



UNIVERSIDADE  
DE SÃO PAULO

**85 ANOS**  
1934 · 2019



# Vacina Genética para prevenção de SARS-CoV-2

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Departamento de Genética  
USP Ribeirão Preto-FMRP

# Roteiro:

## Conceitos Gerais

1. Vacinas e objetivos
2. Vacina de mRNA
3. Sistemas de entrega da molécula mRNA

## Moderna

4. Vacina de mRNA que codifica proteína spike

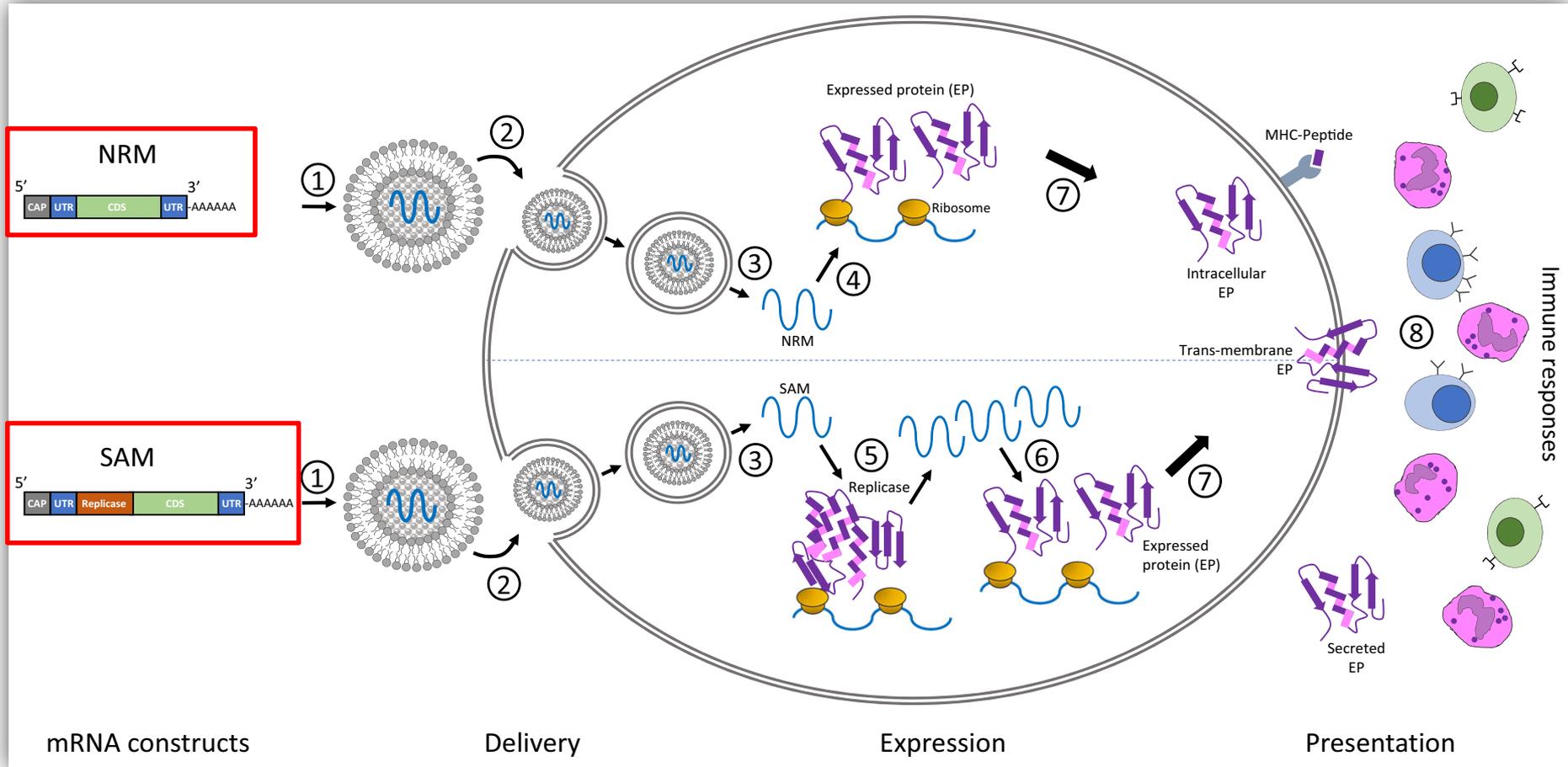
## Pfizer

5. Vacina de mRNA que codifica proteína spike

6. Perspectivas: vacinas mRNA

# 1. Vacinação e Objetivos do desenvolvimento de vacinas

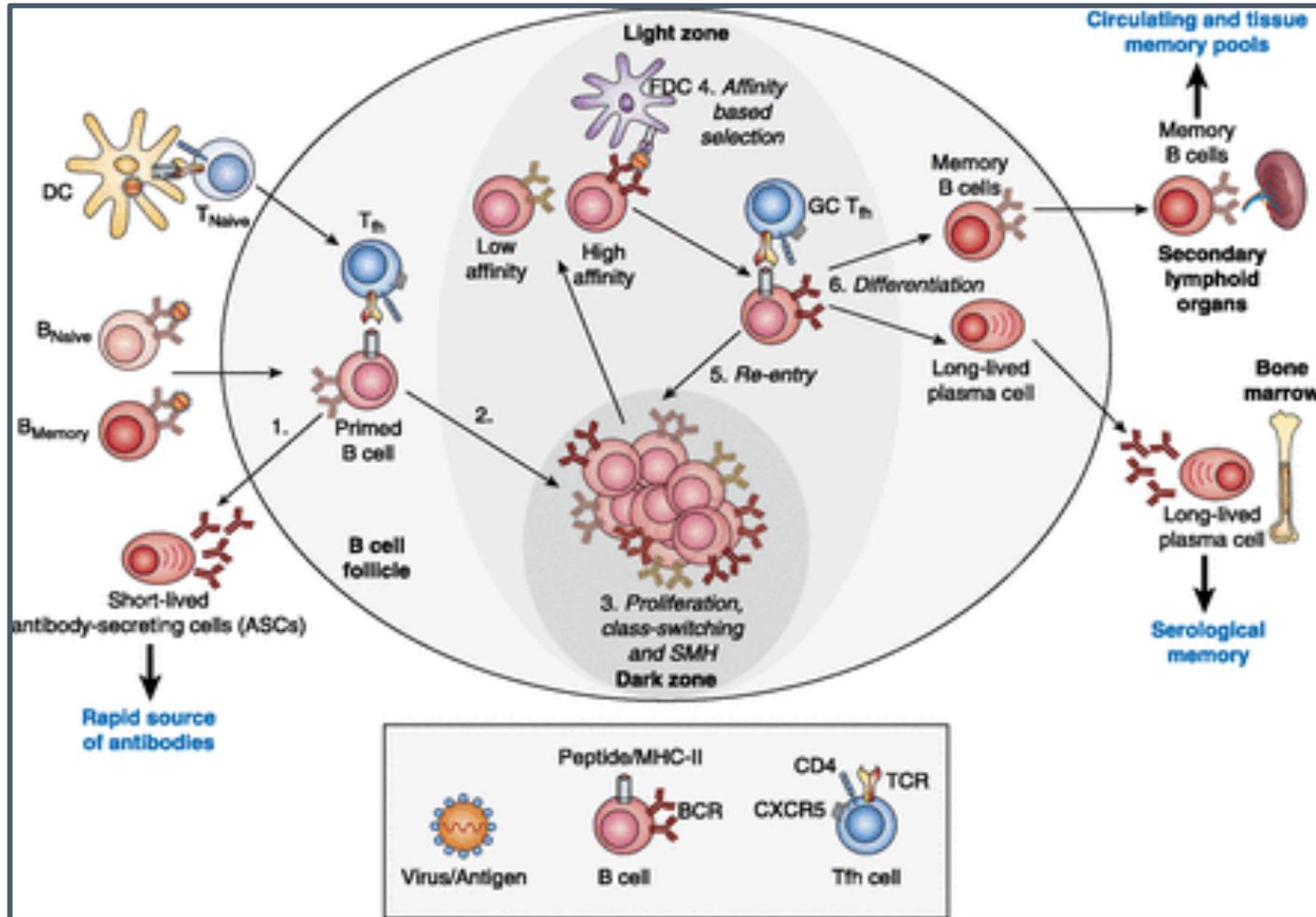
## 2 Tipos de vacina de mRNA:



