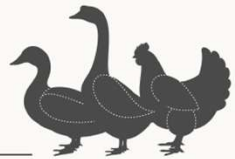
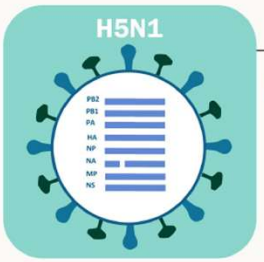
Development of RNA vaccines for outbreak response

Background H5N1

Emergence and Evolution of H5N1 BIRD FLU



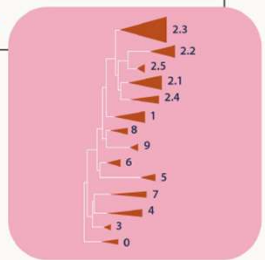


1996-1997 H5N1 bird flu virus first detected

In 1996, highly pathogenic avian influenza H5N1 virus is first identified in domestic waterfowl in Southern China. The virus is named A/goose/Guangdong/1/1996. In 1997, H5N1 poultry outbreaks happen in China and Hong Kong with 18 associated human cases (6 deaths) in Hong Kong. This virus would go on to cause more than 860 human infections with a greater than 50% death rate.

H5N1 spreads 2003-2005

For several years, H5N1 viruses were not widely detected; however, in 2003, H5N1 re-emerges in China and several other countries to cause widespread poultry outbreaks across Asia. In 2005, wild birds spread H5N1 to poultry in Africa, the Middle East and Europe. The hemagglutinin (HA) gene of the virus diversifies into many genetic groups (clades). Multiple genetic lineages (genotypes) are detected.



https://www.cdc.gov/bird-flu/communication-resources/index.html

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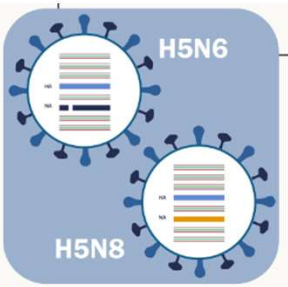
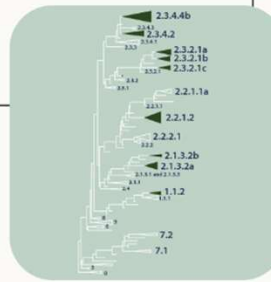


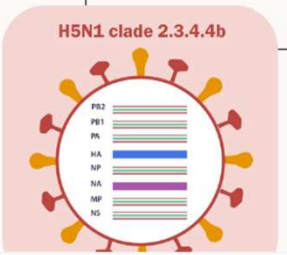
2014-2016 H5N6 and H5N8 viruses emerge

Gene-swapping of H5 viruses from poultry and wild birds leads to emergence/detection of H5N6 and H5N8 virus subtypes. HA diversifies further into clade 2.3.4.4 in Asia, Africa, Europe, the Middle East and North America. H5 viruses with various neuraminidase (NA) genes continue to be detected, including in U.S. wild birds and poultry.

2.3.4.4b viruses spread widely 2018-2020

H5N6 and H5N8 viruses become predominant globally, replacing the original H5N1 viruses. As of 2022, there have been more than 70 H5N6 human infections and 7 H5N8 human infections reported. The H5 HA diversifies further into clade 2.3.4.4b which becomes predominant in Asia, Africa, Europe, and the Middle East.





2021-2023 H5N1 found in Canada, US

A new H5N1 virus belonging to clade 2.3.4.4b with a wild bird adapted N1 NA gene emerges. Clade 2.3.4.4b H5N1 viruses become predominant in Asia, Africa, Europe, and the Middle East by the end of 2021. The virus is detected in wild birds in Canada and the United States in late 2021. In February 2022, the virus begins causing outbreaks in U.S. commercial and backyard poultry. Rare, sporadic human infections with this H5N1 virus are detected, as well as sporadic infections in mammals.

