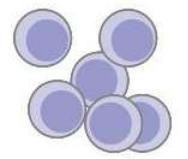
High-Throughput screening of primary B cells for antibody binding & function

MASSIVE IgG+ B LYMPHOCYTES ISOLATION, ACTIVATION & AMPLIFICATION

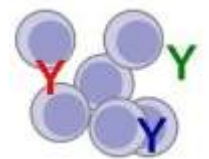
POOLS OF B LYMPHOCYTES SECRETING FUNCTIONAL ANTIBODIES



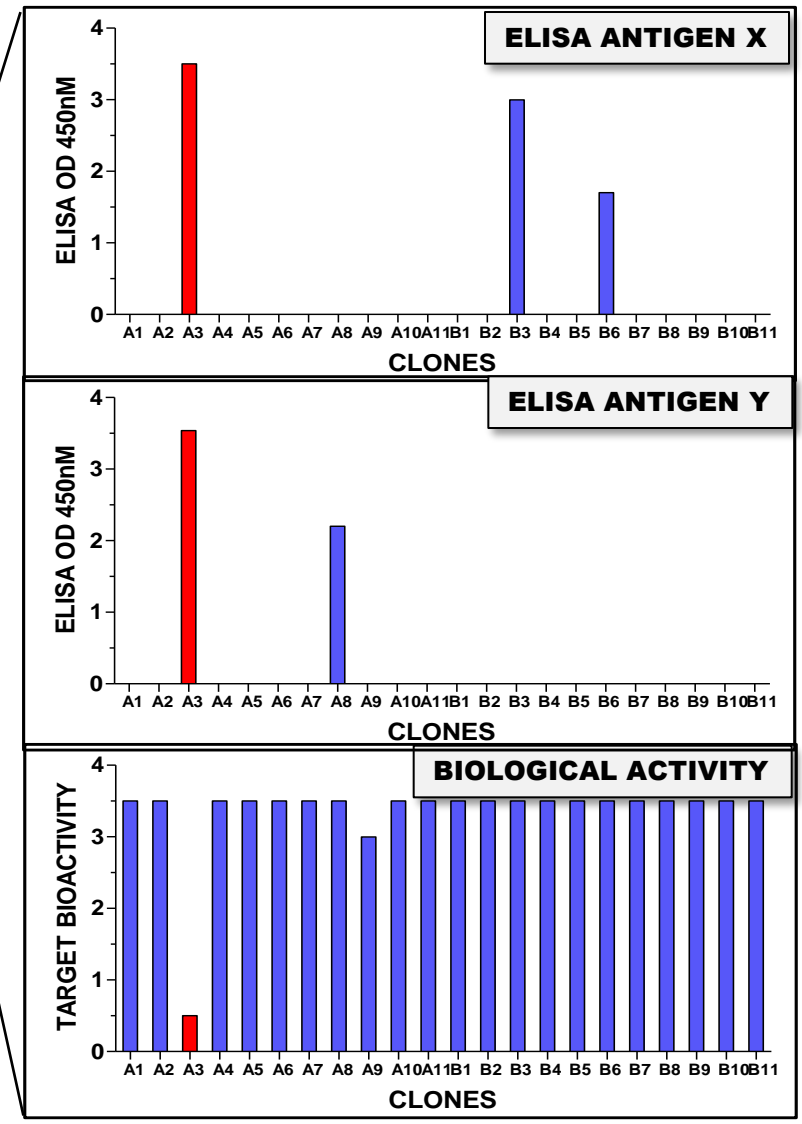
PBMCs
(100-200 millions)



Activated B cells
(100 cells/well)

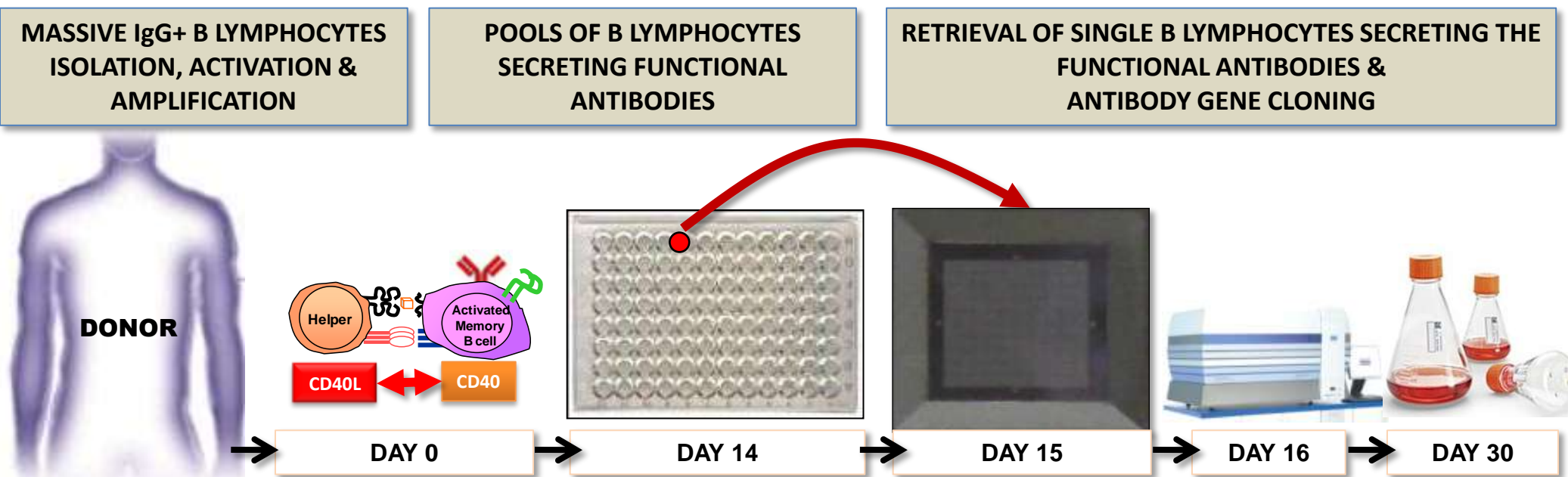


B cells secreting the antibody of interest
(~2x10⁵ cells/well)



VIVA | SCREEN™ Antibody Discovery Platform

High-Throughput massive single cell screening of B lymphocytes



HEALTHY DONOR

- 600 millions PBMCs
- 35 millions B cells (~5% PBMC)