# 1. Vacinação e Objetivos do desenvolvimento de vacinas

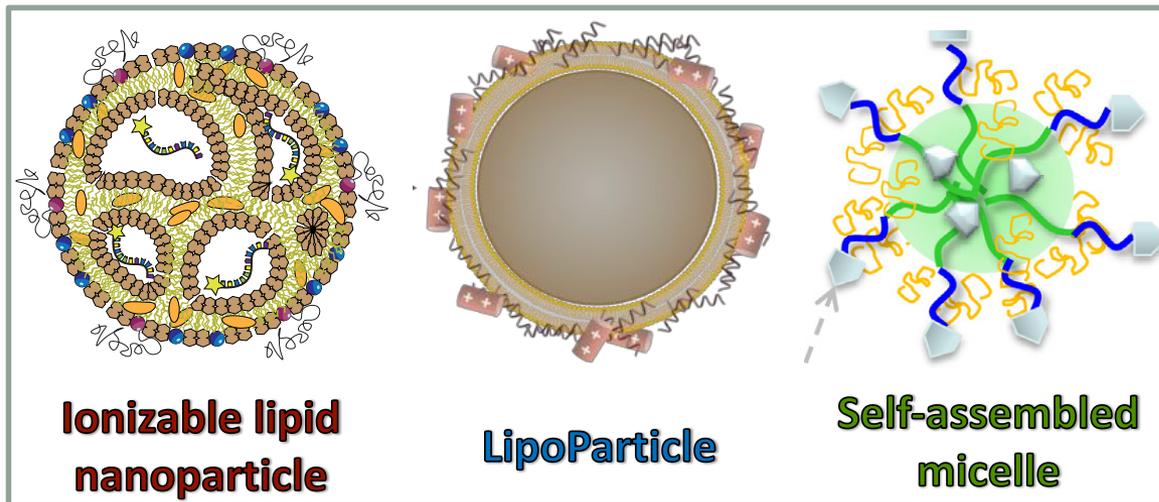
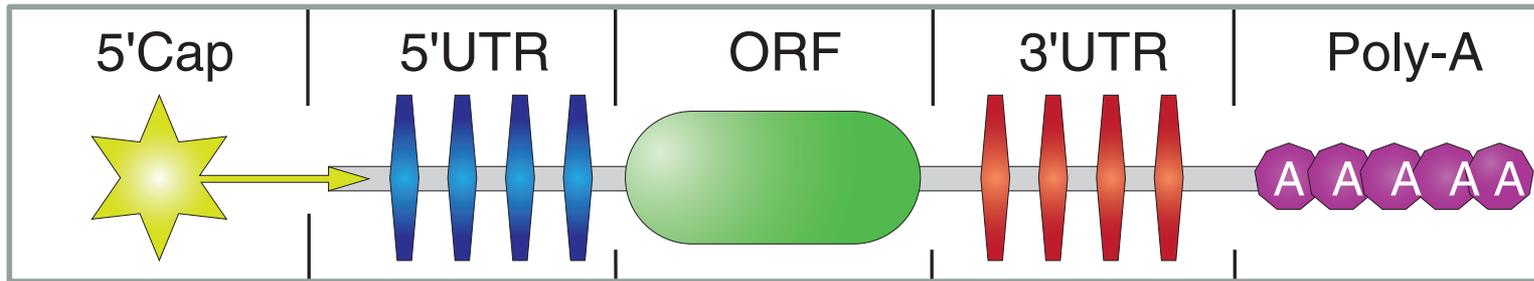
## Como induzir a imunidade duradoura?