Product development

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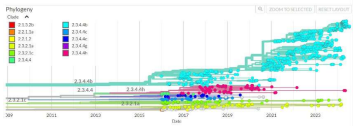




Disease target	Strain selection	Antigen selection	Coding región optimization & NCR selection.	pDNA &mRNA synthesis	in vitro Test	In vivo test (small animal model)

Strains selection: pandemic influenza

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GISAID repository:

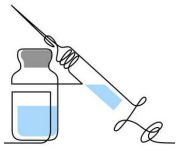
-H5N1 A/Vietnam/1194/2004 (#EPI2017310)→ clade 1

-H5N1 A/American Wigeon/South Carolina/USDA-000345-001/2021 (#EPI2709137) Clade 2.3.4.4b

-H5N1 A/Texas/37/2024 (#EPI3171488)

Vietnam: Pandemic Influenza **Model strain**

Used in commercial vaccines available



GSK Seqirus A CSL COMPANY

Good access to different reagents to conduct animal studies and analytical methods.

South Carolina & Texas: more **prevalent strains**

World Health Organization

Genetic and antigenic characteristics of zoonotic influenza A viruses and development of candidate vaccine viruses for pandemic preparedness

February 2023

During the vaccine composition meeting that occurred in February 2023, the antigenic prototype A/American wigeon/South Carolina/22-000345-001/2021-like (clade 2.3.4.4b) was defined as a candidate virus in preparation by CDC

Technical Update: Summary Analysis of Genetic Sequences of Highly Pathogenic Avian Influenza A(H5N1) Viruses in Texas

Optimization parameters

	#	Vendor	Optimization strategy	Influenza strain	poliA tail	MFE value	CAI value	
1st round	1	A	wt	Vietnam	110nt segmented	-522	0,69	High MFE, Low CAI
	2	A	VB	Vietnam	110nt segmented	-631	0,96	Intermediate MFE, High CAI
	3	A	CPU-UrDep	Vietnam	110nt segmented	-590	0,86	
	4	B	wt	Vietnam	100nt non segmented	-522	0,69	
	5	B	CAI-UrDep	Vietnam	100nt non segmented	-654	0,98	
	6	B	CPU-UrDep	Vietnam	100nt non segmented	-590	0,86	
2nd round	7	C	wt	Vietnam	110nt non segmented	-513	0,69	
	8	C	MFE & CAI	Vietnam	110nt non segmented	-943	0,94	Low MFE, High CAI
	9	C	Wt	South Carolina	110nt non segmented	-484	0,71	High MFE, Low CAI
	10	C	MFE & CAI	South Carolina	110nt non segmented	-955	0,95	
	11	C	Wt	Texas	110nt non segmented	-480	0,70	
	12	C	MFE & CAI	Texas	110nt non segmented	-1120	0,75	
	13	C	Wt	Texas	110nt non segmented	-486	0,70	
	14	C	MFE & CAI	Texas	110nt non segmented	-1128	0,75	Low MFE, Intermediate CAI

14 different mRNA candidates to work with

Wide range of optimization parameters values, including sequences with all posible combinations

