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Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA

IMMUNE RESPONSE ANALYSIS AND OPTIMIZATION OF A PSEUDOVIRUS NEUTRALIZATION ASSAY FOR EVALUATING COVID-19 VACCINATION STRATEGIES

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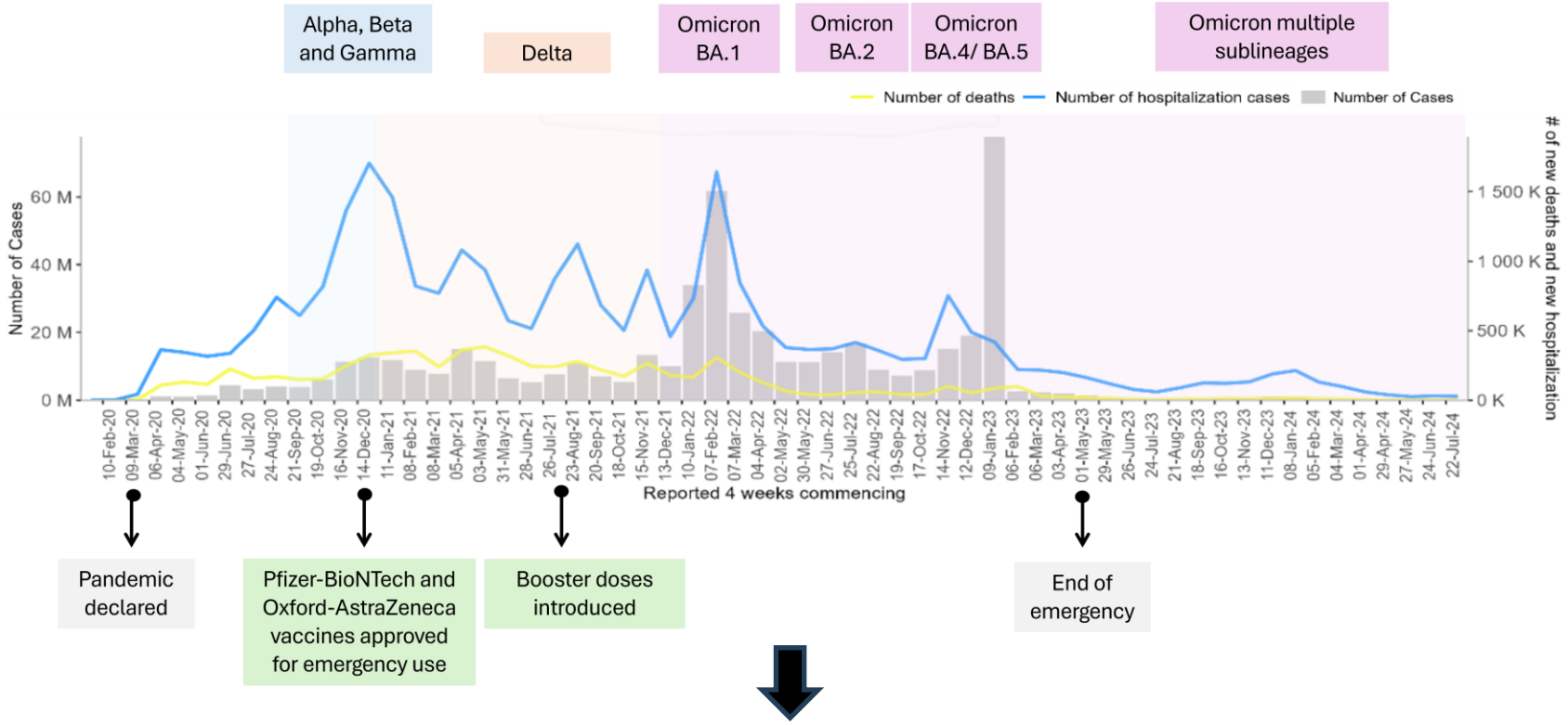
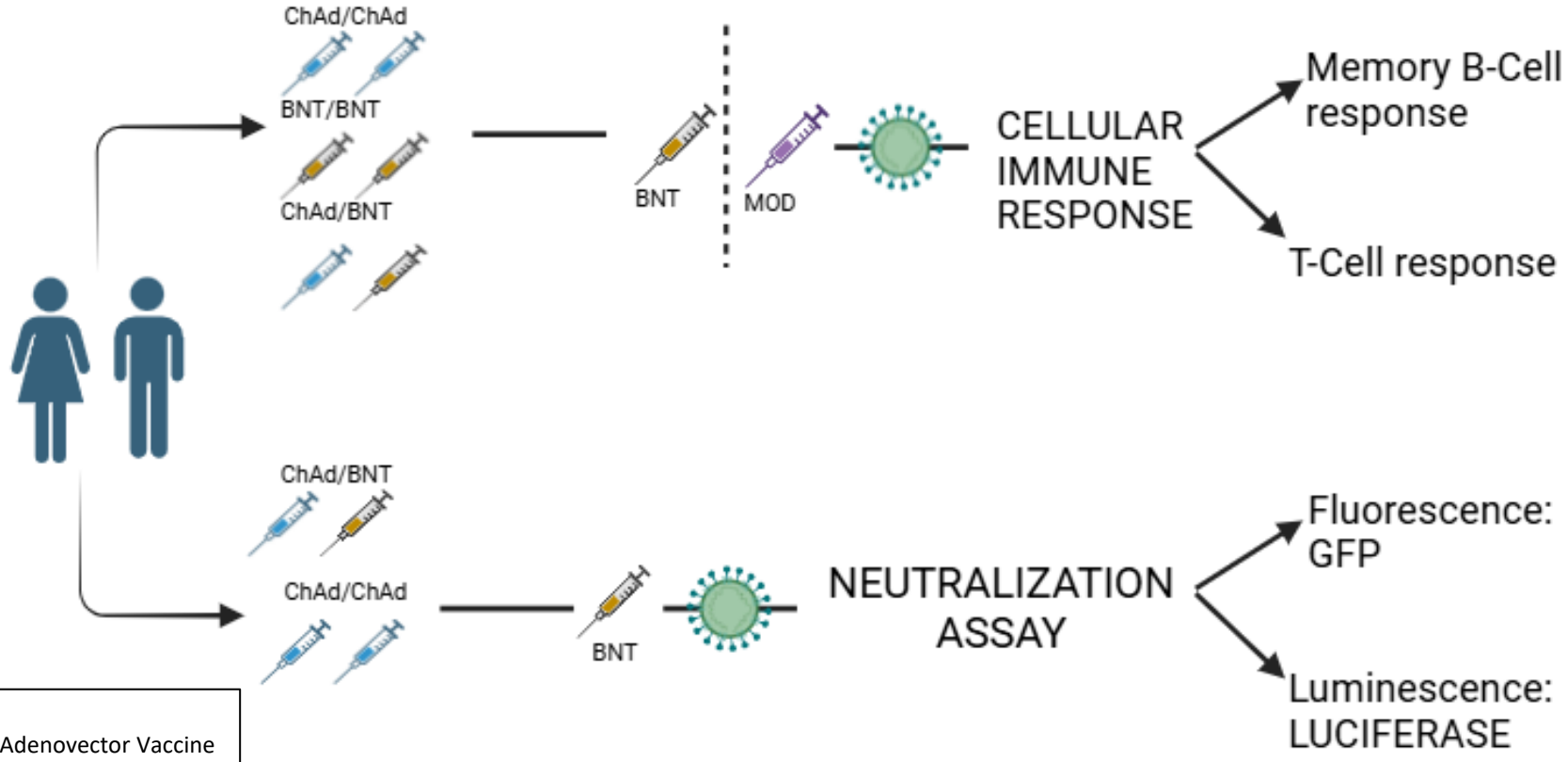
INTRODUCTION