Doria-Rose N, Suthar MS, Makowski M, et al. 2021. Antibody persistence through 6 months after the second dose of mRNA-1273 vaccine for Covid-19. *N. Engl. J. Med.* 384:2259–61



## 2. Vacina de mRNA

### Componentes principais da vacina de mRNA:



## 2. Vacina de mRNA

**Molécula de mRNA: estratégias para aumentar a eficiência da tradução**

**5' Cap**

**5' UTR**

**CDS otimizada**

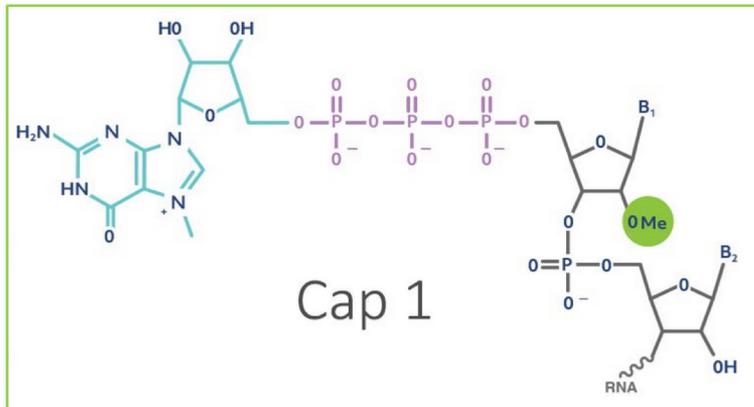
**3' UTR**

**Imunogenicidade**

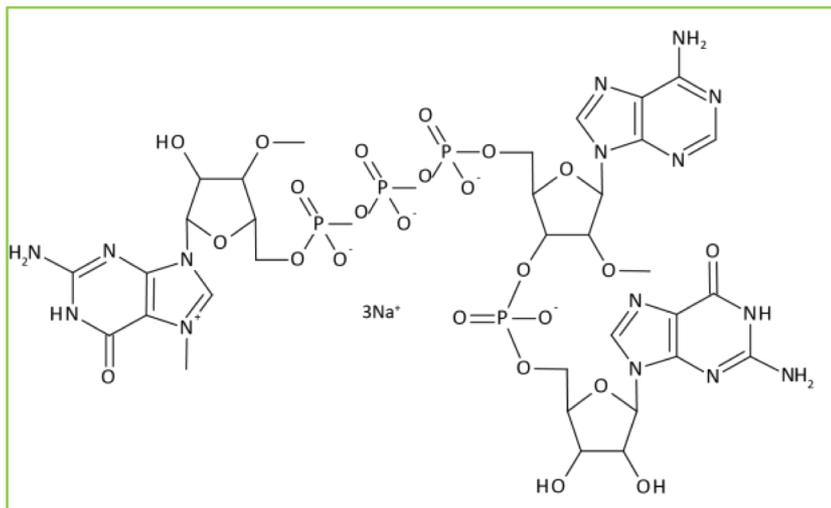
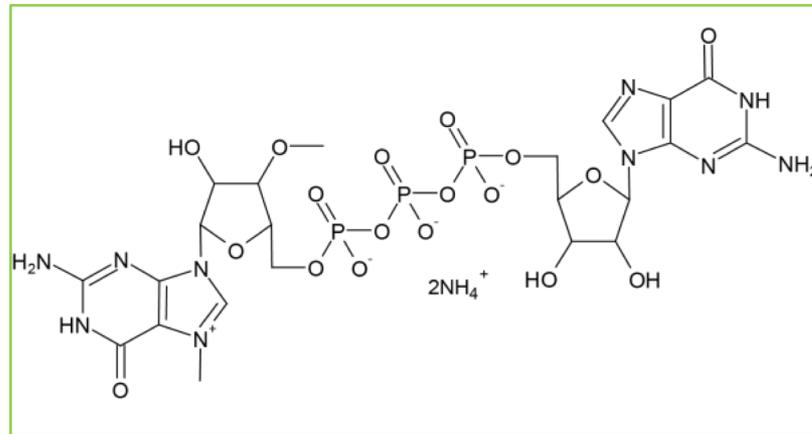
**Nucleosídeos modificados**

# 2. Vacina de mRNA

## 5'Cap:



1ª geração



mRNAs *uncapped* ou *capped* incorretamente podem ser reconhecidos pelos PRRs (ex. RIG-1 e IFIT), induzir interferon tipo 1 e bloquear a tradução do mRNA.