In-silico predicted secondary structures

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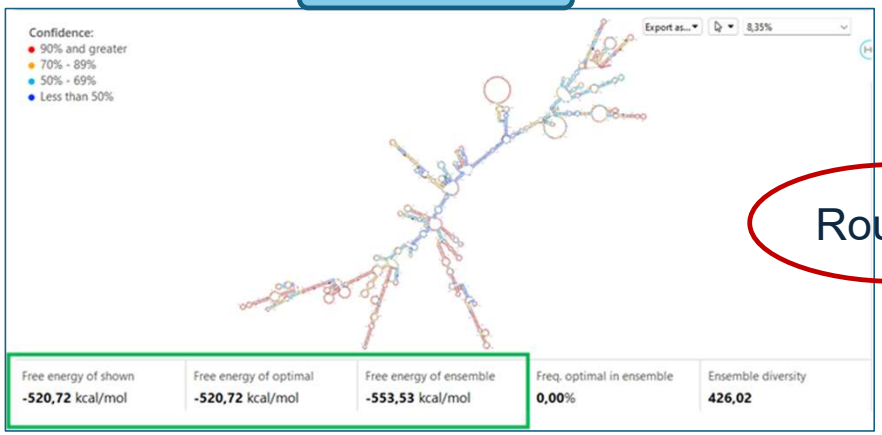
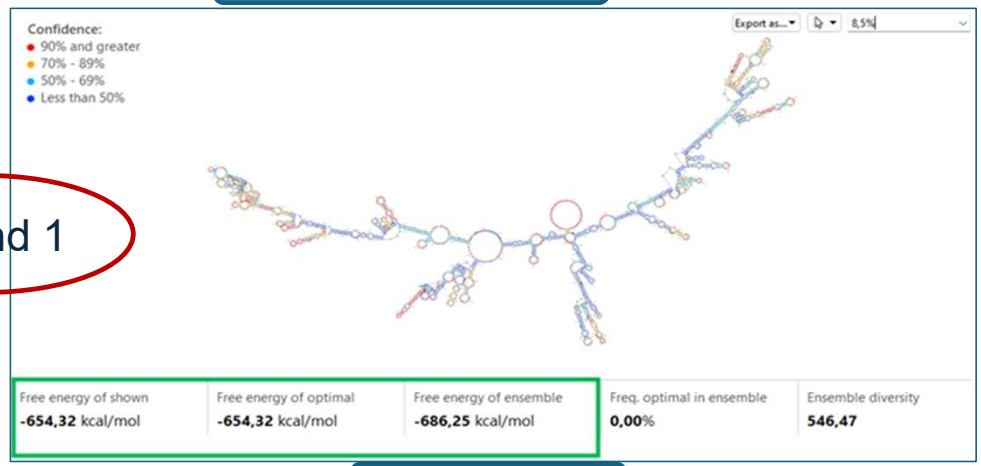
Vietnam wt

