# Comparing immunogenicity of VZV gE antigen in mRNA, subunit and attenuated vaccine in primates

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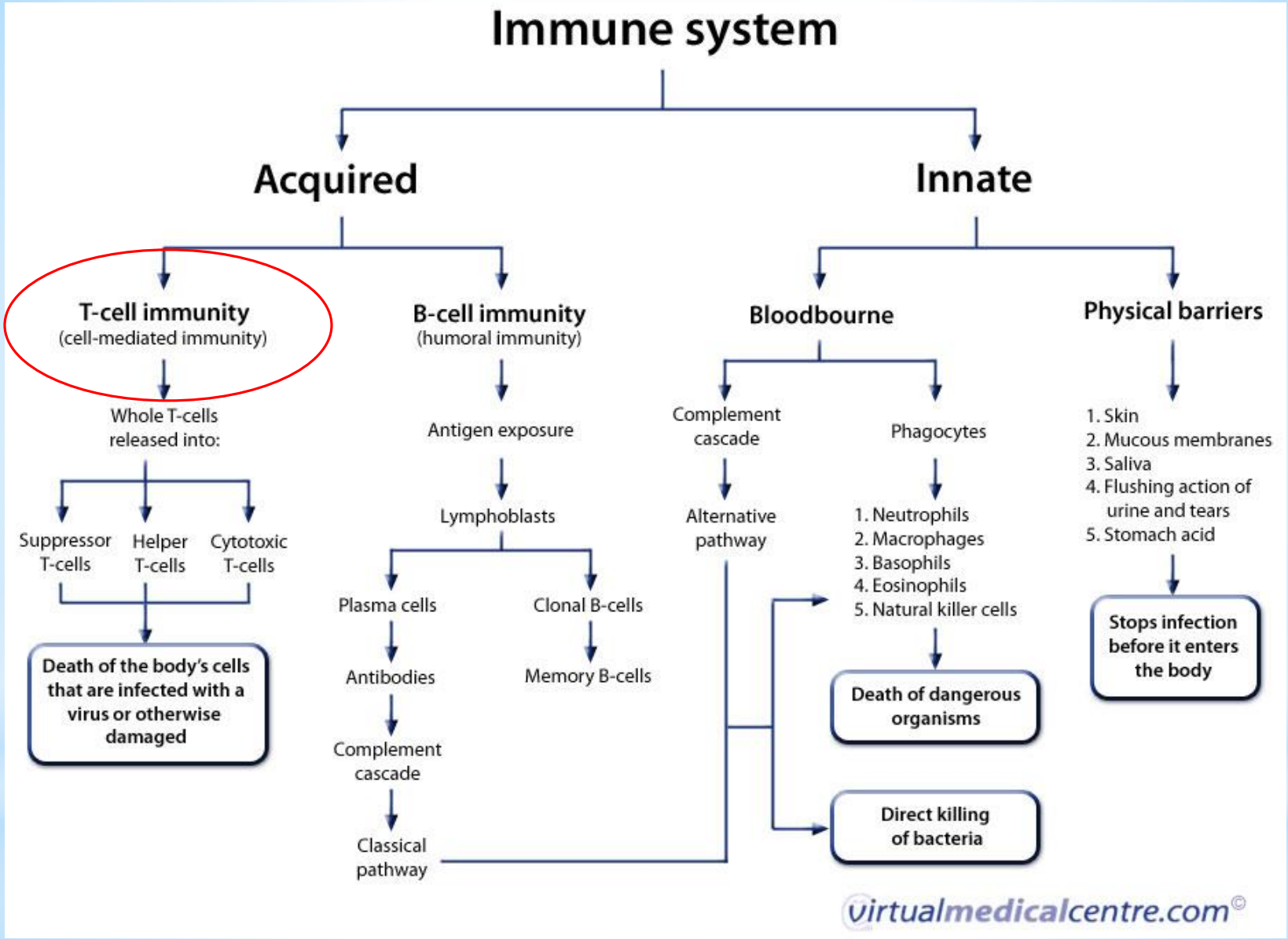
# General information

- ✓ Shingles is a painful, blistering rash caused by varicella-zoster virus(VZV)
- ✓ Affected one in three people during the lifetime
- ✓ Most occurs in elderly , immunocompromised individuals
- ✓ Virus remained latent in sensory ganglia after primary VZV infection(chickenpox)

- ✓ Most common serious complication is postherpetic neuralgia(PHN)
- ✓ PHN causes debilitating pain in region for weeks to years after rash resolving
- ✓ Biggest risk factor is age
- ✓ Incidence rates increase dramatically at 50-60 years of age
- ✓ Concomitant with age-associated decline in cell-mediated immune(CMI) response

