



DURABILITY OF COVID-19 VACCINE IMMUNITY UP TO 21 MONTHS:

**Integrated Humoral & Cellular Readouts with Pseudovirus
Neutralization**

Maria Laura Berloni
Università degli Studi di Urbino Carlo Bo





Finanziato
dall'Unione europea
NextGenerationEU



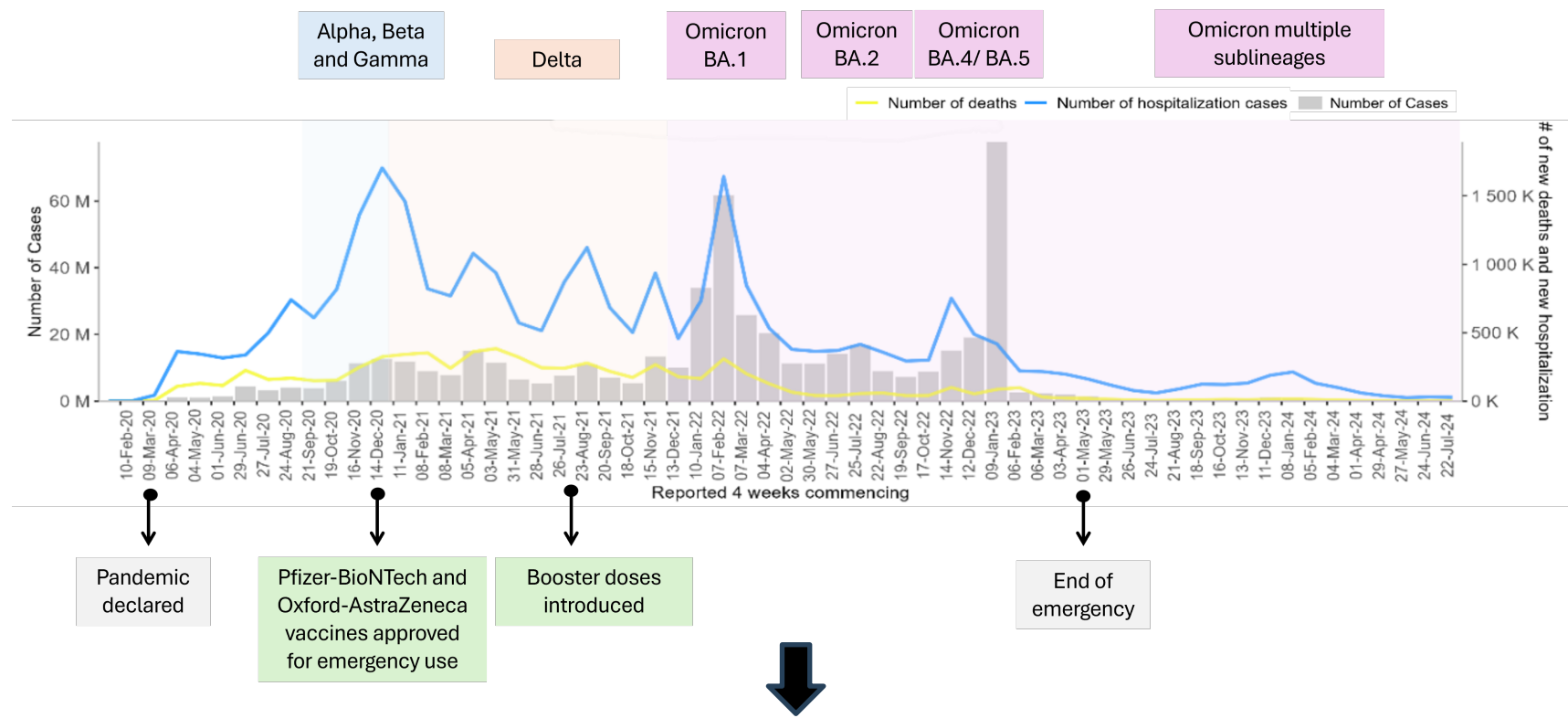
Ministero
dell'Università
e della Ricerca



Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA

INTRODUCTION

COVID-19 trends: variants and vaccination impact



Need for continuous evaluation of vaccine efficacy



Finanziato
dall'Unione europea
NextGenerationEU

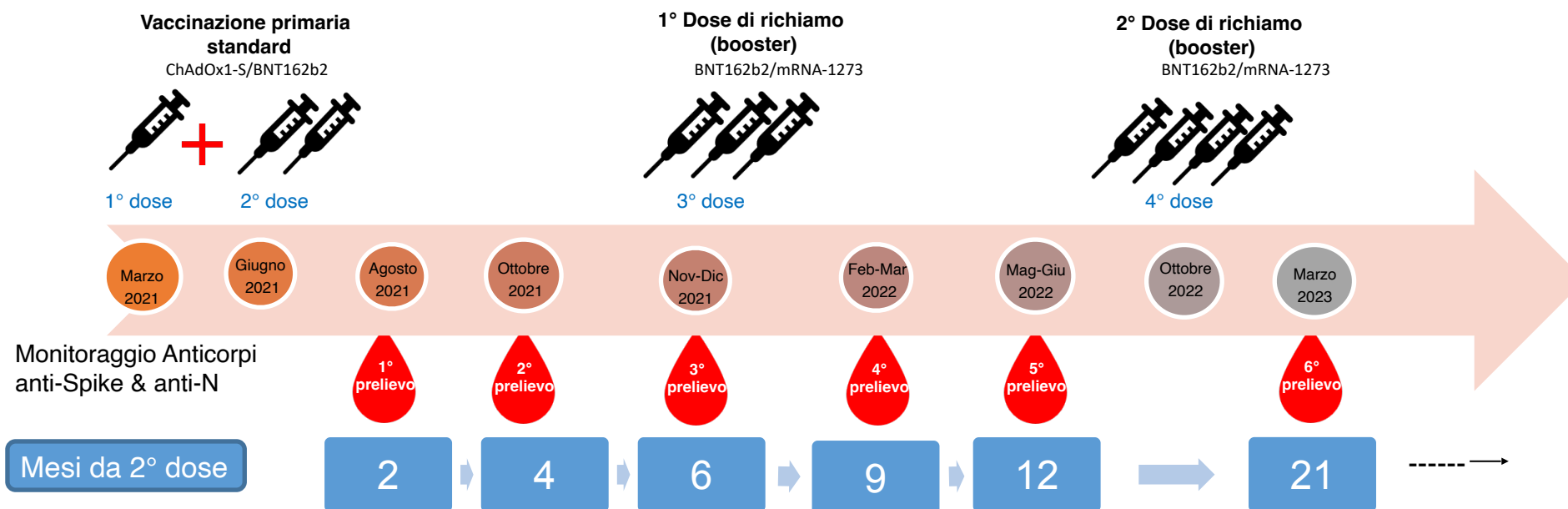


Ministero
dell'Università
e della Ricerca



Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA

TIMELINE DELLO STUDIO: Monitoraggio anticorpale anti-Spike e anti-Nucleocapside post vaccinazione anti COVID -19 sul siero di volontari (operatori sanitari e dipendenti dell'Università) fino a 2 anni dopo somministrazione della vaccinazione primaria



vaccines



Brief Report

Evaluation of Two-Month Antibody Levels after Heterologous ChAdOx1-S/BNT162b2 Vaccination Compared to Homologous ChAdOx1-S or BNT162b2 Vaccination

Simone Barocci ^{1,†}, Chiara Orlandi ^{2,†}, Aurora Diotallevi ², Gloria Buffi ¹, Marcello Ceccarelli ¹, Daniela Vandini ¹, Eugenio Carlotti ³, Luca Galluzzi ², Marco Bruno Luigi Rocchi ², Mauro Magnani ² and Anna Casabianca ^{2,*}



viruses



Article

Comparing Heterologous and Homologous COVID-19 Vaccination: A Longitudinal Study of Antibody Decay

Chiara Orlandi ^{1,2,†}, Giuseppe Stefanetti ^{1,†}, Simone Barocci ³, Gloria Buffi ¹, Aurora Diotallevi ¹, Ettore Rocchi ⁴, Marcello Ceccarelli ¹, Sara Peluso ⁵, Daniela Vandini ³, Eugenio Carlotti ⁶, Mauro Magnani ^{1,2}, Luca Galluzzi ¹ and Anna Casabianca ^{1,2,*}



Frontiers in Immunology

TYPE Original Research
PUBLISHED 02 May 2025
DOI 10.3389/fimmu.2025.1579163

Analysis of humoral and cellular immune activation up to 21 months after heterologous and homologous COVID-19 vaccination