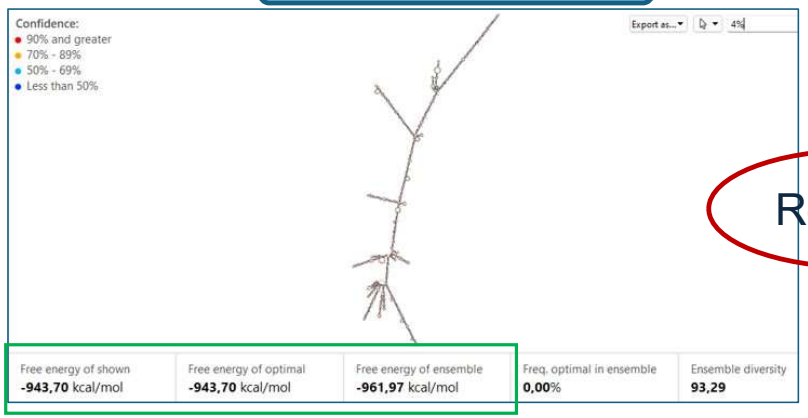


Vietnam CAI-UrDep

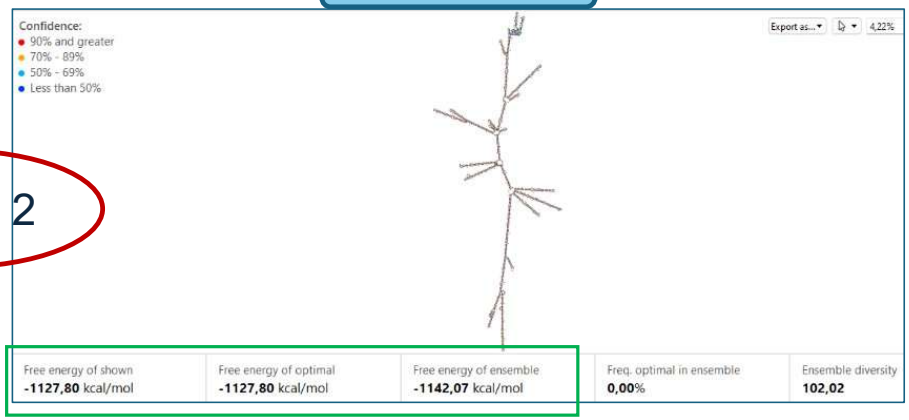


Round 1

Vietnam MFE & CAI



Texas MFE



Round 2

Structure stability

Preliminary results: round 1

-H5N1 A/Vietnam/1194/2004 clade 1

Sept, 2024

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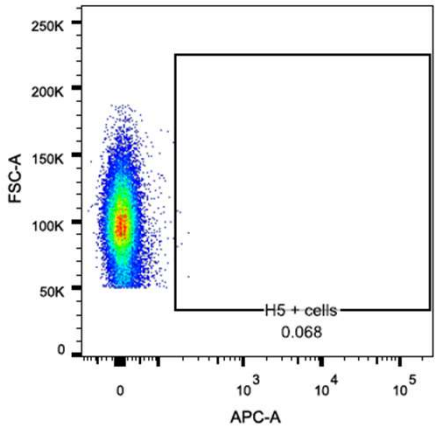
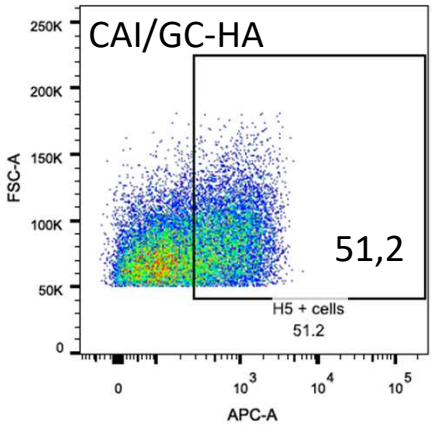
Round 1: In-vitro translation

Cells W/O transfection

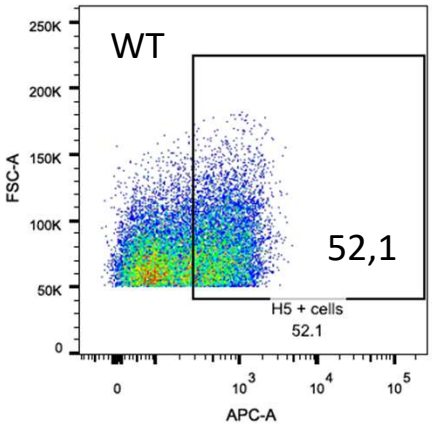


Superficie_sup ST.fcs
FSC-A, SSC-A subset
25062

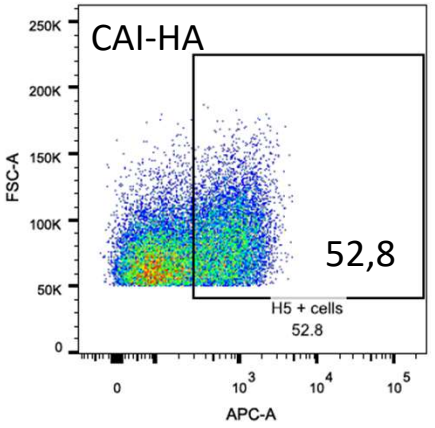
Cells + HA-mRNA-LNP



Permeabilizados_Perm NUU.fcs
FSC-A, SSC-A subset
18892

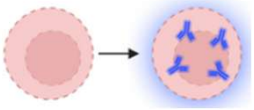


Permeabilizados_Perm TVG.fcs
FSC-A, SSC-A subset
16548



Permeabilizados_Perm CAI.fcs
FSC-A, SSC-A subset
18045

Intracellular antigen
(Permeabilized cells)