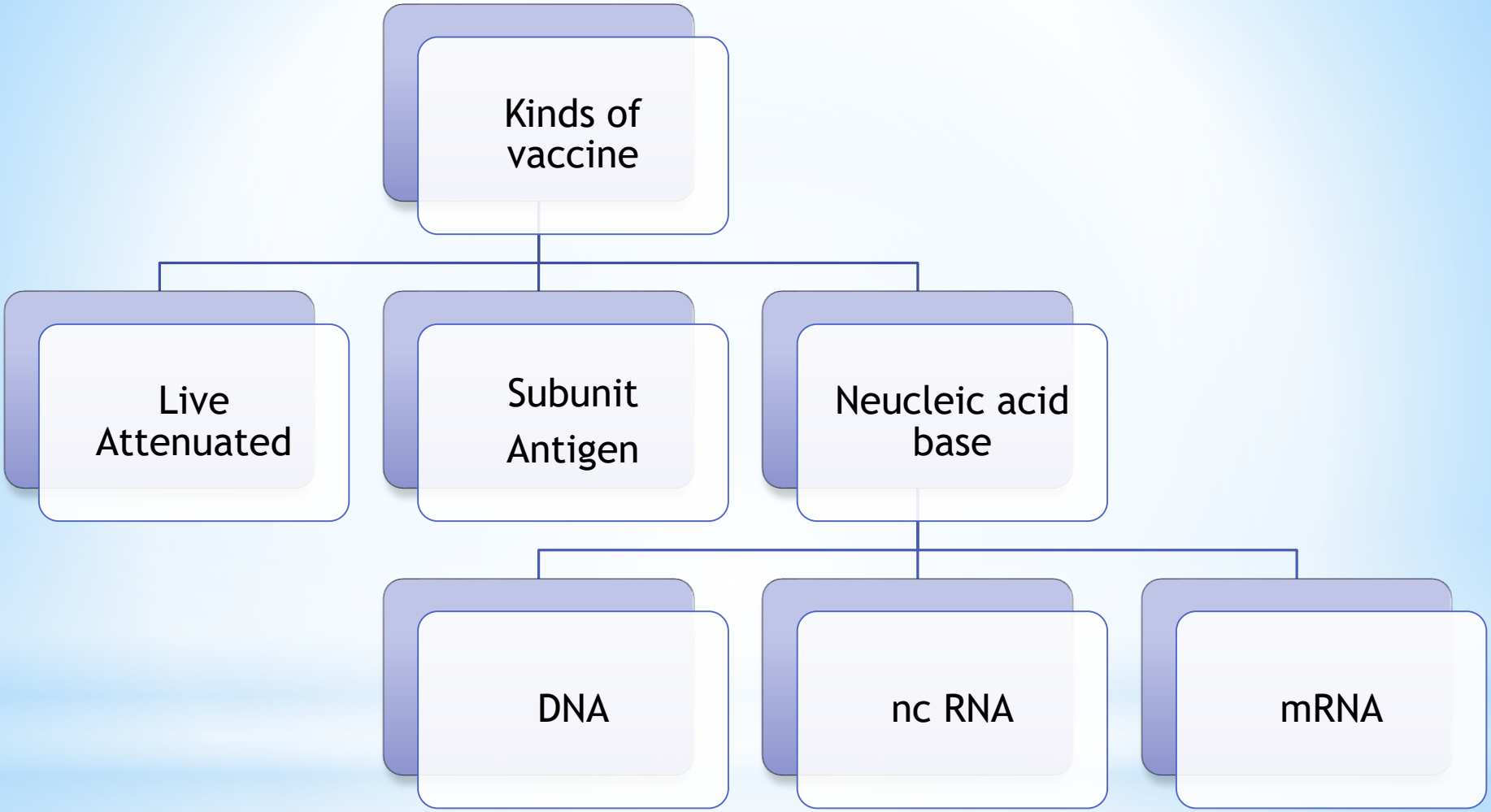
# General information




# Immune system

# Objectives

- ✓ Utilized non-human primates(NHP) to evaluate humoral and cellular immune response by three vaccine :
- ✓ Lipid nanoparticle (LNP) formulated mRNA encoding VZV gE antigen (VZV gE mRNA/LNP)
- ✓ Live attenuated VZV (VZV LAV)
- ✓ Adjuvanted VZV gE subunit protein (VZV gE protein/adjuvant)




# Kinds of vaccine

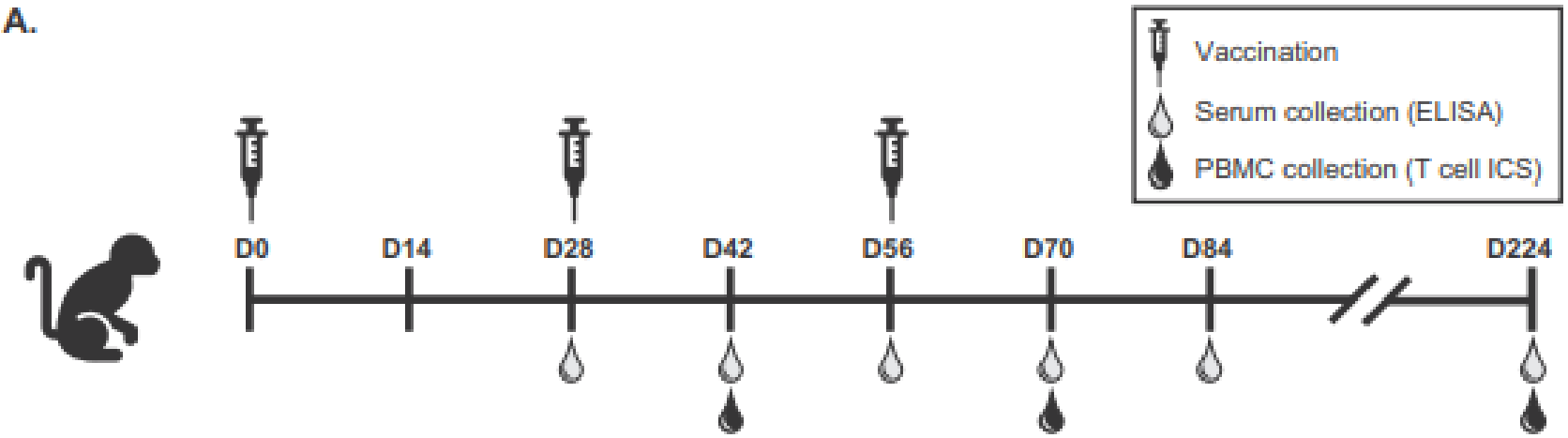
| ZOSTAVAX | Shingrix | Moderna |
|---|---|---|
| Live attenuated | gE subunit | mRNA |
| Single Dose | Two doses | |
| Subcotaneous | Intramascular | |
| 64% (60-69 years)<br>18%(>80) | 97.4% (60-69years)<br>>90% all age | |
| Four-fold in humoral and CMI responces | Twenty-fold for CMI responces | |

# Current vaccines

- ✓ Live attenuated vaccine: viruse per dose19400 forming unit
- ✓ Subunit vaccine: 539 aa, carboxyl-terminal with His tag and thrombin cleavage site was expressed in Expi 293 F
  - Protein captured by HisTrap chromatography
  - Purified by size exclusion chromatography
  - Formulated in adjuvant and monophosphoryl lipid A
- ✓ mRNA vaccine: mRNA encoding 573 aa in cationic LNP , Y569A mutation in C-terminal modulate subcellular trafficking

# Materials and methods

A.