COVID-19 trends: variants and vaccination impact



Need for <u>continous evaluation</u> of vaccine efficacy

EVALUATION OF COVID-19 VACCINATION STRATEGIES



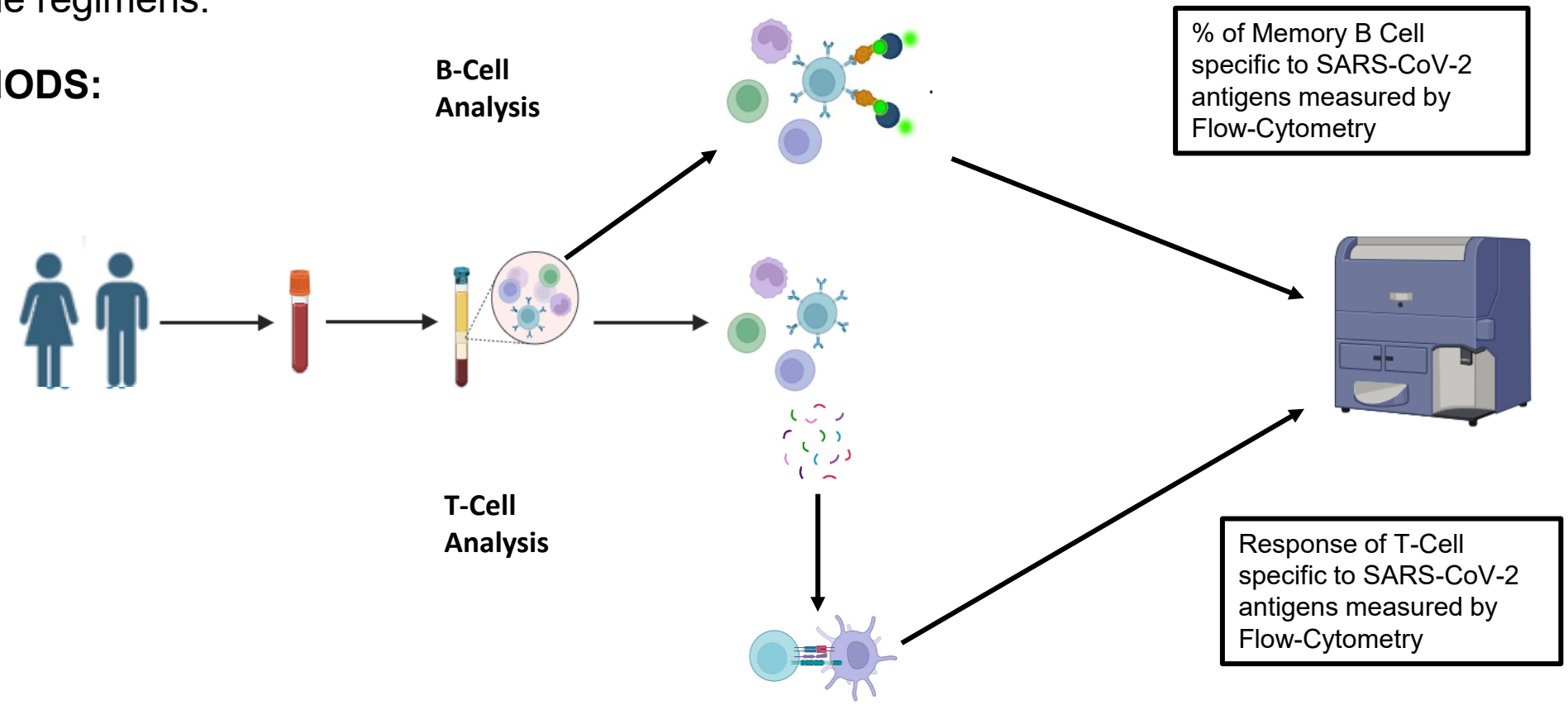
ChAdOx1-S (**ChAd**)-Astrazeneca= Adenovector Vaccine
BNT162b2 **(BNT)**-Pfizer BioNTech= mRNA Vaccine
mRNA1273 (**MOD**)-Moderna= mRNA Vaccine

CELLULAR IMMUNE RESPONSE

OBJECTIVE:

Evaluation of cellular immune response against Sars-CoV-2 elicited in patients subjected to different vaccine regimens.