Davide Torre ^{1†}, Chiara Orlandi ^{2,3†}, Ilaria Conti ¹, Simone Barocci ⁴, Eugenio Carlotti ⁵, Mauro Magnani ^{1,3}, Anna Casabianca ^{2,3*} and Giuseppe Stefanetti ^{1,2*}

¹Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy; ²Department of Biomolecular Sciences, Section of Biochemistry and Biotechnology, University of Urbino Carlo Bo, Fano, Italy; ³Laboratorio Covid, University of Urbino Carlo Bo, Fano, Italy; ⁴Department of Clinical Pathology, Azienda Sanitaria Territoriale (AST) di Pesaro-Urbino, Urbino, Italy; ⁵Department of Prevention, Azienda Sanitaria Territoriale (AST) di Pesaro-Urbino, Urbino, Italy



FUTURE PAPERS:

- Under revision: «Long term immunity after BNT/BNT vaccination»
- In preparation: «Evaluation of functional activity after COVID-19 vaccination»



Finanziato
dall'Unione europea
NextGenerationEU



Ministero
dell'Università
e della Ricerca



Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA

HUMORAL RESPONSE (9–12–21 MONTHS) – ChAd/BNT VS ChAd/ChAd and impact of third doses

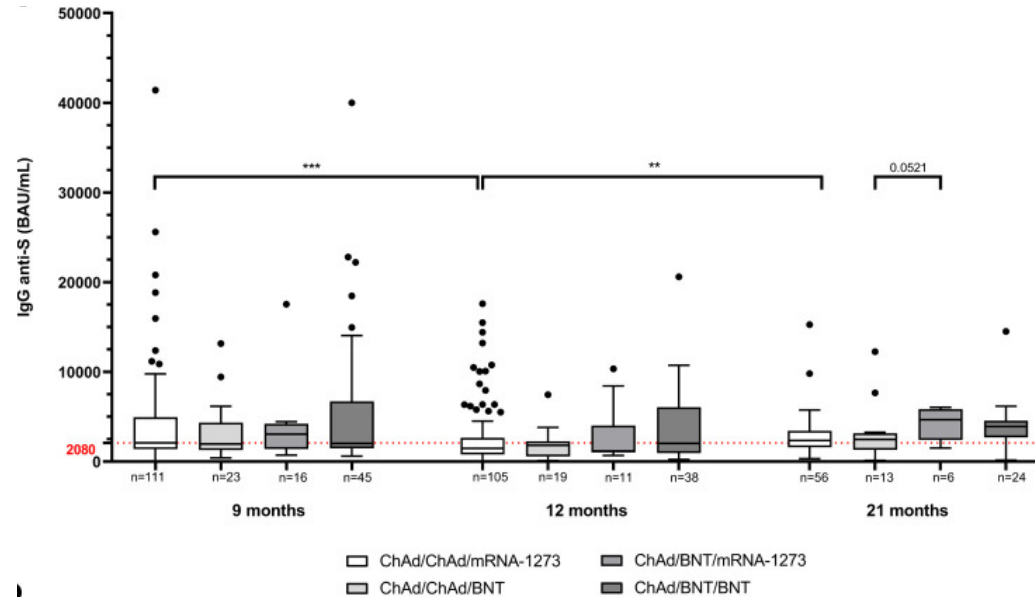
frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 02 May 2025
DOI 10.3389/fimmu.2025.1579163

Analysis of humoral and cellular immune activation up to 21 months after heterologous and homologous COVID-19 vaccination

Davide Torre^{1†}, Chiara Orlandi^{2,3†}, Ilaria Conti¹, Simone Barocci⁴,
Eugenio Carlotti⁵, Mauro Magnani^{1,3}, Anna Casabianca^{2,3*†}
and Giuseppe Stefanetti^{1,2*†}

¹Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy; ²Department of Biomolecular Sciences, Section of Biochemistry and Biotechnology, University of Urbino Carlo Bo, Fano, Italy; ³Laboratorio Covid, University of Urbino Carlo Bo, Fano, Italy; ⁴Department of Clinical Pathology, Azienda Sanitaria Territoriale (AST) di Pesaro-Urbino, Urbino, Italy; ⁵Department of Prevention, Azienda Sanitaria Territoriale (AST) di Pesaro-Urbino, Urbino, Italy



- Anti-Spike IgG levels declined at 12 months but rebounded by 21 months
- Heterologous regimens (especially ChAd/BNT/mRNA-1273 and ChAd/BNT/BNT) showed higher titers vs homologous
- Participants with prior infection showed higher Ab titers than infection-naïve at all timepoints (*not shown*)