B.

| | | Study 1 Vaccinations | | |
|---|---|---|---|---|
| Group | # of Animals | Day 0 | Day 28 | Day 56 |
| 1 | 4 | Live, attenuated VZV ≥19,400 pfu | Live, attenuated VZV ≥19,400 pfu | Live, attenuated VZV ≥19,400 pfu |
| 2 | 5 | Live, attenuated VZV ≥19,400 pfu | VZV gE subunit protein/adjuvant 50 µg | VZV gE subunit protein/adjuvant 50 µg |
| 3 | 5 | Live, attenuated VZV ≥19,400 pfu | VZV gE mRNA/LNP 200 µg | VZV gE mRNA/LNP 200 µg |
| 4 | 5 | Live, attenuated VZV ≥19,400 pfu | VZV gE mRNA/LNP 100 µg | VZV gE mRNA/LNP 100 µg |
| 5 | 5 | Live, attenuated VZV ≥19,400 pfu | VZV gE mRNA/LNP 50 µg | VZV gE mRNA/LNP 50 µg |

First study: five groups of 4-5 male monkeys

1. Were immunized three times at 28 days intervals
2. Received two doses of vaccine
3. Collecting serum and peripheral blood mononuclear cell(PBMC)

   14 days post vaccination
4. Final collection six months after third vaccination
5. All samples were cryopreserved

# Animal study 1

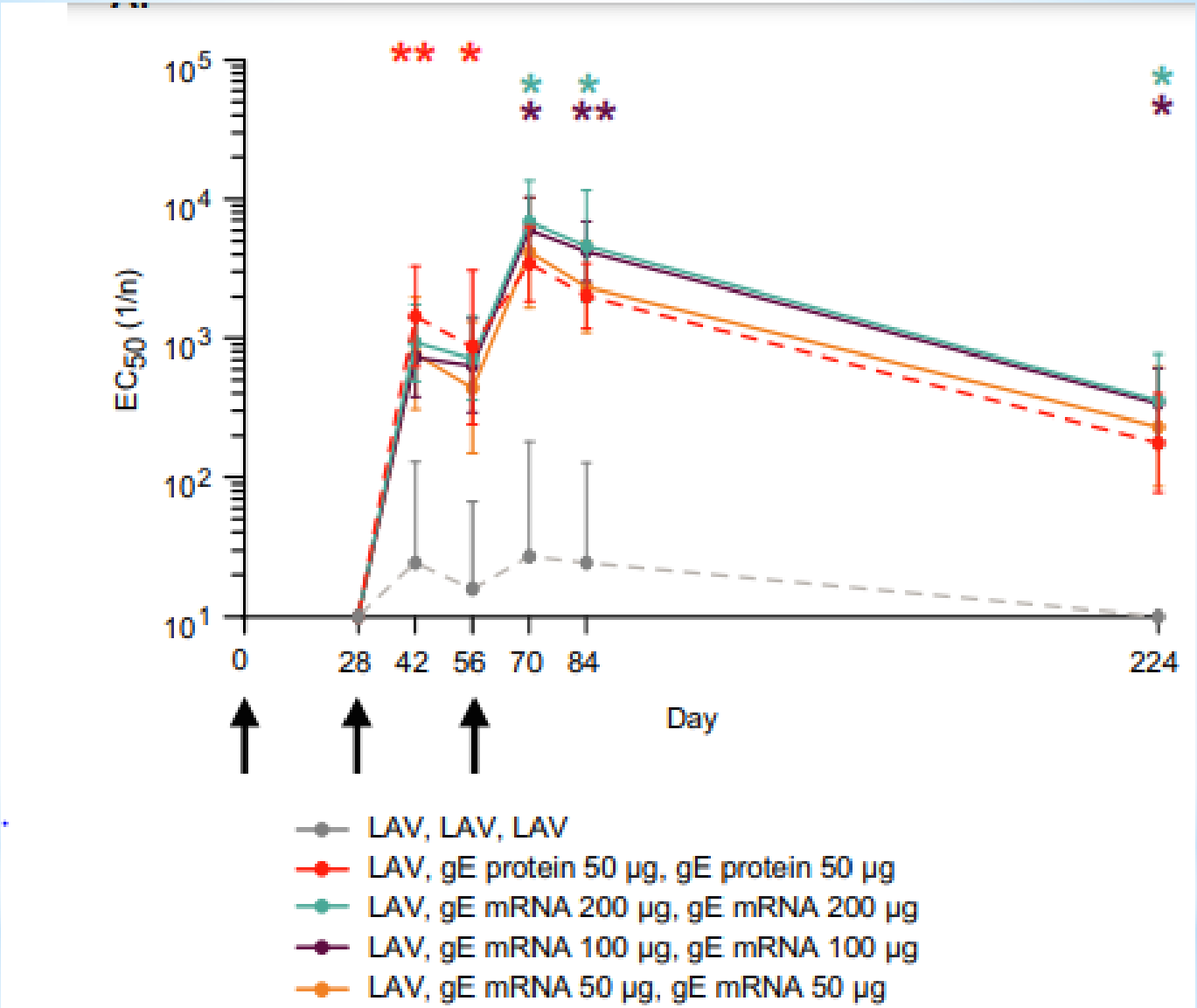
**Antibody binding titers against VZV gE quantified by ELISA**

1. 96-well nickel-coated plates were coated overnight at 4˚C with 1μg/ml His-tag recombinant protein
2. Washed plate six times with PBS
3. blocked in room temperature(RT) with blocking buffer for 1 hour
4. NHP sera was diluted 5-fold serially
5. Transfer to plates and incubated for 1.5 h at RT

# ELISA and avidity assay

6. Plates were washed six times with PBS
7. HRP conjugated goat anti-human Ig Fc was diluted 1:6000
8. Adding to plates and incubated for additional 1 hour at RT
9. SuperBlu-Turbo TMB for five minutes at RT
10. Elisa stop solution for TMB added
11. Absorbance was read at OD450 nm