METHODS:



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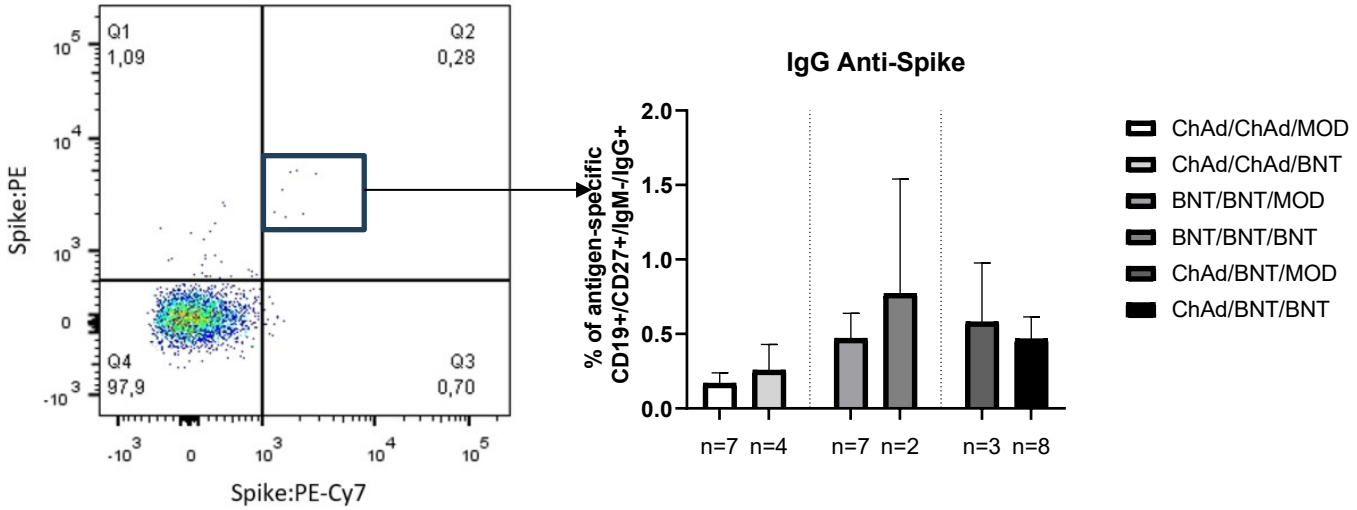
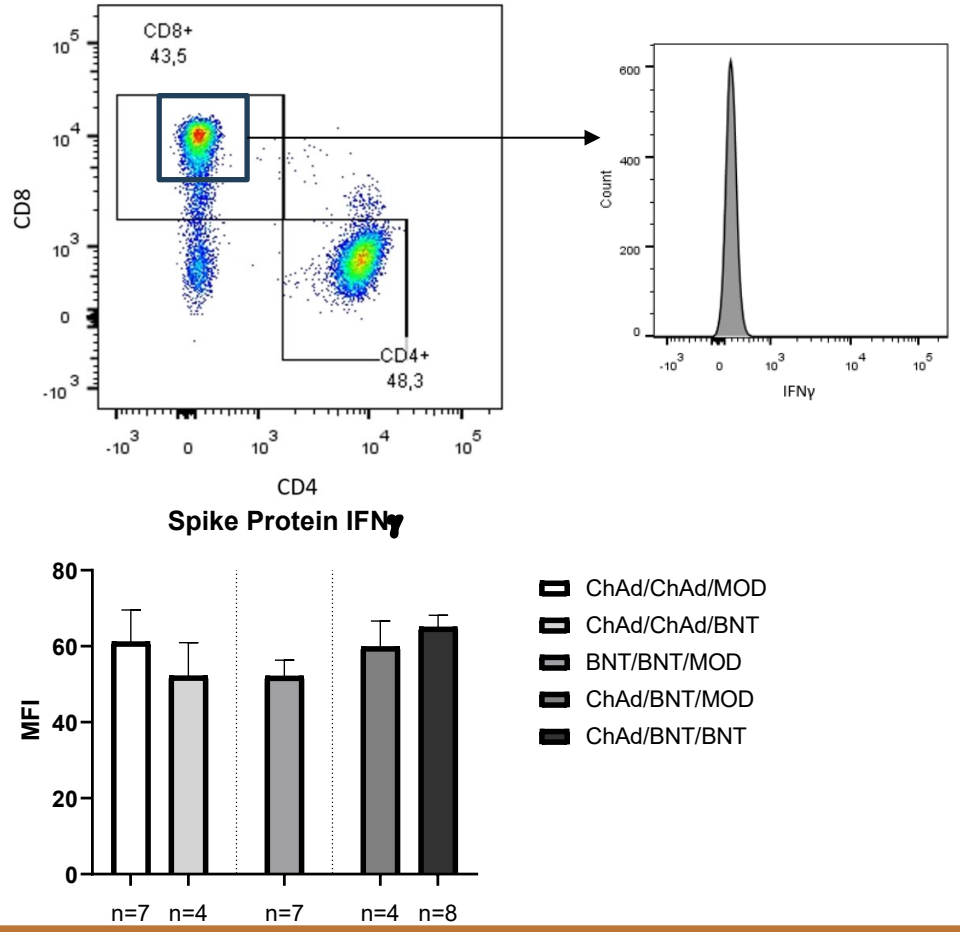
CELLULAR IMMUNE RESPONSE RESULTS