ChAdOx1-S (**ChAd**)-Astrazeneca= Adenovector Vaccine
BNT162b2 (**BNT**)-Pfizer BioNTech= mRNA Vaccine
mRNA1273 (**MOD**)-Moderna= mRNA Vaccine



Finanziato
dall'Unione europea
NextGenerationEU



Ministero
dell'Università
e della Ricerca



Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA

CELLULAR RESPONSE AT 21 MONTHS – ChAd/BNT VS ChAd/ChAd

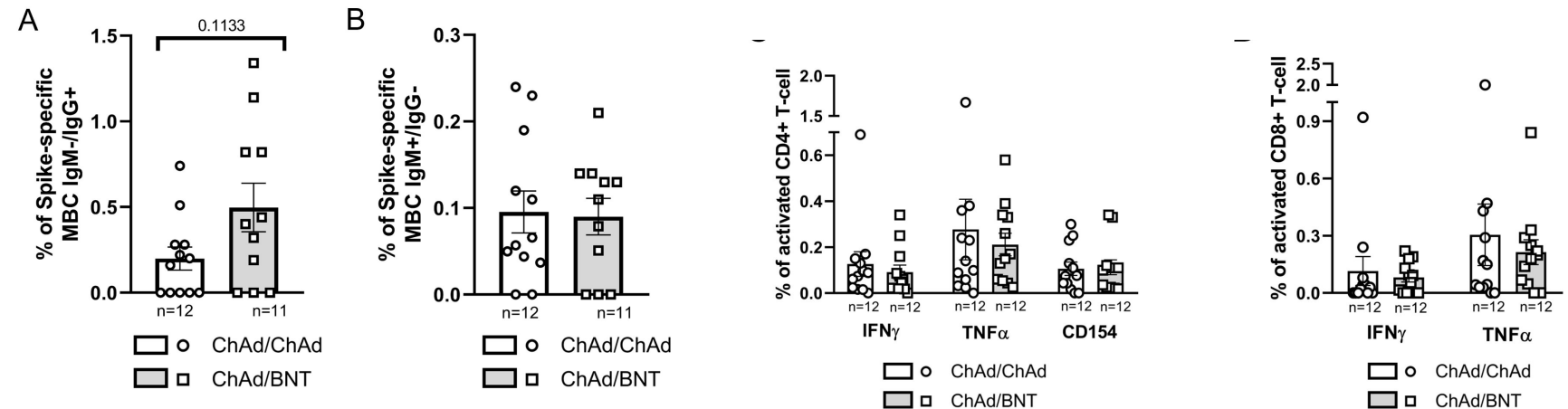
frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 02 May 2025
doi 10.3389/fimmu.2025.1579163

Analysis of humoral and cellular immune activation up to 21 months after heterologous and homologous COVID-19 vaccination

Davide Torre^{1†}, Chiara Orlandi^{2,3†}, Ilaria Conti¹, Simone Barocci⁴, Eugenio Carlotti⁵, Mauro Magnani^{1,3}, Anna Casabianca^{2,3*†} and Giuseppe Stefanetti^{1,2*†}

¹Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy, ²Department of Biomolecular Sciences, Section of Biochemistry and Biotechnology, University of Urbino Carlo Bo, Fano, Italy, ³Laboratorio Covid, University of Urbino Carlo Bo, Fano, Italy, ⁴Department of Clinical Pathology, Azienda Sanitaria Territoriale (AST) di Pesaro-Urbino, Urbino, Italy, ⁵Department of Prevention, Azienda Sanitaria Territoriale (AST) di Pesaro-Urbino, Urbino, Italy



- Spike-specific IgG⁺ MBCs and T cells detectable up to 21 months, beyond the 6–8 months usually reported.
- MBC levels trend higher after ChAd/BNT vs ChAd/ChAd
- Natural infection further boosts MBCs and CD8⁺ cytokine production(not shown)

ChAdOx1-S (**ChAd**)-Astrazeneca= Adenovector Vaccine
BNT162b2 (**BNT**)-Pfizer BioNTech= mRNA Vaccine
mRNA1273 (**MOD**)-Moderna= mRNA Vaccine