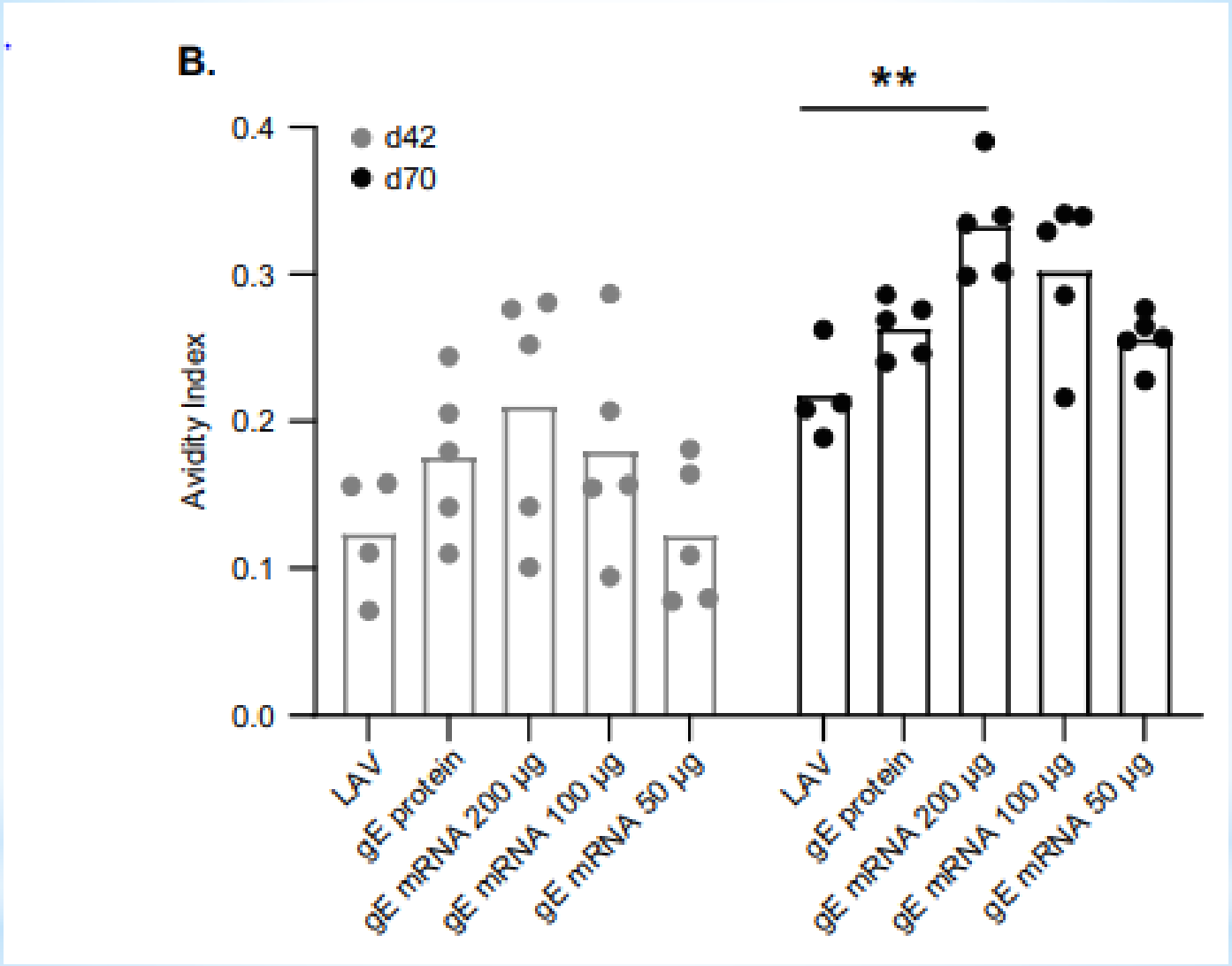
# ELISA and avidity assay




# Humoral responses results

- ✓ Follwing serum incubation, ELISA plates washed six times with PBS
- ✓ 8M Urea diluted in  PBS was added for 5 minute in RT
- ✓ Matched control wells were incubated for 5 minutes at RT
- ✓ Plates were washed six times with PBS
- ✓ Rest of ELISA
- ✓ Avidity index(AI) is calculated by EC50 of wells treated with urea divided to EC5o of control wells treated with PBS