Memory B-Cell



CD8+ T-Cell



Each vaccine regimen tested elicits cellular immune responses

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NEUTRALIZATION ASSAY

OBJECTIVE:

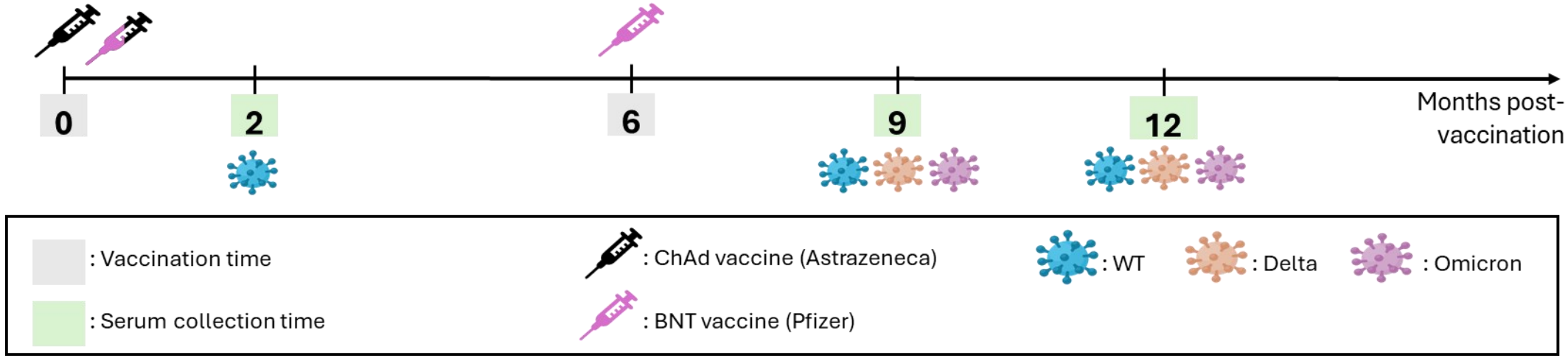
- **Optimization of a pseudovirus neutralization assay**

Comparing GFP (Green Fluorescent Protein) and LUC (luciferase) as reporters for quantification of viral infection