Finanziato
dall'Unione europea
NextGenerationEU



Ministero
dell'Università
e della Ricerca



Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA

NEUTRALIZATION ASSAY

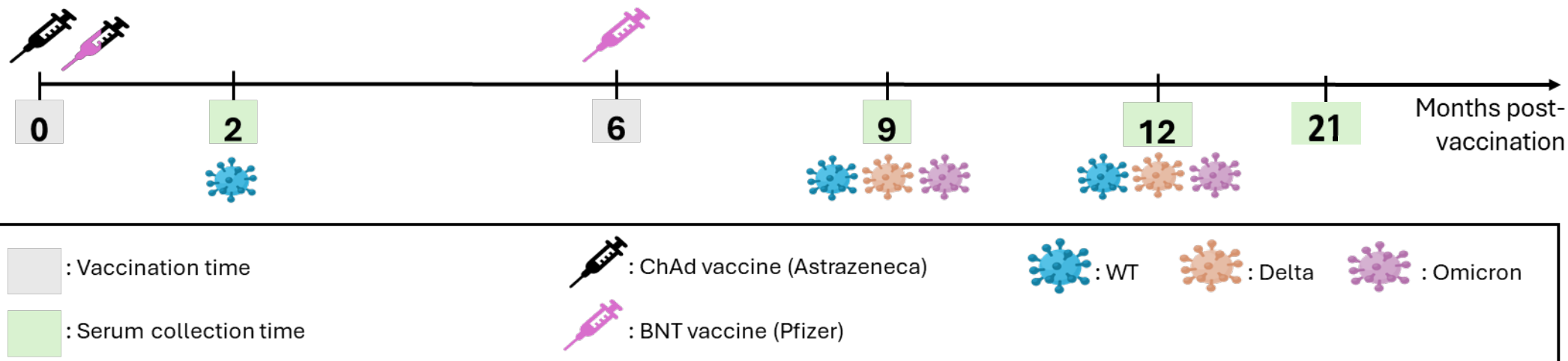
OBJECTIVE:

- **Evaluation of neutralizing ability of sera from vaccinated individuals**

Comparing two vaccination regimens

- homologous (ChAd/ChAd)
- heterologous (ChAd/BNT)

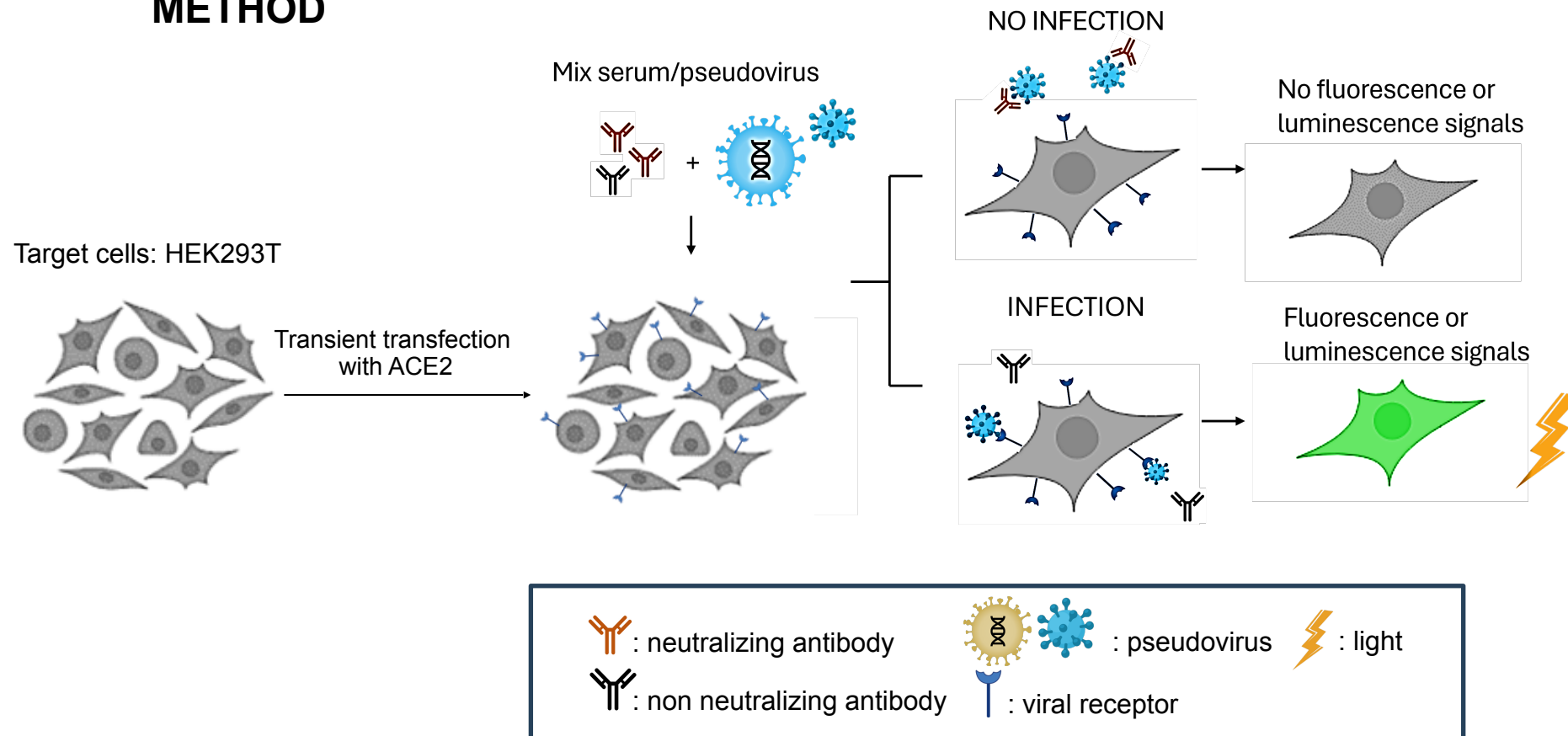
Previously...