# Avidity index(AI)



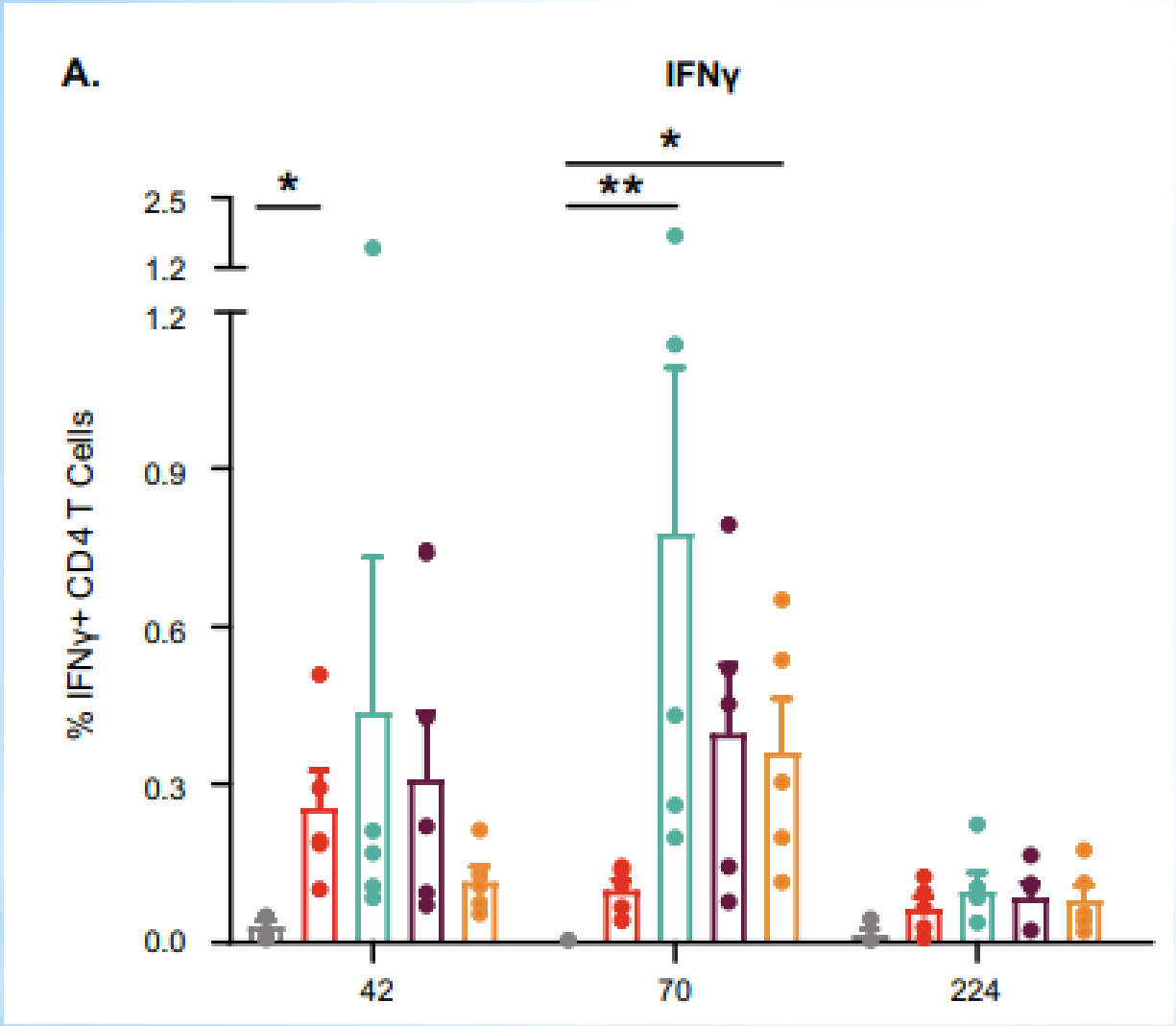

# Antibody avidity results

1. Cryopreserved PMBC were quick-thawed in 37˚C
2. Washed with R10 (10% fetal bovine serum, HEPES, L-glutamin, penicillin-streptomycin, sodium pyruvate)
3. Incubate overnight at 37˚C, 5% CO2
4. Distributing in plates with VZVgE protein and CD28/CD49
5. Plates incubated at 37˚C, 5% CO2 30-60 minutes
6. Adding brefeldinA and incubated for 5 h

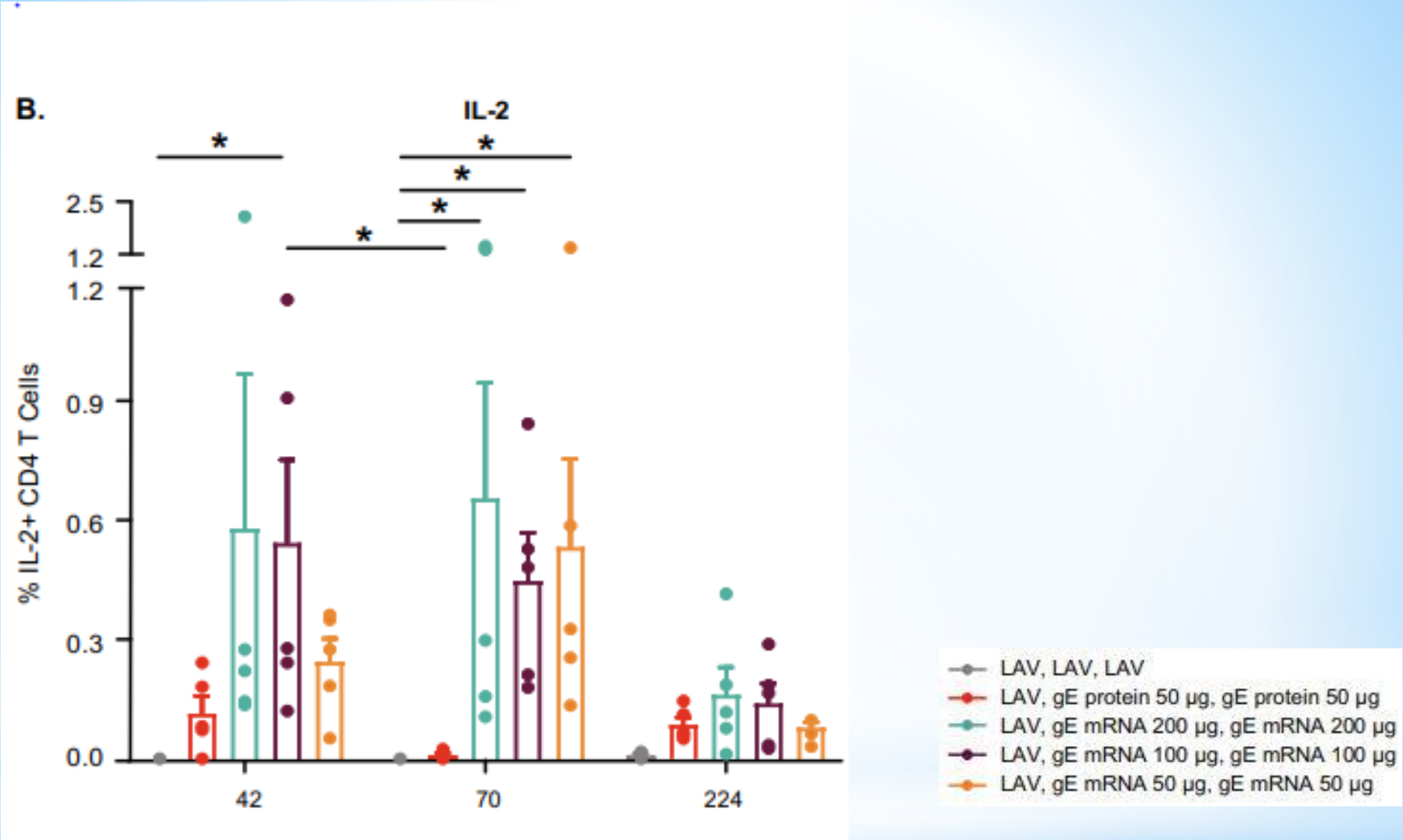
# PBMC cell stimulation

7. Cell washed with PBS
8. Stained with live/dead fixable violet stain
9. Washed with FACS wash buffer
10. Incubated with fluorescently-labeled antibodies for 30 min
11. Washed with FACS wash buffer
12. Incubated with BD ctoflix fixation
13. Cell washed twice with BD buffer
14. Incubated with fluorescent–labeled anti bodies for 1 h to detect intracellular cytokine expression