N° ANTIBODIES

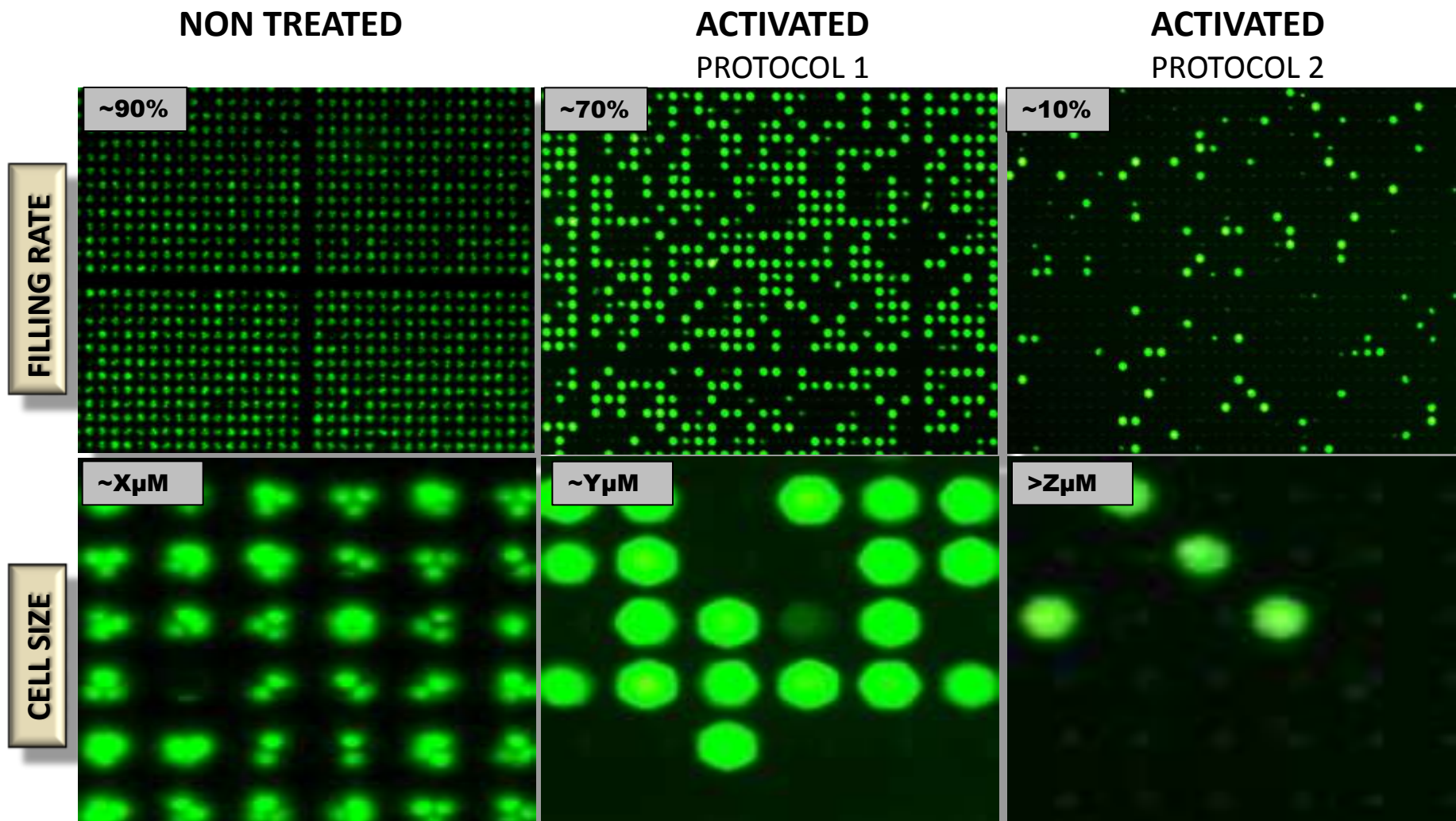
- ELISA HITS : 933
- BIOLOGICAL HITS : 23

N° RECOMBINANT FUNCTIONAL mAbs

19

VIVA | SCREEN™ Antibody Discovery Platform

Optimization of activation protocol & size of microwells:





From cells to therapeutics **Vivalis**[®]

Examples of Applications

Infectious diseases & Infected donors

Human Donors

Native antibodies against self antigens: Infectious diseases

Neutralizing antibodies discovered against a very broad range of pathogens

VIRUSES

- INFLUENZA VIRUS
- HEPATITIS B
- CYTOMEGALOVIRUS
- RABIES
- CHIKUNGUNYA VIRUS
- UNDISCLOSED TARGET A
- ...