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

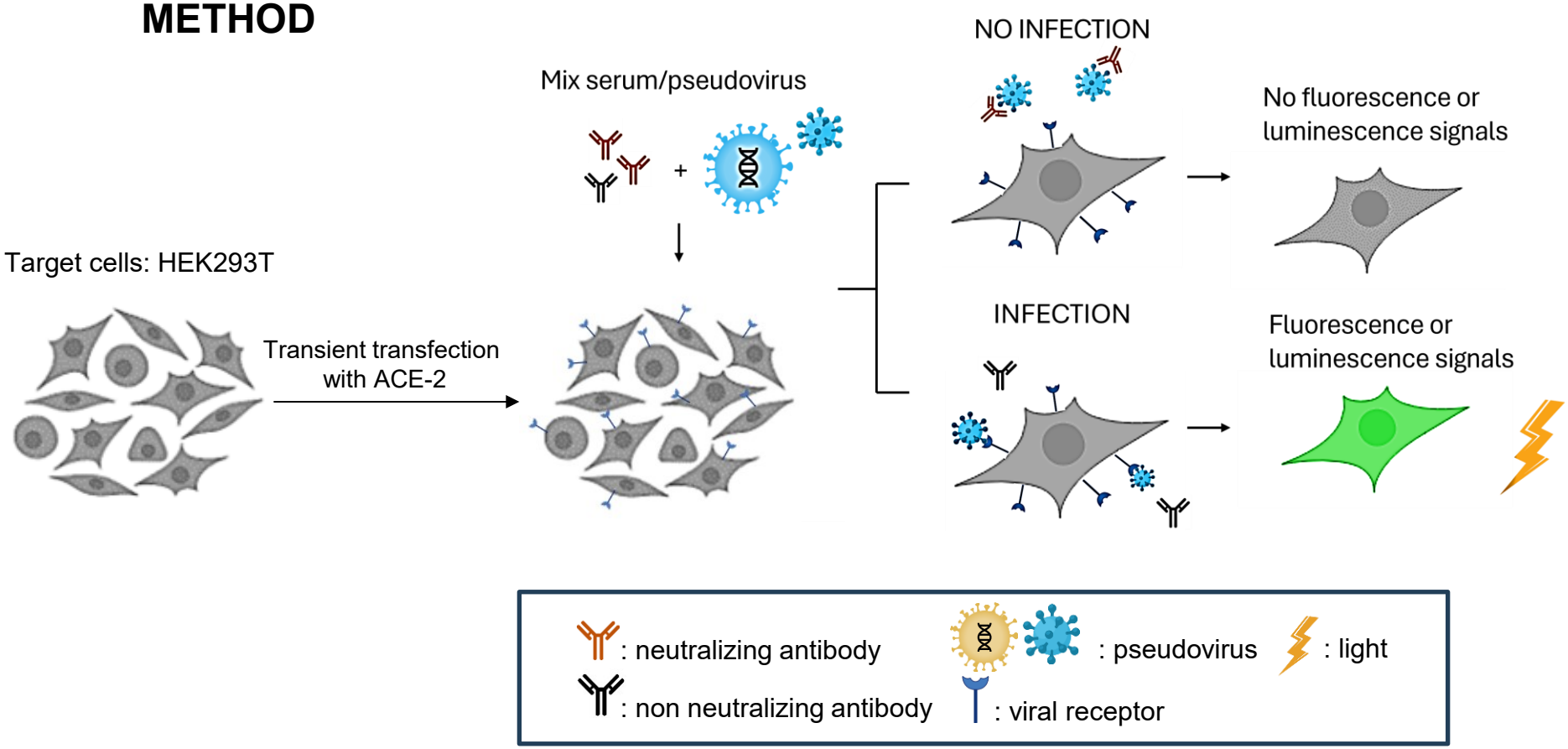
Comparing two vaccination regimens

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



PSEUDOVIRUS-BASED NEUTRALIZATION ASSAY

METHOD



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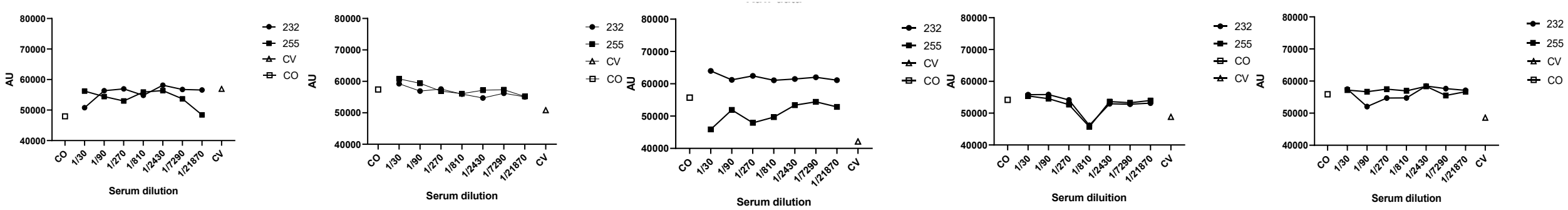
GFP EXPRESSION