# PBMC cell stimulation




# Cellular response results

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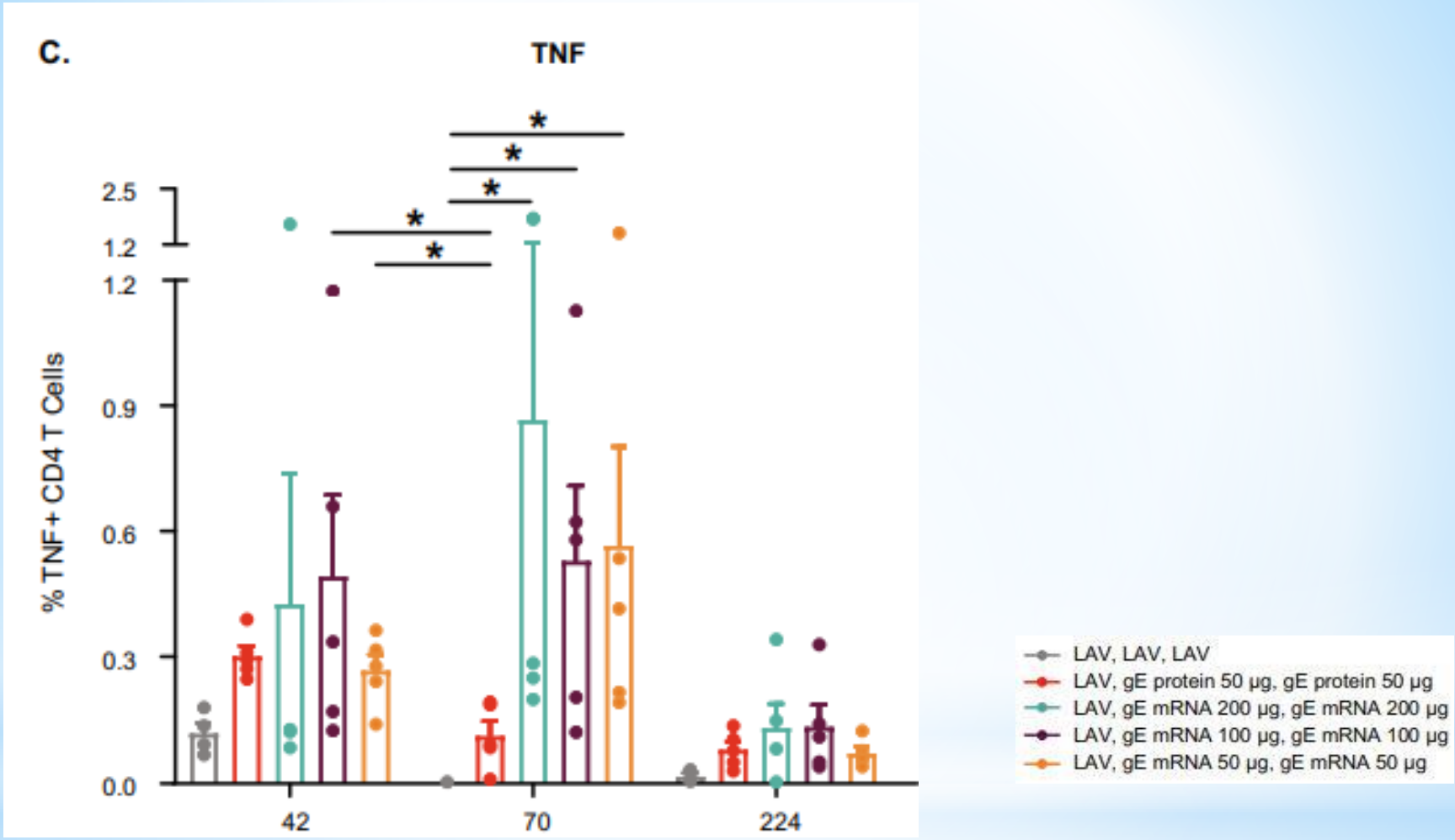
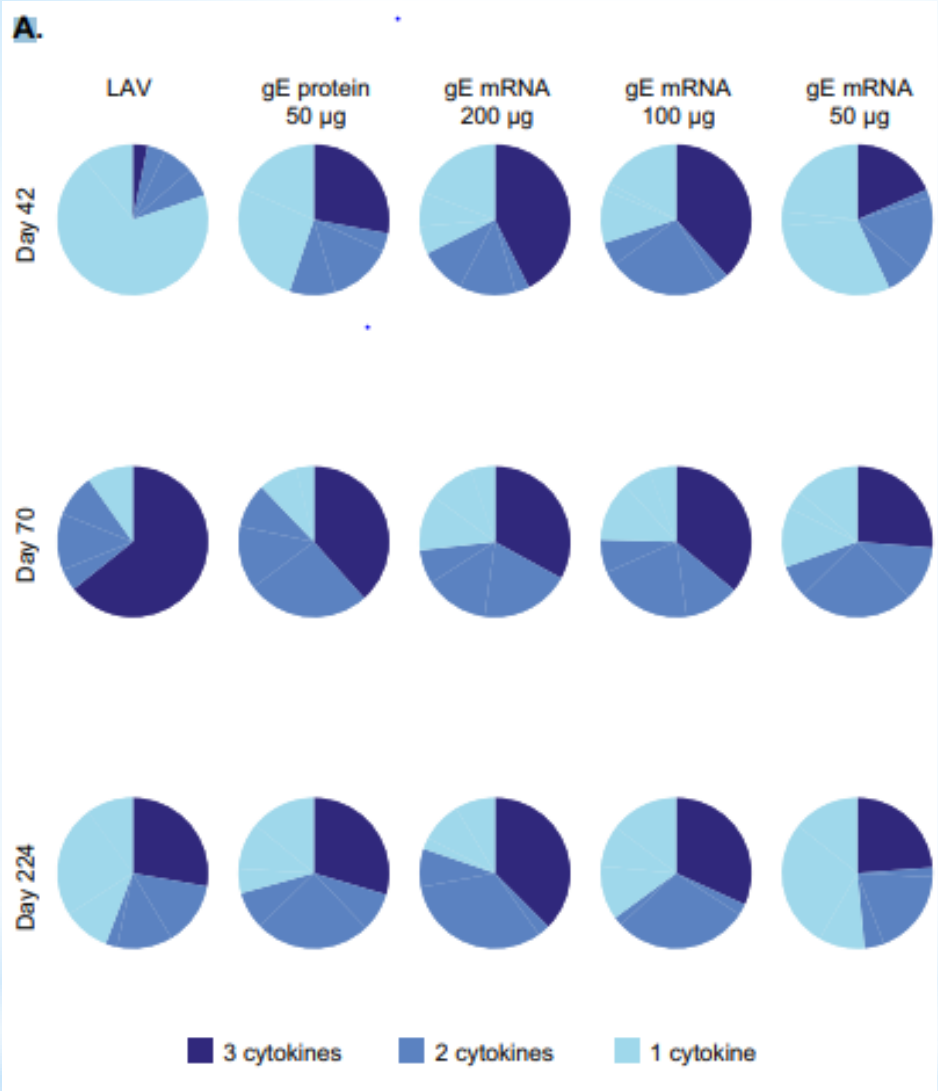
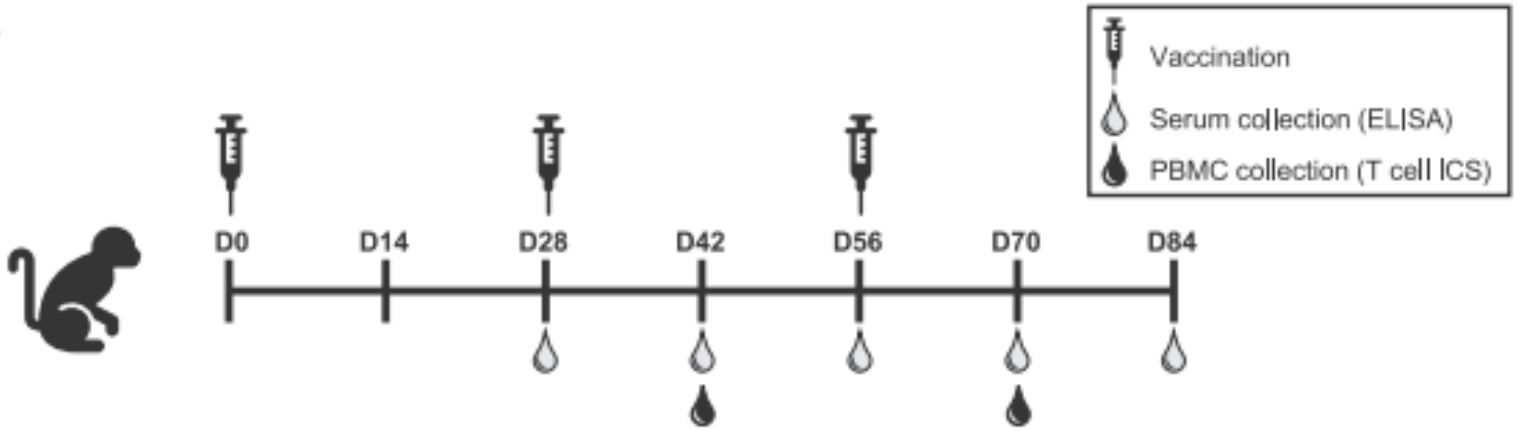