BACTERIA

- NOSOCOMIAL BACTERIA
- UNDISCLOSED TARGET A
- UNDISCLOSED TARGET B
- UNDISCLOSED TARGET C
- UNDISCLOSED TARGET D

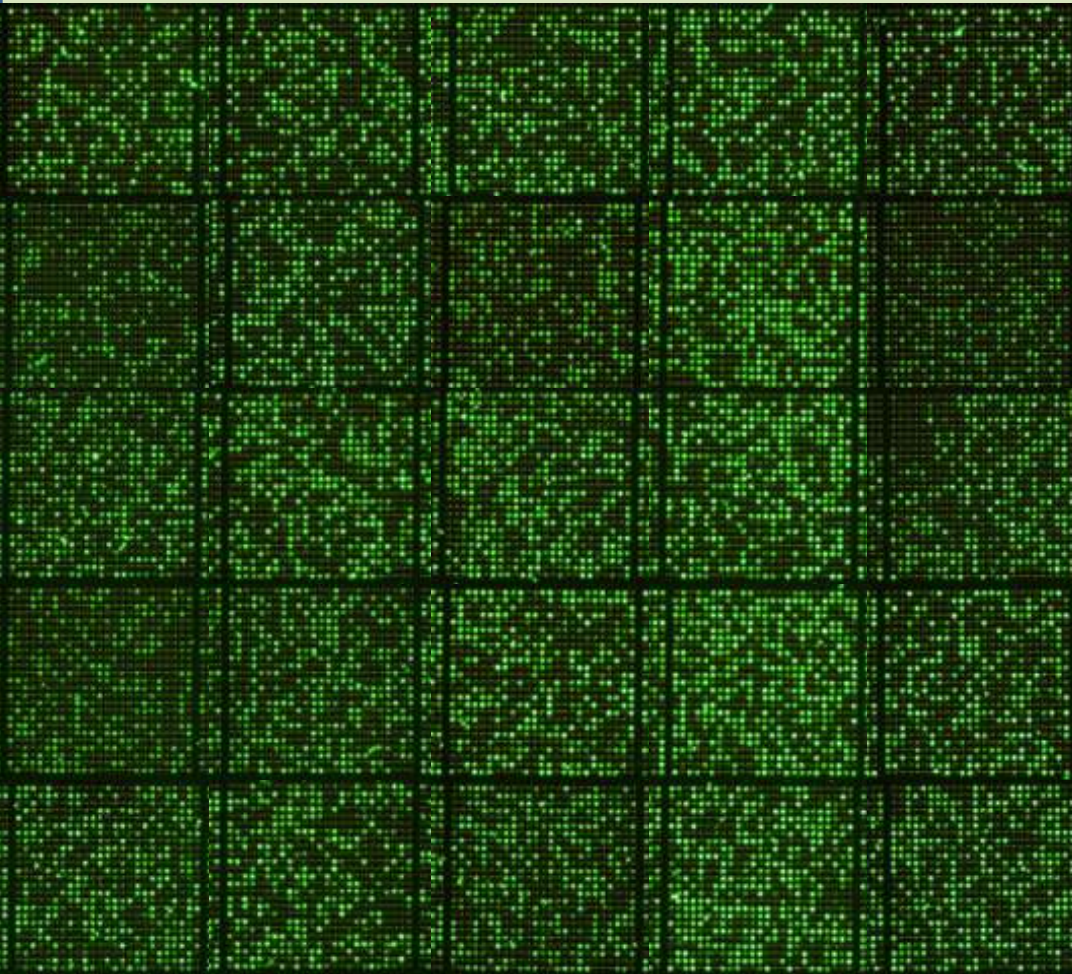
Antibody Discovery

Example of Influenza virus, infected donor

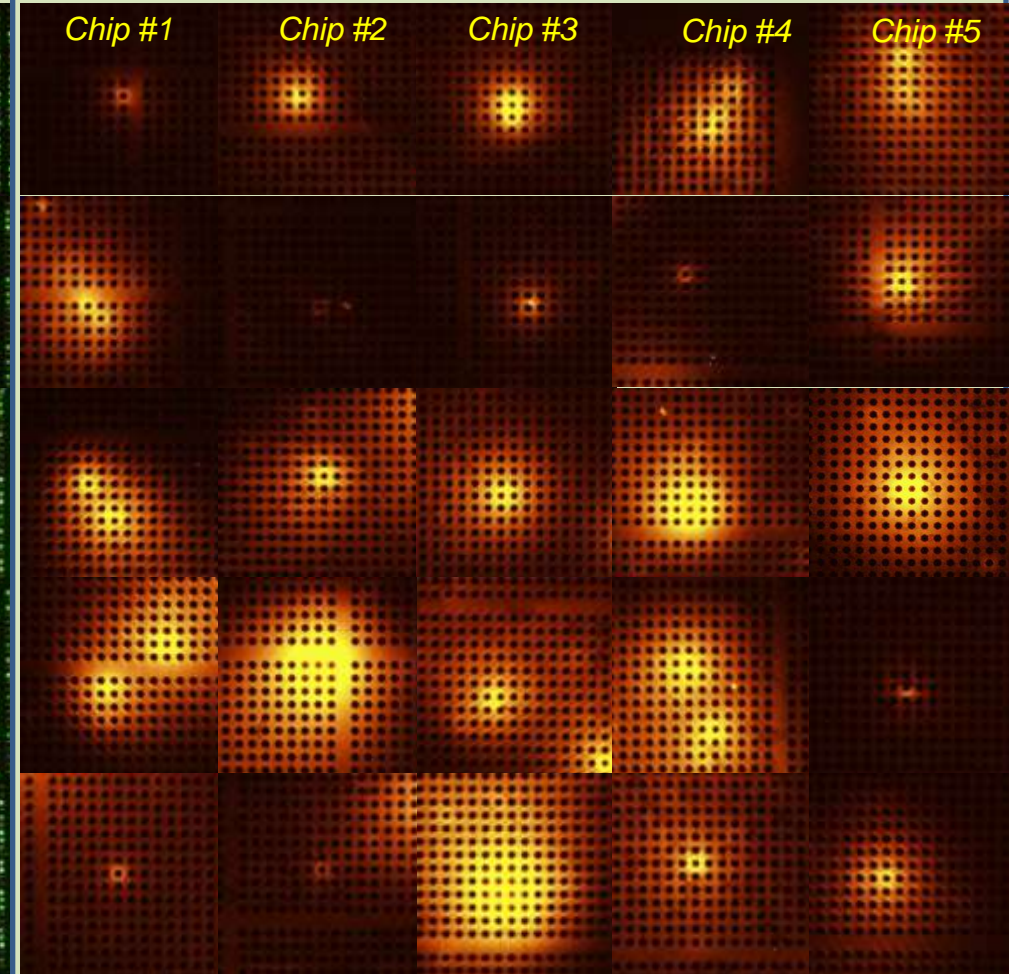


NEURAMINIDASE

Detection cells by Oregon green 488 staining



Detection of mAbs-secreting cells by anti-hlgGFc-Cy3 staining

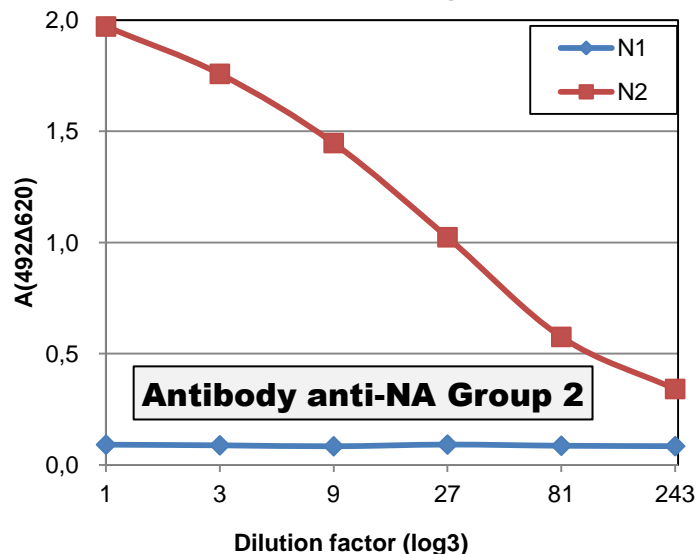
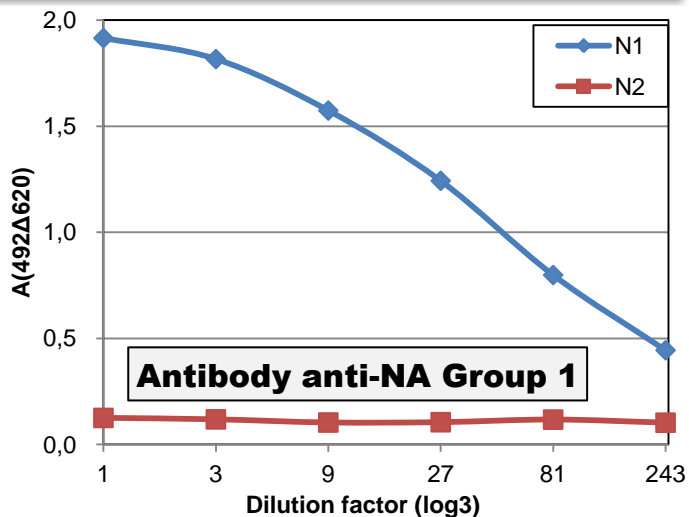


Antibody Discovery

A wide panel of antibodies with different specificities



NEURAMINIDASE: GROUP 1 & 2



HEMAGGLUTININ & MATRIX PROTEINS

Binding of inactivated influenza viruses type^a

Antibody	H1N1	H3N2	H5N1	B	Antigen ^b
InV-03	+	+	+	-	non-HA/M2e
InV-07	-	-	-	+	B-HA
InV-10	+	+	+	-	M2e
InV-15	-	-	-	+	non-HA/M2e
InV-16	+	-	-	-	H1-HA
InV-17	+	+	+	-	non-HA/M2e
InV-19	-	-	-	+	B-HA
InV-22	-	-	-	+	B-HA
InV-32	+	+	+	-	non-HA/M2e
InV-33	+	+	+	-	non-HA/M2e
InV-34	+	+	+	-	non-HA/M2e
InV-43	+	+	+	+	non-HA/M2e
InV-47	+	+	+	-	non-HA/M2e
InV-49	+	+	+	-	non-HA/M2e
InV-50	+	-	-	-	non-HA/M2e
InV-58	+	+	+	+	non-HA/M2e
InV-61	+	+	+	-	non-HA/M2e

ND, not determined.

^aBinding of viruses was examined by ELISA with the following inactivated viruses: H1N1, A/New Caledonia/20/99 strain; B, B/Tokio/53/99 strain. ^bAntigen specificity was examined by ELISA with recombinant hemagglutinin (HA) from B virus (B/Jilin/20/2003), or M2e peptide. ^cAffinity was analyzed with surface plasmon resonance analysis. ^dNeutro in vitro neutralization of A/New Caledonia/20/99 strain and B/Malaysia/2506/2004 strain using Madin-Darby canine kidney

From cells to therapeutics **Vivalis**[®]

Examples of Applications

Healthy donors with very low circulating antigen specific B cells

VIVA | Screen™ Antibody Discovery Platform

Example of undisclosed targets in healthy donors



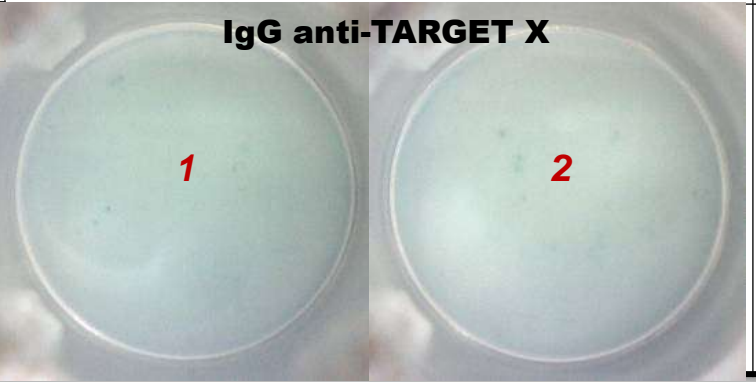
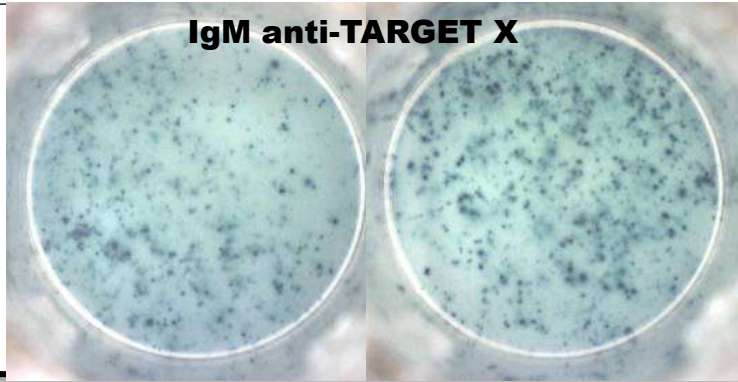
- **DONORS**
 - No patients available
 - 3000 healthy donors, with no history of specific disease
- **SCREENING OF DONORS**
 - ELISA
 - Neutralization assays
 - ELISPOT