Shorter post-transfection incubation time		Extended post-transfection incubation time	Increased transfection reagents	
24 hpt		72 hpt	72 hpt	
24 hpi	48 hpi	48hpi	48 hpi	72 hpi



AU: arbitrary unit
CO: cells only control
CV: cells plus pseudovirus without serum control
232, 255 : 21-month sera post ChAd/BNT

Inconsistency

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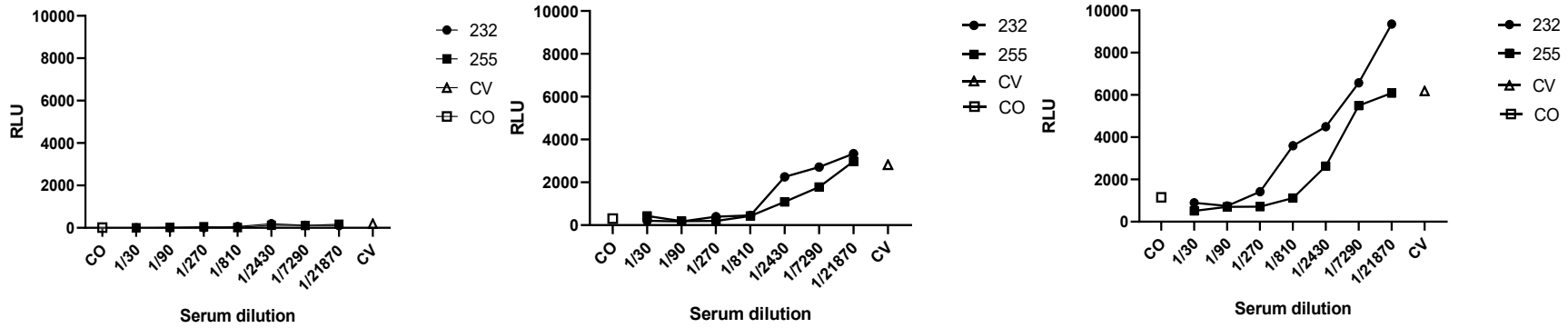
LUCIFERASE ACTIVITY

Shorter post-transfection incubation time		Extended post-transfection incubation time
24 hpt		72 hpt
24 hpi	48 hpi	48hpi



RLU: relative light unit
CO: cells only control
CV: cells plus pseudovirus without serum control
232, 255 : 21-month sera post ChAd/BNT

- Robustness and consistency
- 72 hpt + 48 hpi optimal timing

EARLY IMMUNE RESPONSE (2-MONTH)

		WT	
Vaccination regimen	**Serum sample**	**LogIC50**	**IC50**
ChAd/ChAd	**31**	0.97	9.29
	53	3.72	5,237.44
	117	3.21	1,639.83
ChAd/BNT	**190**	4.12	13,238.03
	195	3.62	4,206.22
	206	3.70	4,996.74

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

BOOSTER EFFECT AND SUSTAINED IMMUNITY (9 MONTH)

		WT		Delta		Omicron	
Vaccination regimen	**Serum sample**	**LogIC50**	**IC50**	**LogIC50**	**IC50**	**LogIC50**	**IC50**
ChAd/ChAd	**31**	4.828	67,332	Unstable	Unstable	1.880	75.80
	53	6.390	24,571,766	2.971	934.5	2.480	302.1
	117	10.64	4,323,672,30	2.869	739.1	3.162	1,454
ChAd/BNT	**190**	5.418	262,080	3.746	5,577	3.133	1,358
	195	5.630	426,831	5.204	160,008	2.536	343.4
	206	4.342	21,983	2.014	103.2	2.238	172.8

Booster dose enhanced immune response

LONG-TERM EFFICACY (12-MONTH)

		WT		Delta		Omicron	
Vaccination regimen	**Serum sample**	**LogIC50**	**IC50**	**LogIC50**	**IC50**	**LogIC50**	**IC50**
ChAd/ChAd	**31**	3.134	1,360	-310,202	0.000	-3.16	0.001
	53	7.871	74,369,582	1.651	44.80	1.74	54.968
	117	7.361	22,976,580	2.905	804.3	3.34	2,163.297
ChAd/BNT	**190**	4.754	56,862.39	1.966	92.39	3.27	1,841.817
	195	5.065	116,039.43	1.668	46.58	2.24	171.908
	206	3.122	1,325.17	1.466	29.25	1.82	66.187