# Cellular response results




# Combinatorial analyses

A.




B.

| Group | # of Animals | Study 2 Vaccinations | | |
|---|---|---|---|---|
| | | Day 0 | Day 28 | Day 56 |
| 1 | 5 | Live, attenuated VZV ≥19,400 pfu | VZV gE mRNA/LNP 400 µg | VZV gE mRNA/LNP 400 µg |
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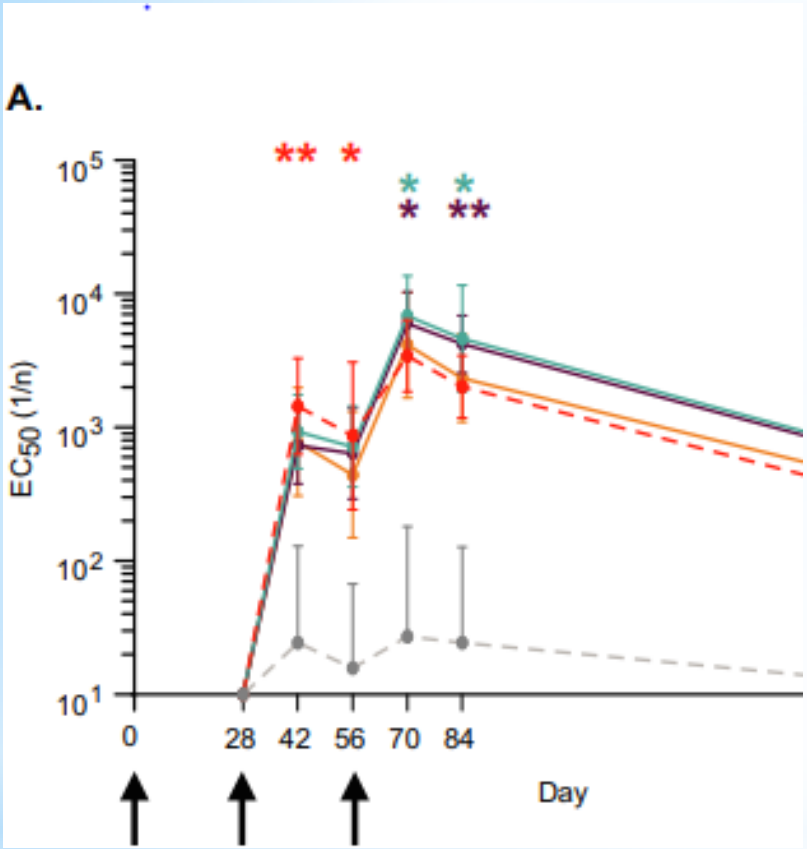
| 3 | 5 | Live, attenuated VZV ≥19,400 pfu | VZV gE mRNA/LNP 100 µg | VZV gE mRNA/LNP 100 µg |

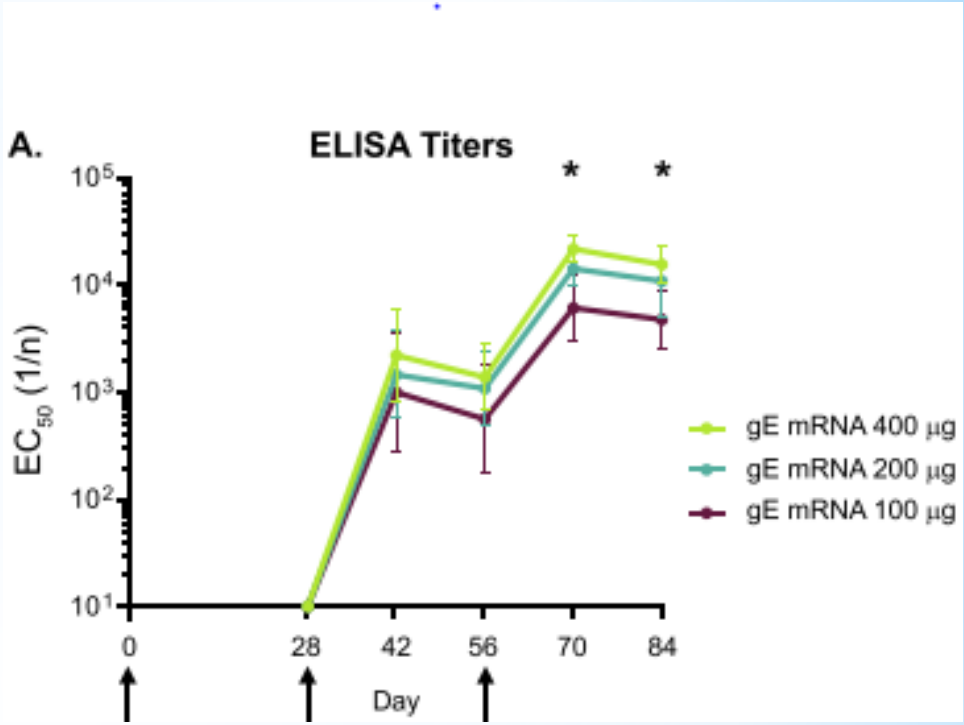
Second study: three groups of 4-5 male and female monkeys