- **RESULTS**

BIOLOGICAL ACTIVITY (NEUTRALIZATION AGAINST)	N° POSITIVE DONORS	FREQUENCY
TARGET X	94/3000	3.13%

VIVA | Screen™ Antibody Discovery Platform

Example of undisclosed targets in healthy donors

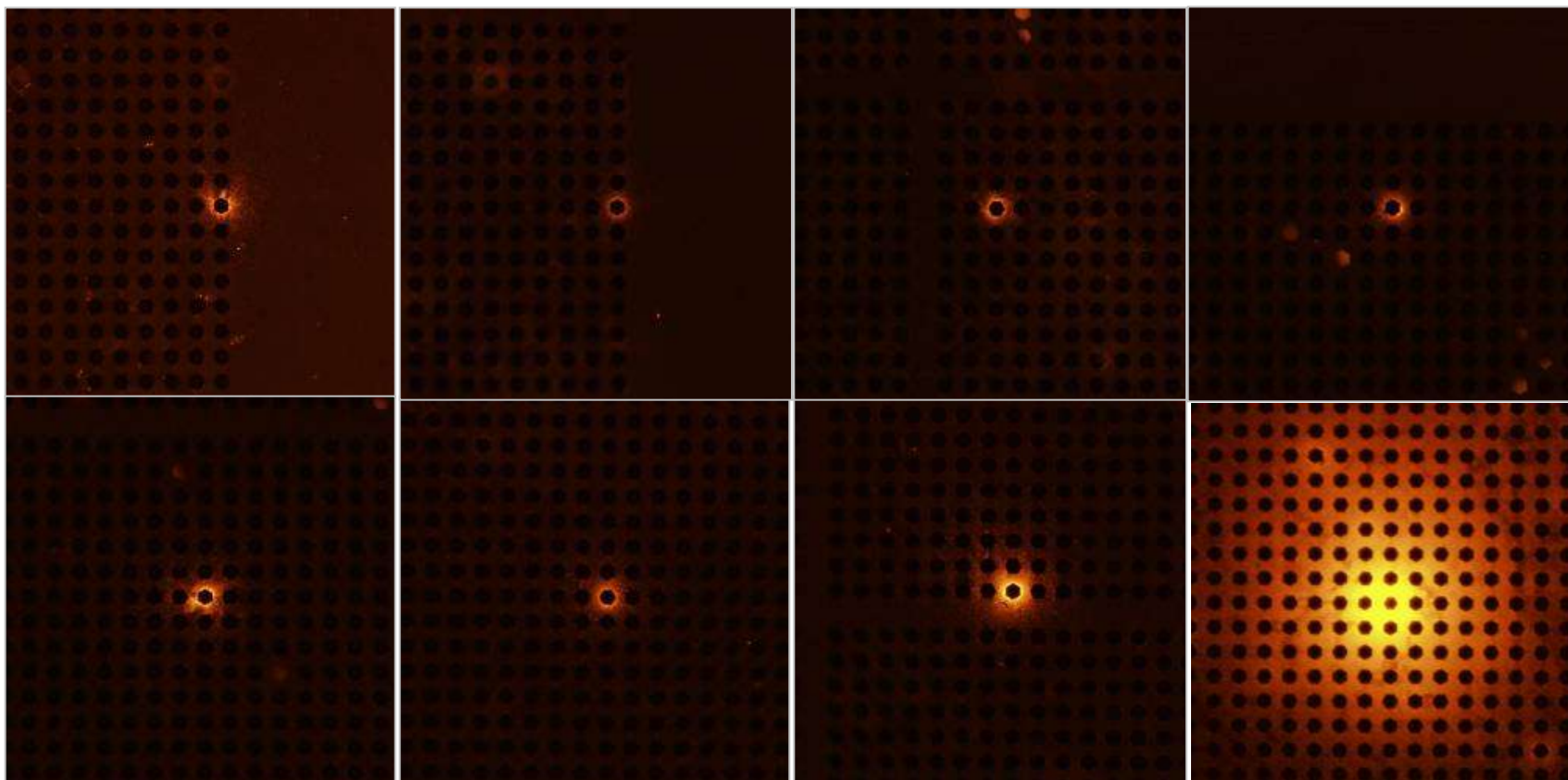
DONOR	ANTIGEN	ELISPOT ASSAY Frequency of positive spots (number / 100,000 B Cells)
HEALTHY DONOR X	TARGET X : IgG	1
	TARGET X : IgM	883
ELISPOT PICTURES	<p style="text-align: center;">IgG anti-TARGET X</p> 	<p style="text-align: center;">IgM anti-TARGET X</p> 
<ul style="list-style-type: none"> ▪ Low frequency of circulating B lymphocytes secreting anti-target IgGs ▪ > 500 fold higher frequency of circulating B lymphocytes secreting anti-targets IgM 		

VIVA | Screen™ Antibody Discovery Platform

Example of undisclosed targets in healthy donors

TARGET ANTIGEN X

(healthy donor with very low circulating B cells secreting biologically active antibodies; < 1/2,000,000 B Cells)