PSEUDOVIRUS-BASED NEUTRALIZATION ASSAY

METHOD





PSEUDOVIRUS NEUTRALIZATION AT ~21 MONTHS (WT & DELTA)

		WT		Delta	
Vaccination regimen	Serum sample	LogIC50	IC50	LogIC50	IC50
ChAd/ChAd	7	3.217	1648	2.443	277.4
	31	2.697	497.4	2.369	234
	53	7.647	44,402,960	2.453	284
ChAd/BNT	190	2.439	274.7	1.459	28.75
	195	2.078	119.7	2.233	170.9
	206	2.197	157.3	1.649	44.59

- Variability in ChAd/ChAd group
- Consistent and stable neutralization in ChAd/BNT group



CONCLUSIONS & OUTLOOKS

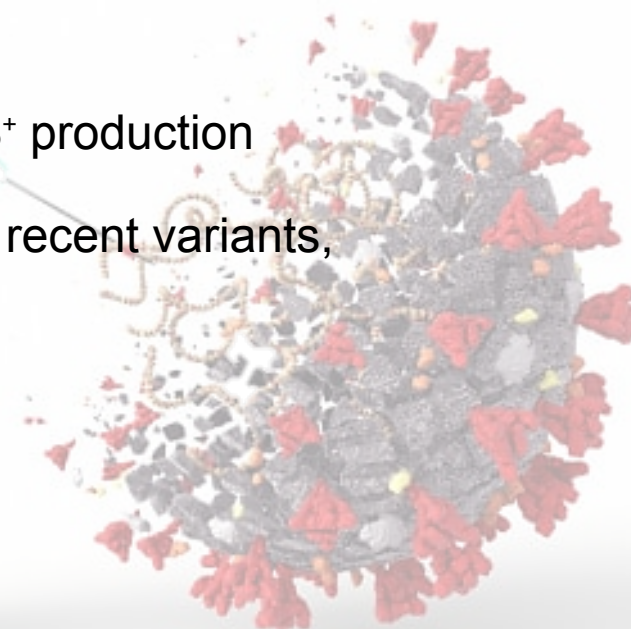
- **Durable multi-layer immunity to 21 months:** IgG, MBC and T cells induction
- **Heterologous advantage:** ChAd/BNT shows higher IgG titers and more consistent pseudovirus neutralization than ChAd/ChAd at late timepoints.
- **Hybrid effect:** Prior infection is associated with higher IgG, MBC and CD8⁺ production



Neutralization data will need to be supplemented with analyses against more recent variants, including Omicron sublineages

Limitations:

Sample size, variant coverage and pseudovirus only





Finanziato
dall'Unione europea
NextGenerationEU



Ministero
dell'Università
e della Ricerca



Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA

Acknowledgements:
Prof. Giuseppe Stefanetti,
Davide Torre; Dr. Ilaria Conti; Nicole D'Ugo



Thank you for your attention-