1. Were immunized three times at 28 days intervals
2. Once with VZV LAV followed by two immunization with 100-400 μg of mRNA vaccine
3. Collecting serum and peripheral blood mononuclear cell(PBMC) 14 days post vaccination
4. All samples were cryopreserved

# Animal study 2

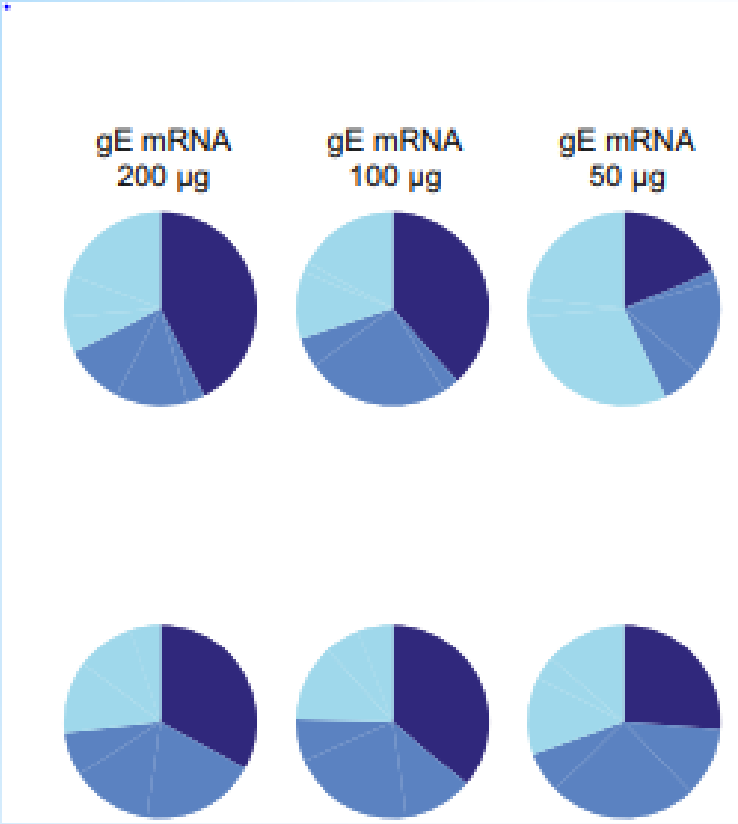



Study 1




Study 2

# Comparinghumoral responses results

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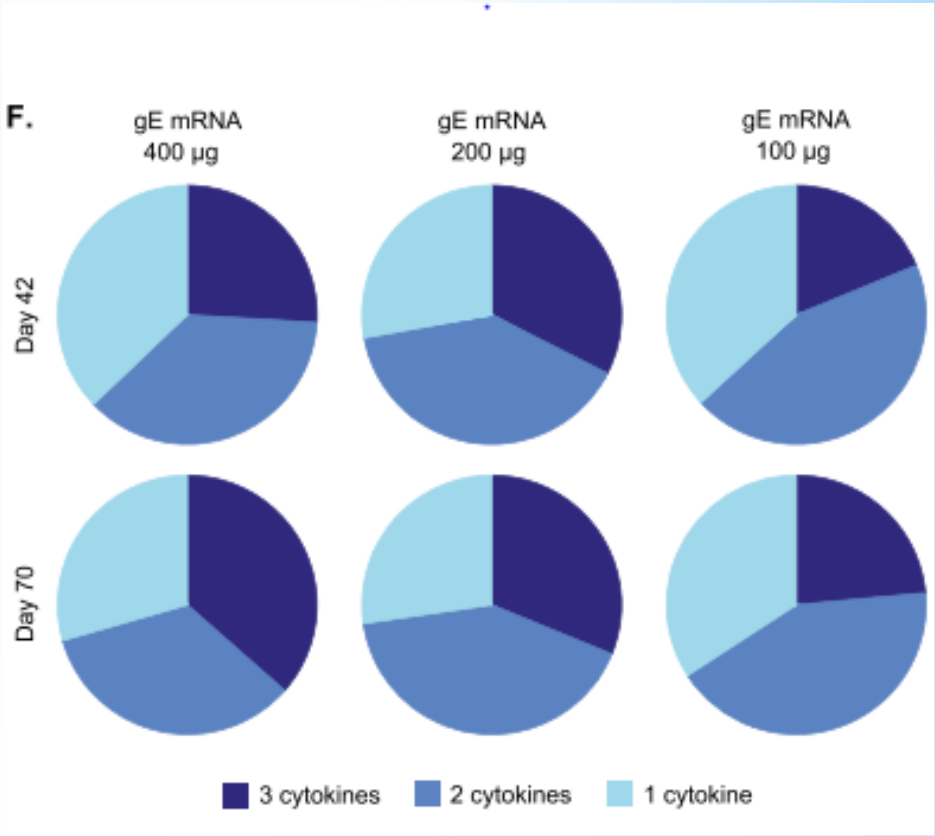
Study1




Study 2

# Comparing combinatorial analyses

- ✓ Easier to manufactures:
  - No need to purify virus
  - No need to express protein
  - No need to formulation adjuvants
- ✓ Virus-like immune stimulation without potential risk of virul replication
- ✓ Efficient stimulation of CD8 T cell with peptid presentation on MHC class 1
- ✓ LNP formulated mRNA tolerability profile

# mRNA vaccine advantages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