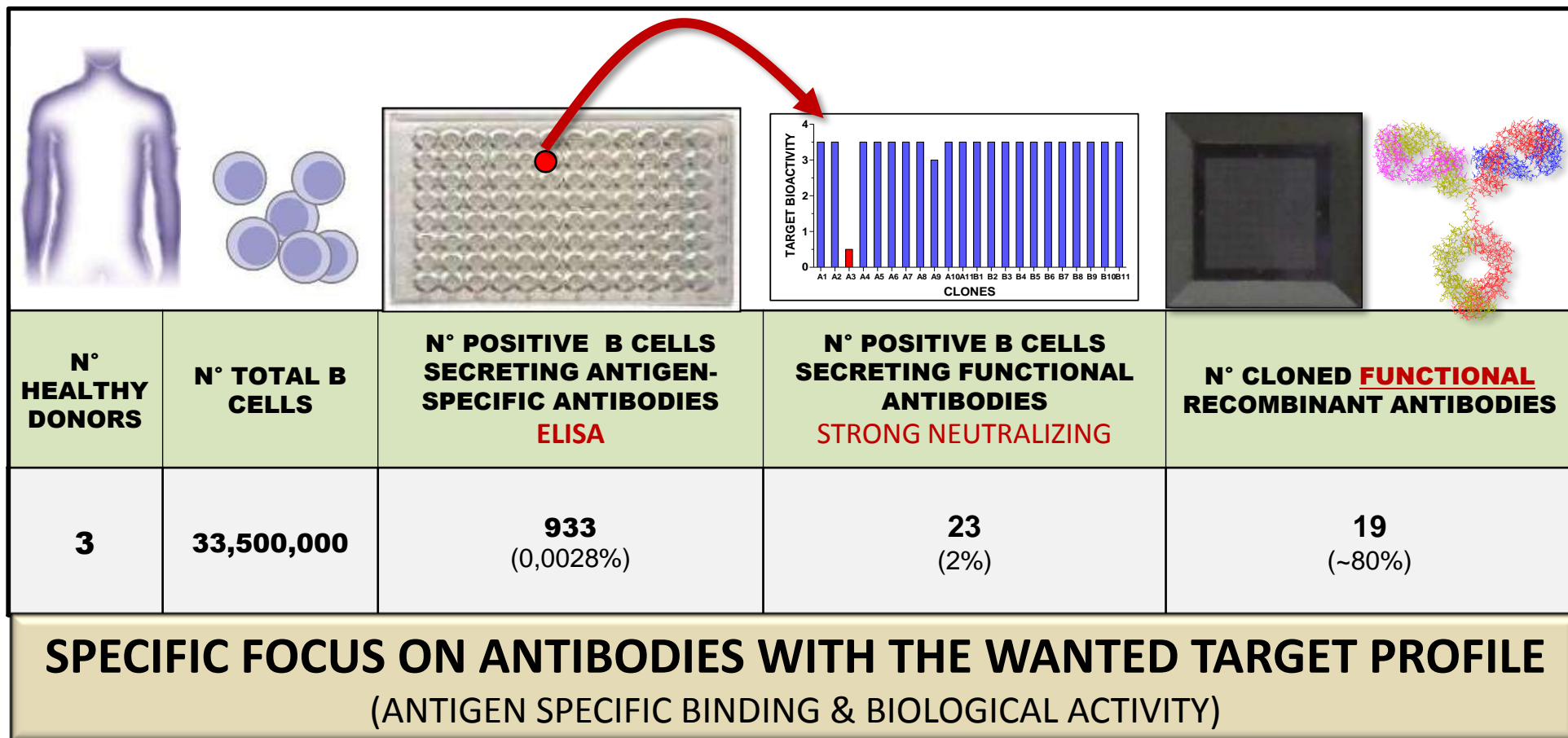
Influenza HA

Vaccinated donor with high frequency of anti-HA circulating B cells

ivalis®

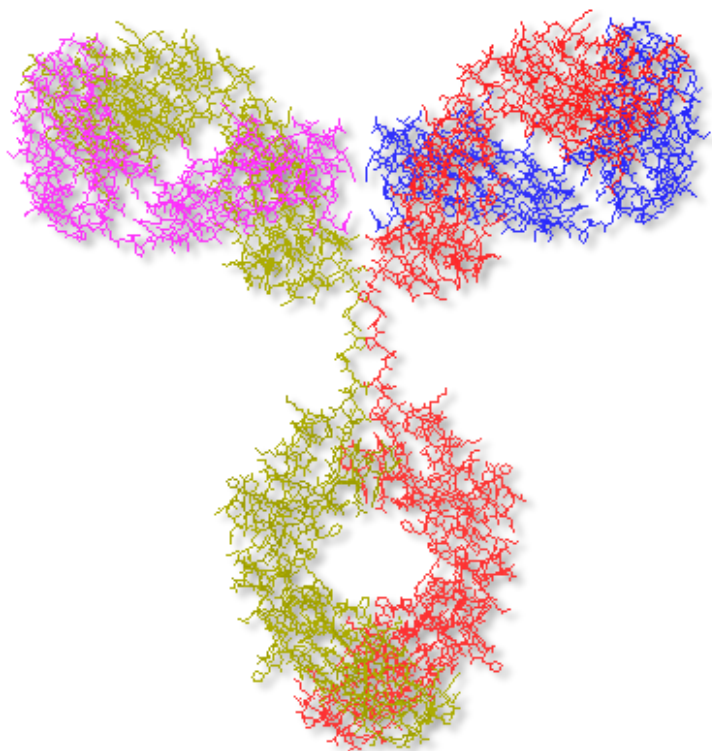
VIVA | SCREEN™ Antibody Discovery Platform

Example of an undisclosed bacterial target X in healthy donors

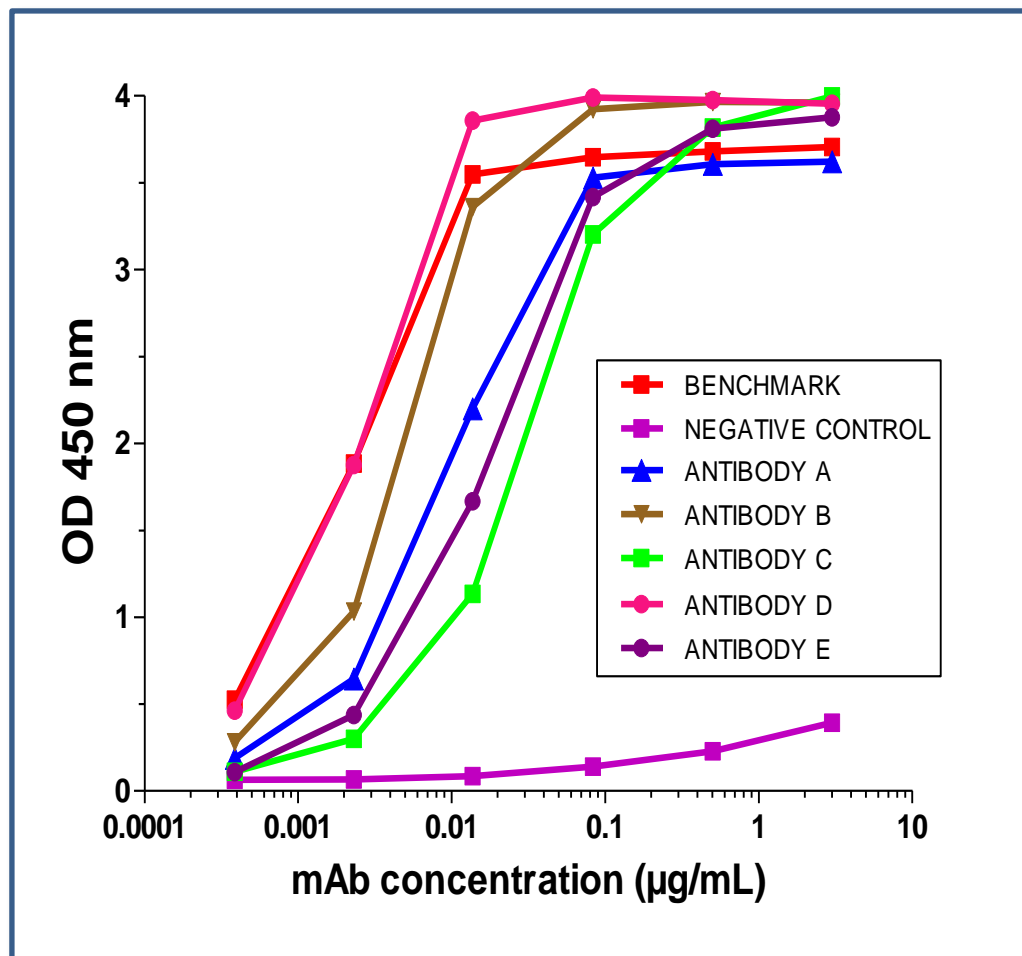


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Example of undisclosed targets in healthy donors

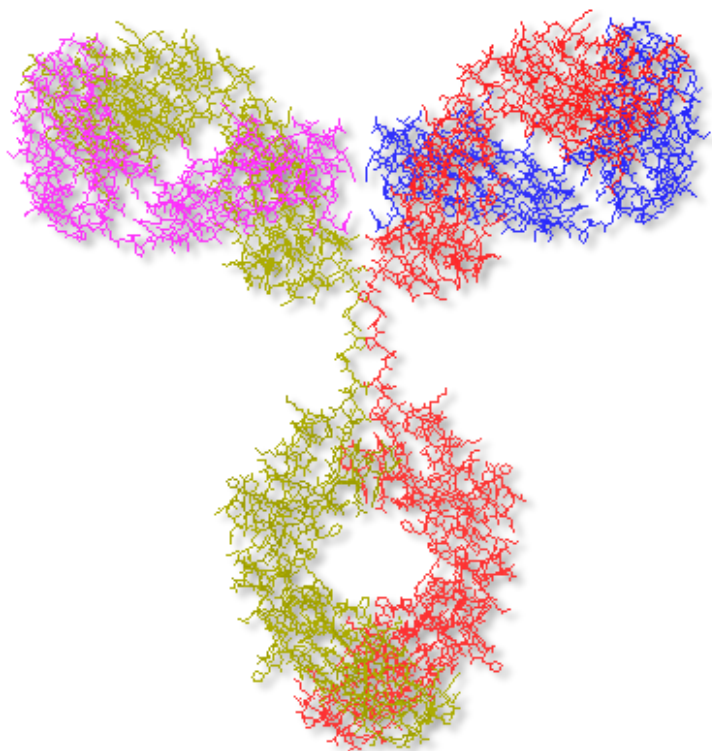


**CONFIRMED STRONG ANTIGEN
SPECIFICITY**



VIVA | Screen™ Antibody Discovery Platform

Example of undisclosed targets in healthy donors



IN VITRO BIOLOGICAL ACTIVITY

ANTIBODIES	AFFINITY (KD)	BIOLOGICAL ACTIVITY (EC ₅₀)
A	0.5 pM	70 pM
B	200 pM	75 pM
C	16 pM	60 pM
D	6 pM	270 pM
E	141 pM	402 pM
F	193 pM	100 pM
G	63 pM	130 pM
H	117 pM	130 pM

From cells to therapeutics **Vivalis**[®]



Examples of Applications

ANTIBODIES FOR CANCER

Human Donors & Cancer

Native antibodies against self antigens: Oncology

EXPERT
REVIEWS
Glycopeptide microarray
for autoantibody
detection in cancer
Expert Rev. Proteomics 2011; 4(1): 431-437 (2011)

TUMOR ASSOCIATED ANTIGENS

p16

P53

p62

cMYc

HER2

NY-ESO-1

Muc1

BRCA2

IMP1

Koc

Cyclin B1

Survivin

PPIA

PRDX2

FKDX2

ISP-60

IGFBP2

TOPO2 α

Livin

ASB-9

SERAC1

RELT

The human system recognizes the autologous Tumor Associated Antigens as « non-self » and makes a humoral immune response very early in the disease process.