Flow cytometry

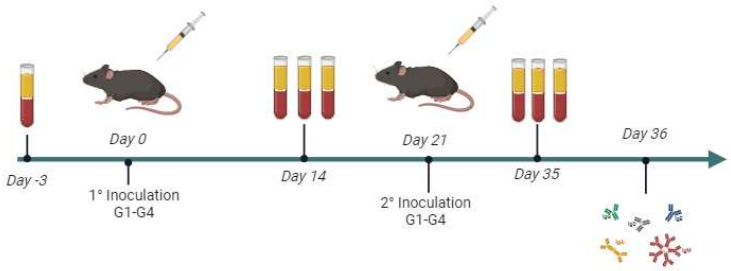
*Laboratorio de Inmunotecnología de Ácidos Nucleicos

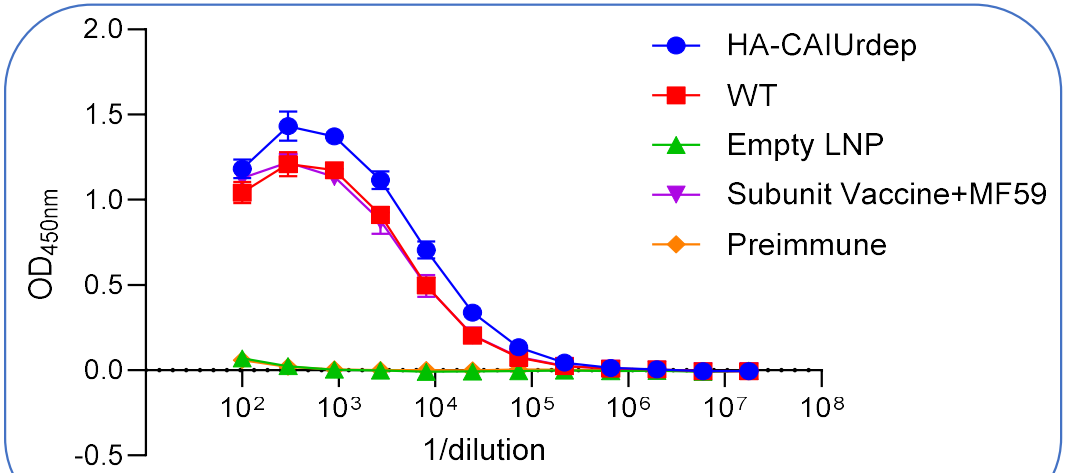
Round 1: Immunogenicity

Proof of concept
H5N1mRNA candidates

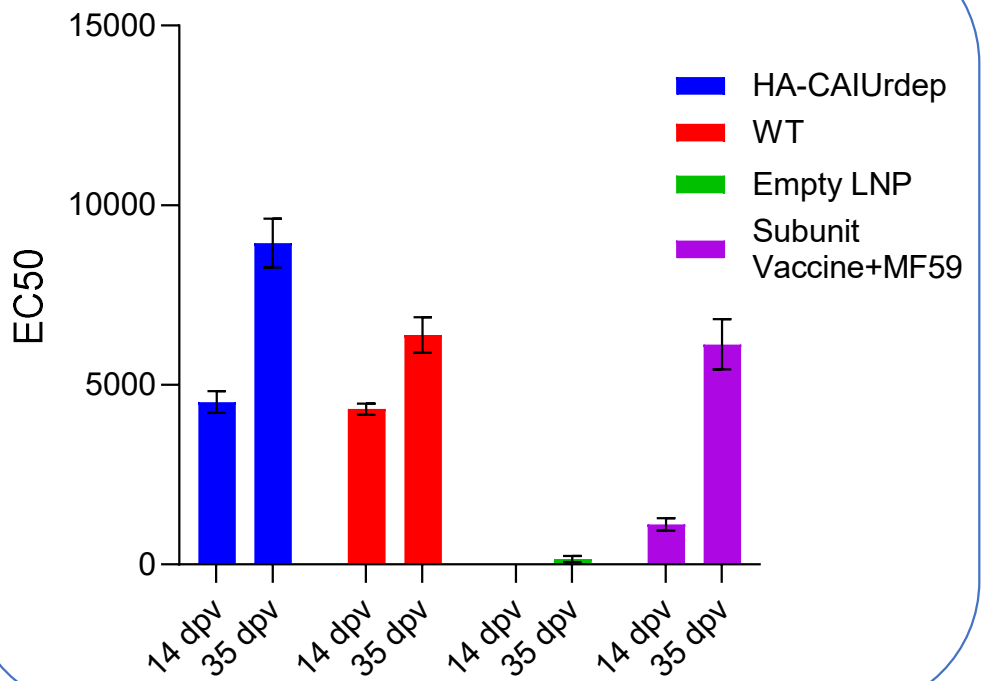
Mouse model C57BL/6

Groups:
G1: WT Candidate 10 µg
G2: Opt candidate10 µg
G3: Empty LNP
G4: Reference H5 protein +Adjuvant Oil-in-water