Decline in neutralizing activity in both groups

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CONCLUSION AND FUTURE DIRECTIONS

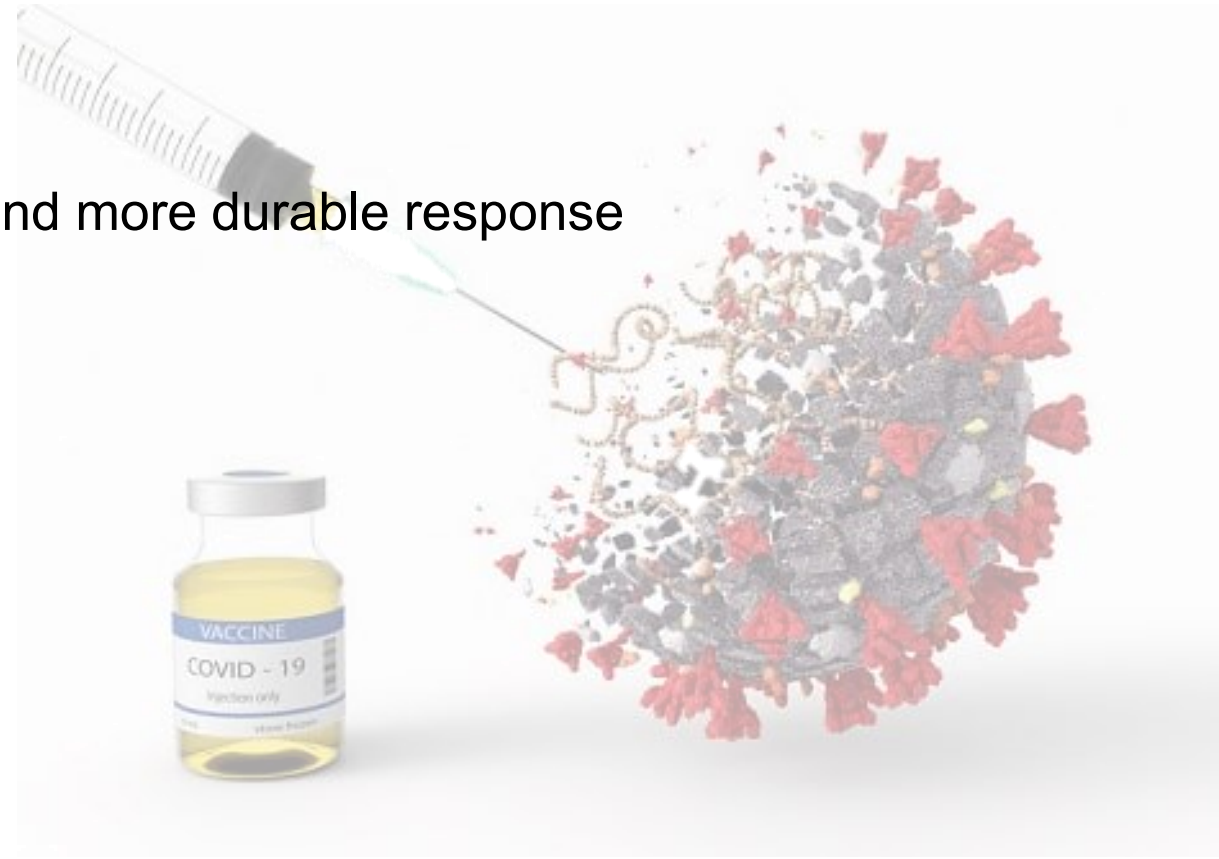
- **GFP** is cost-effective but less reliable than **luciferase** for measuring viral infectivity

  ➡ Alternative reporters or methods

- **ChAd/BNT vaccination** delivers stronger and more durable response than ChAd/ChAd

Limitations:

➡ Larger cohorts, more recent variants and live virus-based assays



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Thank you for your attention