Survival in Early Breast Cancer Patients Is Favorably Influenced by a Natural Humoral Immune Response to Polymorphic Epithelial Mucin

By S. von Mensdorff-Pouilly, A.A. Verstraeten, P. Kenemans, F.G.M. Snijdwint, A. Kok, G.J. Van Kamp, M.A. Paul, P.J. Van Diest, S. Meijer, and J. Hilgers

Review Article

Autoantibodies to Tailor-Made Panels of Tumor-Associated Antigens in Breast Carcinoma

Ettie Piura¹ and Benjamin Piura²

¹ Department of Obstetrics and Gynecology, Sapir Medical Center, Sackler School of Medicine, University of Tel-Aviv, Kfar-Saba 44281, Israel

² Unit of Gynecologic Oncology, Department of Obstetrics and Gynecology, Soroka Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva 84101, Israel

Correspondence should be addressed to Benjamin Piura, piura@bgu.ac.il

Received 31 October 2010; Accepted 14 January 2011

Hum Antibodies. 2007;16(3-4):87-98.

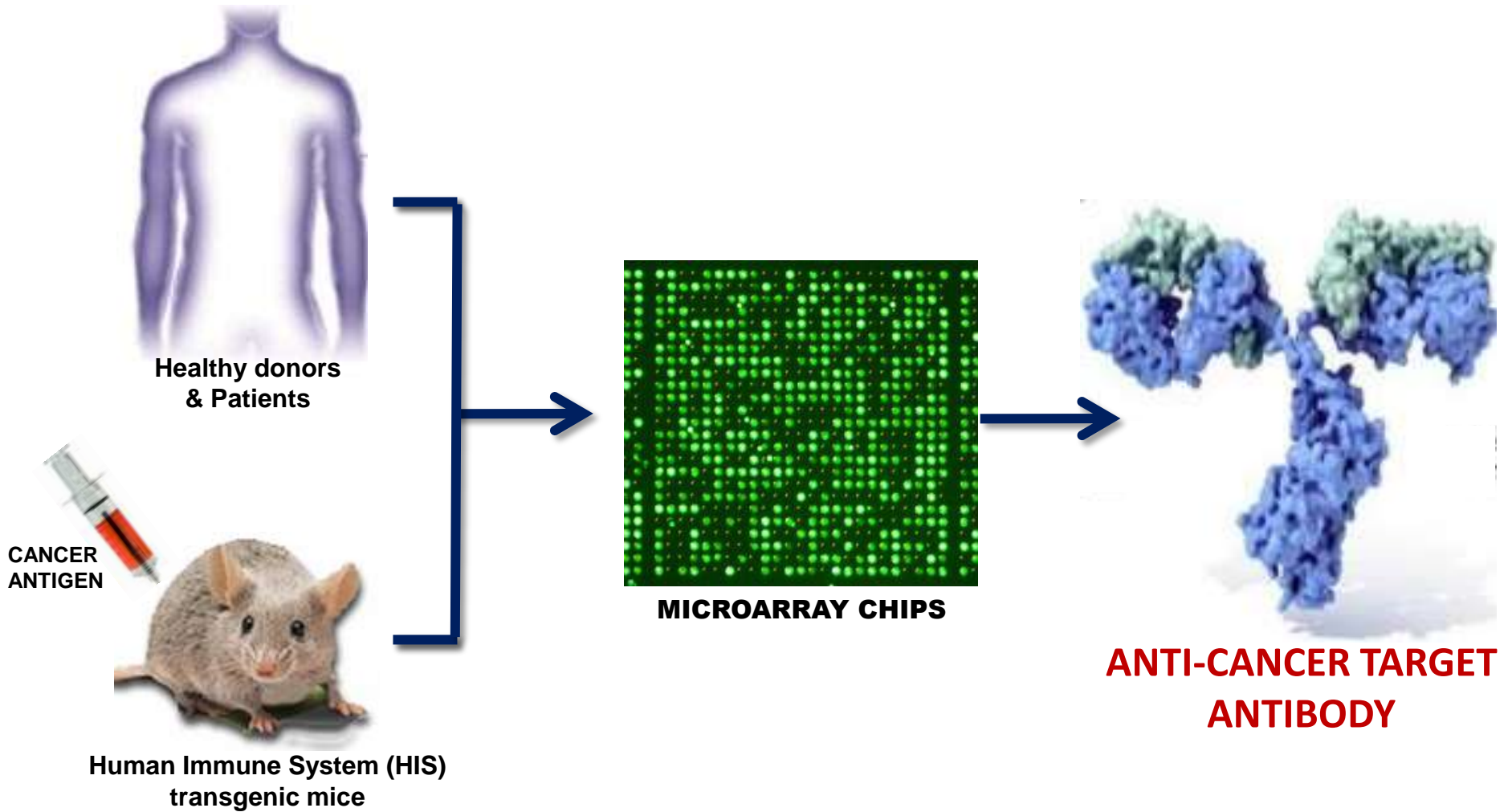
Cocktails of human anti-cancer antibodies show a synergistic effect in nude mouse tumor xenografts.

Glassy MC, McKnight ME, Kotlan B, Glassy EF, Koda K.