Total biding antibodies against H5 A/Vietnam/1194/2004 clade 1

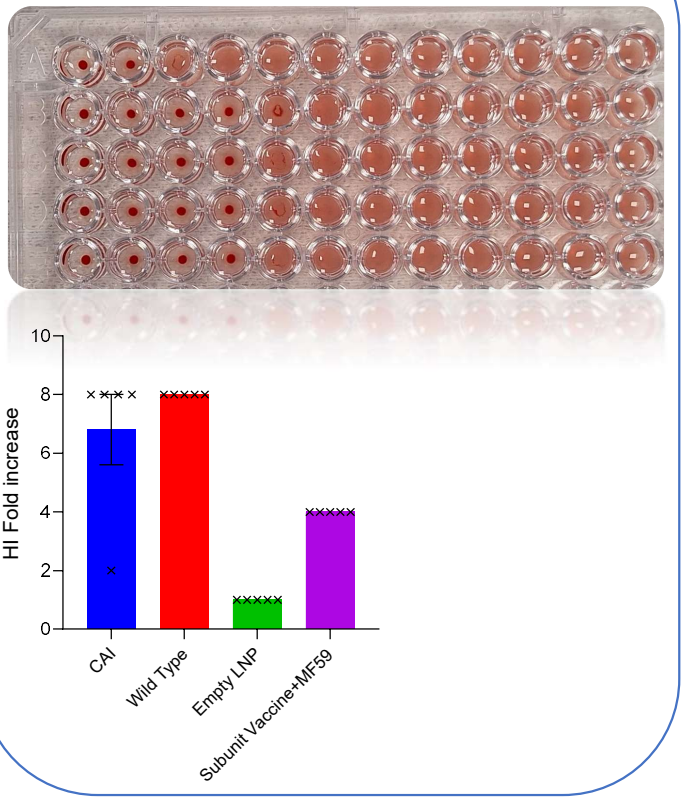


EC50 comparission

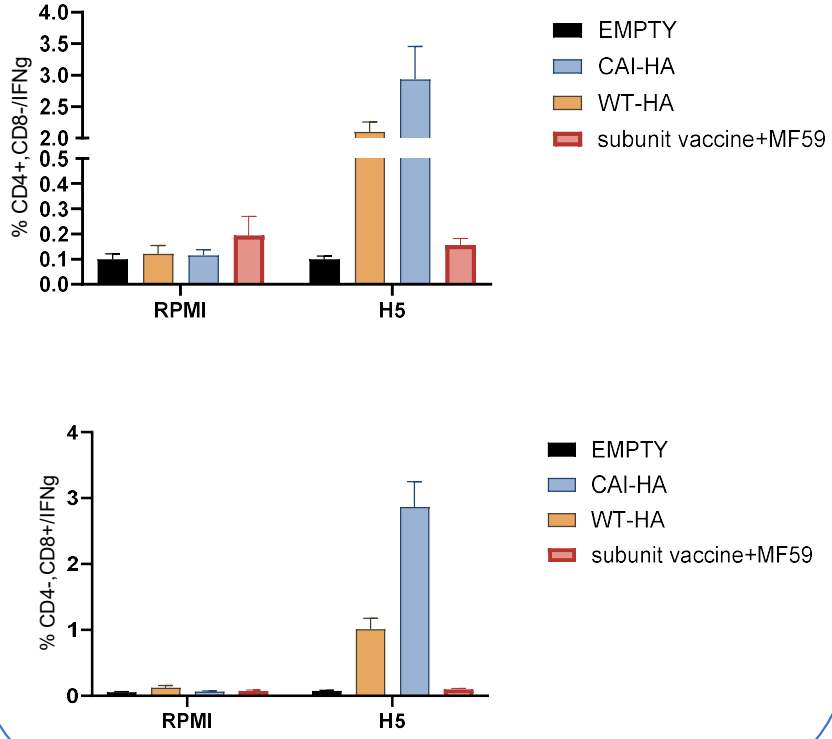
Round 1: Immunogenicity

Functional antibodies

haemagglutination inhibition



Cellular response



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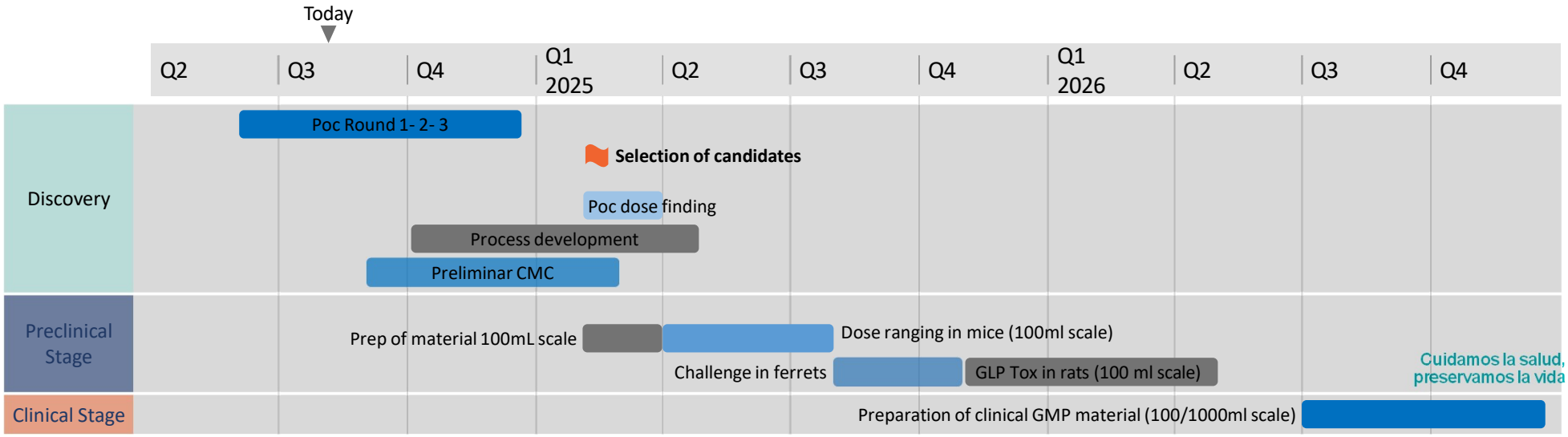
Conclusions

1. **We leveraged the knowledge acquired through the TT program sponsored by WHO/MPP to target new vaccine developments.**
2. **We have applied bioinformatics tools to improve stability and immunogenicity of our vaccine candidates.**
3. **We successfully tested two mRNA vaccine candidates against H5N1 in murine models, demonstrating our capacity to develop effective vaccines in a timely manner.**

Coming soon



Construction: mRNA production facility

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Thank you

Development & Innovation lab
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ARNm
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