Rajko Medenica Research Foundation, San Diego, CA 92126, USA. markglassy@aol.com

Application of VIVA | SCREEN to Cancer Targets

Ongoing project on target X



Anti-cancer Antibodies with enhanced ADCC

Development of a novel cell substrate for antibody production



Avian Post-translational Modification

Presence of low fucose on avian proteins



BIRDS ANTIBODIES DISPLAY LOWER CONTENT OF FUCOSE

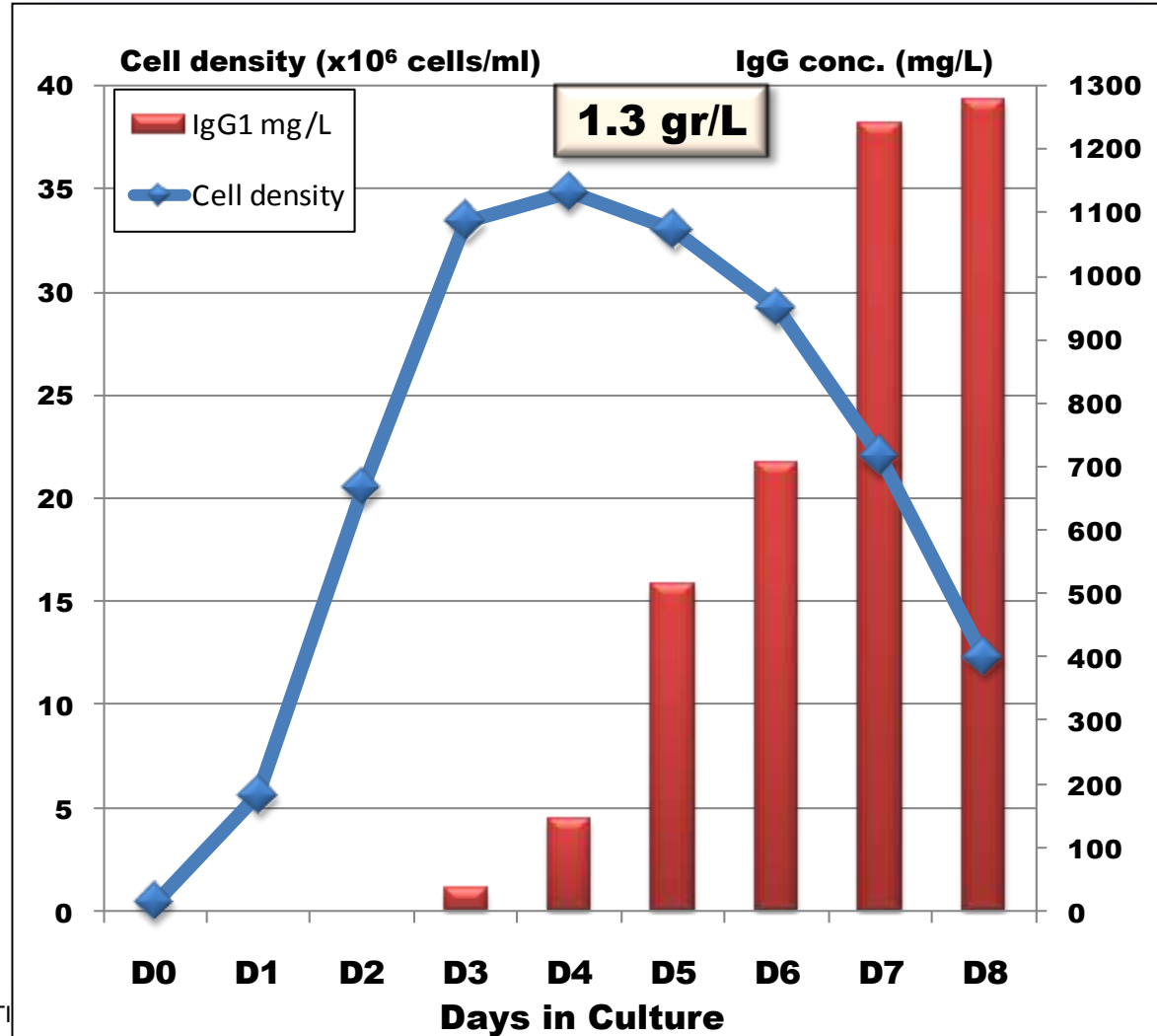
	HUMAN	MURINE	AVIAN
NANA	+++	---	+++
NGNA	---	+	---
Bisecting GlcNac	+++	---	+++
Fucosylation	+++	+++	+
Complex mannose	---	---	---
Gal α 1-3 Gal	---	+	---



DEVELOPMENT OF A DUCK CELL SUBSTATE FOR MANUFACTURING OF ANTIBODIES WITH ENHANCED ADCC

mAb Production in EB66[®] Cells

fedbatch process in chemically defined cell culture medium

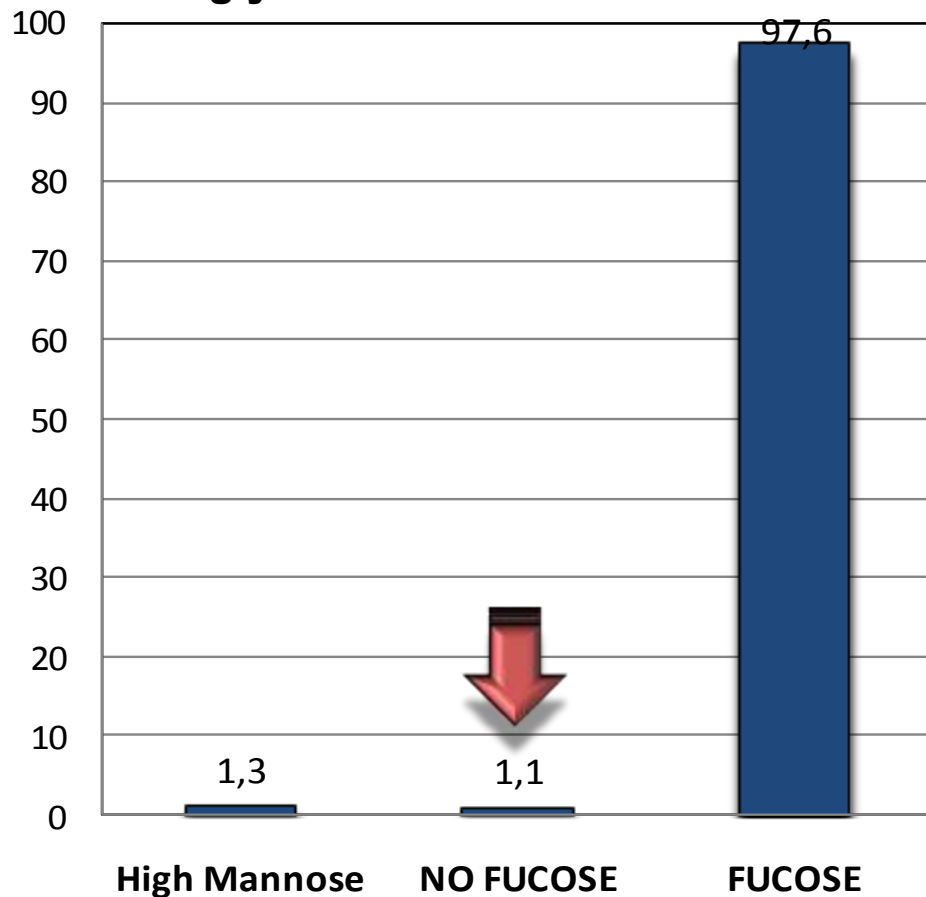


Glycosylation profile of EB66[®]-IgGs

EB66[®] IgG display low fucose & low [high mannose] structures

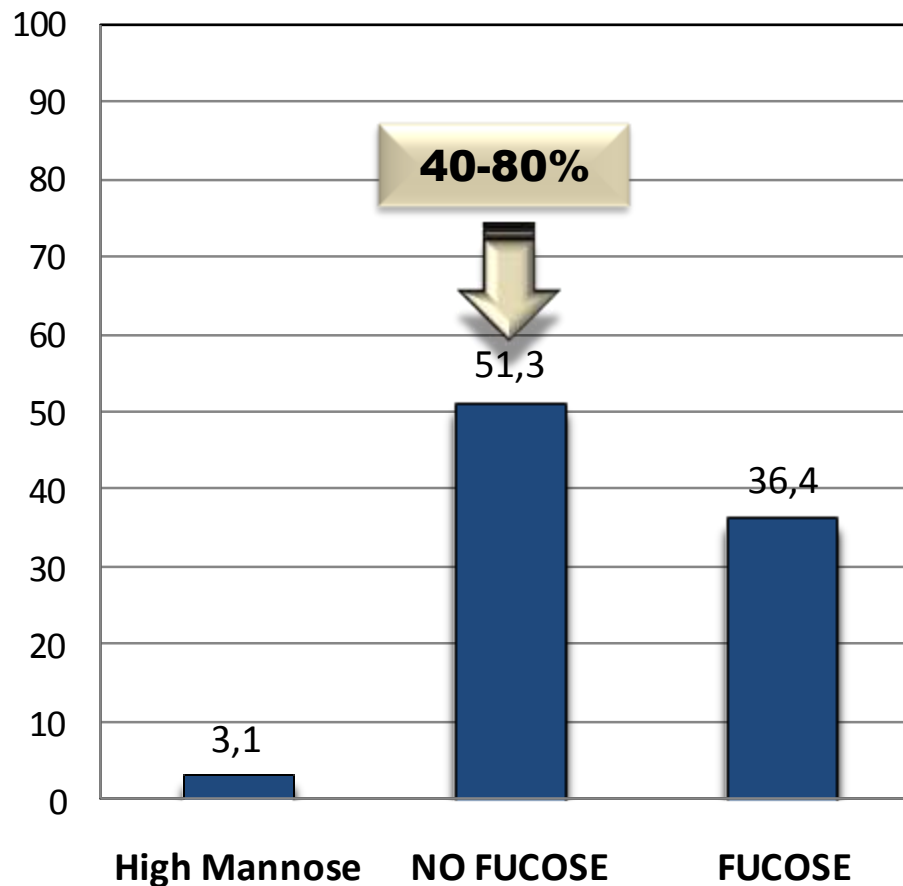
CHO-ANTIBODY

% of glycans



EB66-ANTIBODY

% of glycans

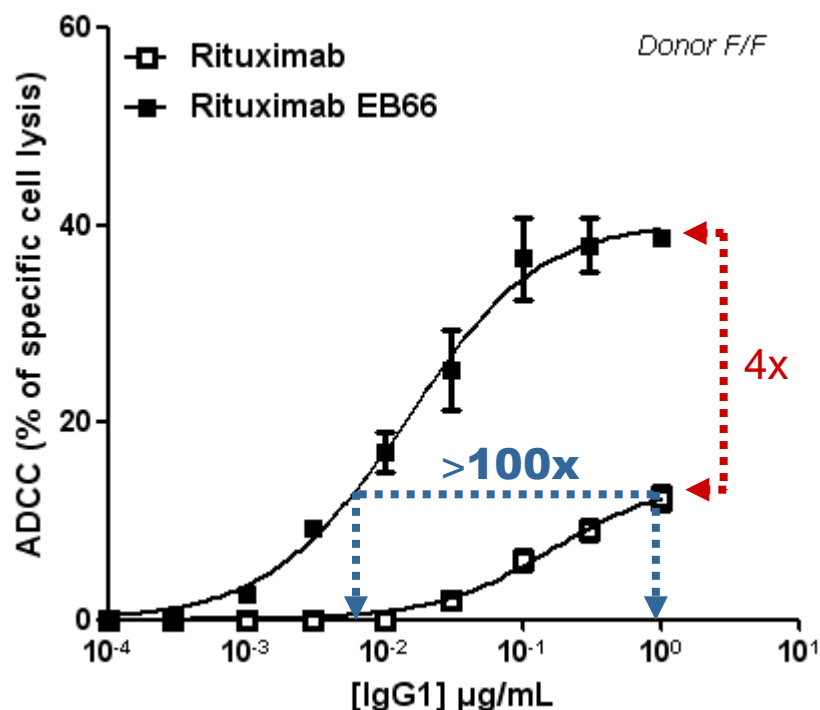


Production of antibodies on EB66[®] cells

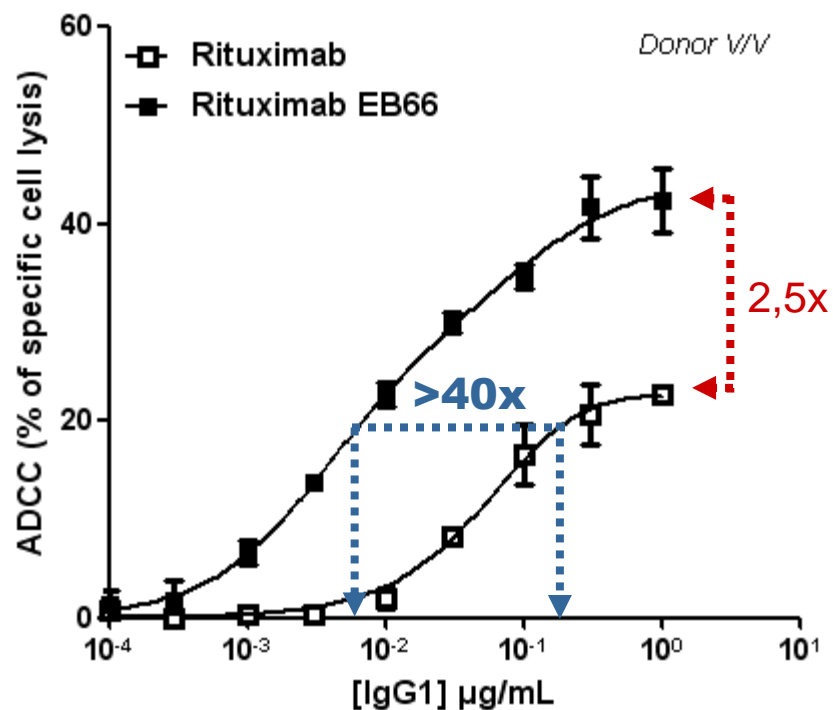
EB66 antibodies display low fucose content & enhanced cytotoxicity

RITUXIMAB (Similar results with 5 independent antibodies)

Homozygous carrier for Fc γ RIIIa 158F



Homozygous carrier for Fc γ RIIIa 158V



BIOACORE STUDIES: INCREASED AFFINITY TOWARDS Fc γ RIIIa

The EB66[®] cell line

A new standard for the production of vaccines and an emerging platform for proteins



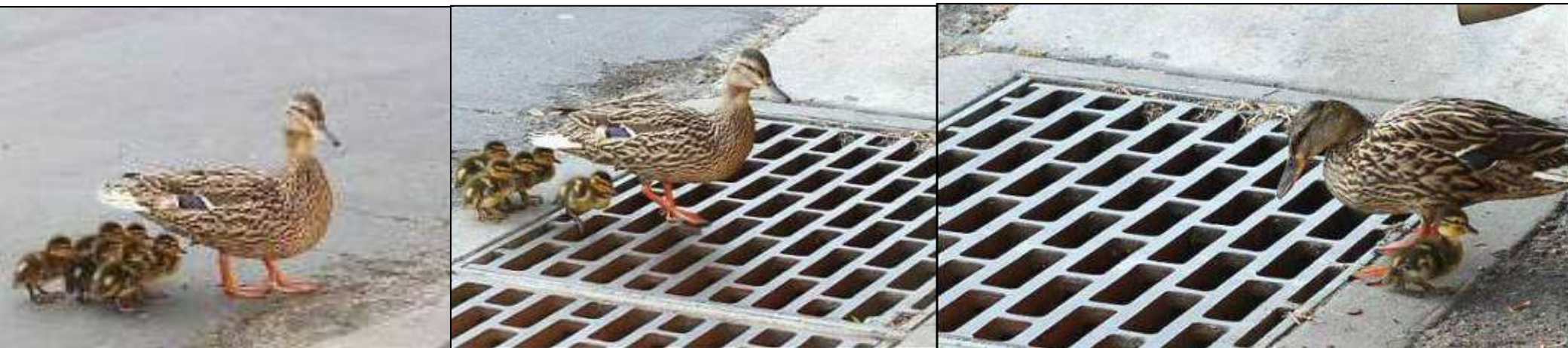
- 18 Commercial licenses + ~12 research licenses
- Phase I & II clinical trials ongoing for flu vaccines in the USA and Japan
- A first veterinary EB66 vaccine marketed in 2013

VIVA | SCREEN™ Antibody Discovery Platform

From donor to patients with native human monoclonal antibody

A TECHNOLOGY WITH MULTIPLE ADVANTAGES

- Access to a large pool of healthy human healthy donors and to patients
- Isolation of native high affinity mAbs, with no need for further engineering & lower risks of off-target specificities
- Access to very rare antibodies (*<1 B cell/10,000,000 B Cells or <1 B cell in 200,000,000 PBMCs*)
- Focus on biologically active antibodies (*cloning of 20 active antibodies instead of 900 antigen-specific candidates*) → major time and resource savings for better antibodies
- Applicable to infectious and autologous targets, including tumor associated antigens
- Endorsed by the industry: collaborations with several pharmaceutical companies



VIVALIS

LYON (F)

- Nicola Beltraminelli
- Riad Abes

TOYAMA (JPN)

- Shinya Toba
- Sachiko Takami

NANTES (F)

- Fabrice Le Gall
- Stéphane Olivier

TOYAMA UNIVERSITY

- Hiroyuki Kishi
- Atsushi Muraguchi

EFS-LYON

- Dominique Rigal

Merci
Thank You
ありがとうございました