## 2. Vacina de mRNA

### Imunogenicidade:

O sistema imunológico pode reconhecer *motifs* denominados PAMPs (padrões moleculares associados com patógenos) pelos receptores de reconhecimento de padrão (PRRs).

Esses receptores são expressos em elevada quantidade nas células apresentadoras de antígenos (células dendríticas) presentes no endossomo ou citoplasma.

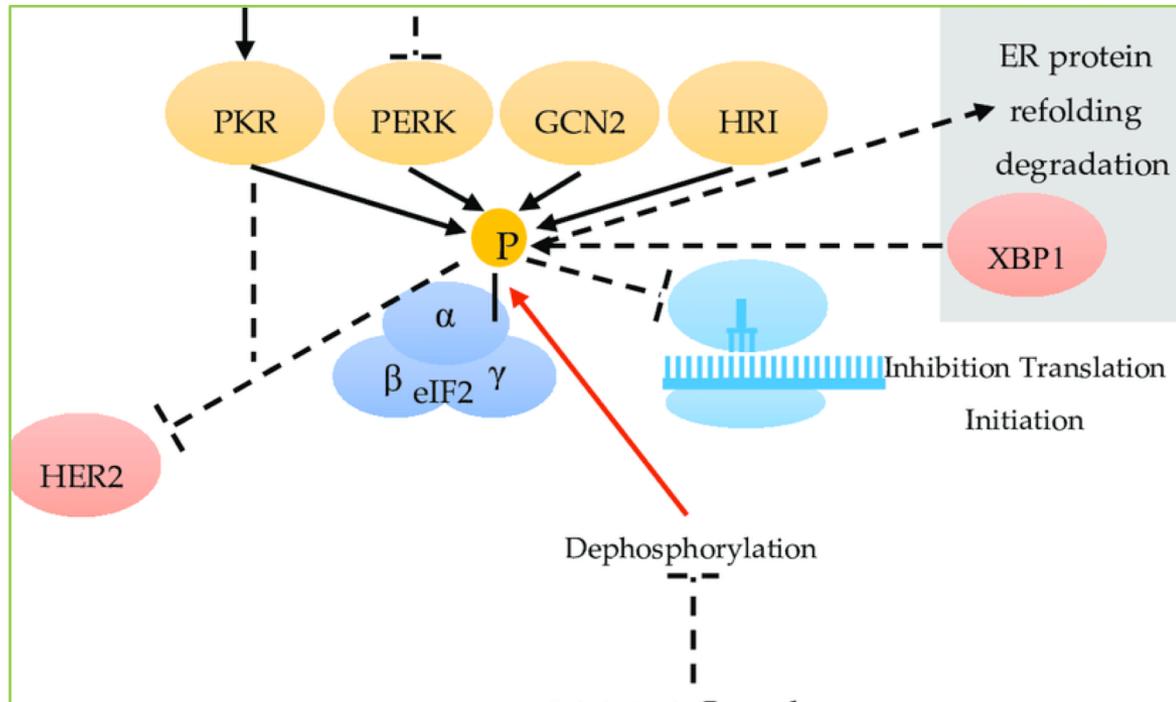
Receptores presentes no endossoma: TLR-7 e TLR-8 que ativam MyD88, interferon tipo-1 e a secreção de citocinas inflamatórias.

Receptores presentes no citoplasma: RIG-I-like (MDA-5) e OAS (*oligoadenylate synthetase*) e enzima *kinase* dependente de RNA (PKR).

## 2. Vacina de mRNA

### Imunogenicidade:

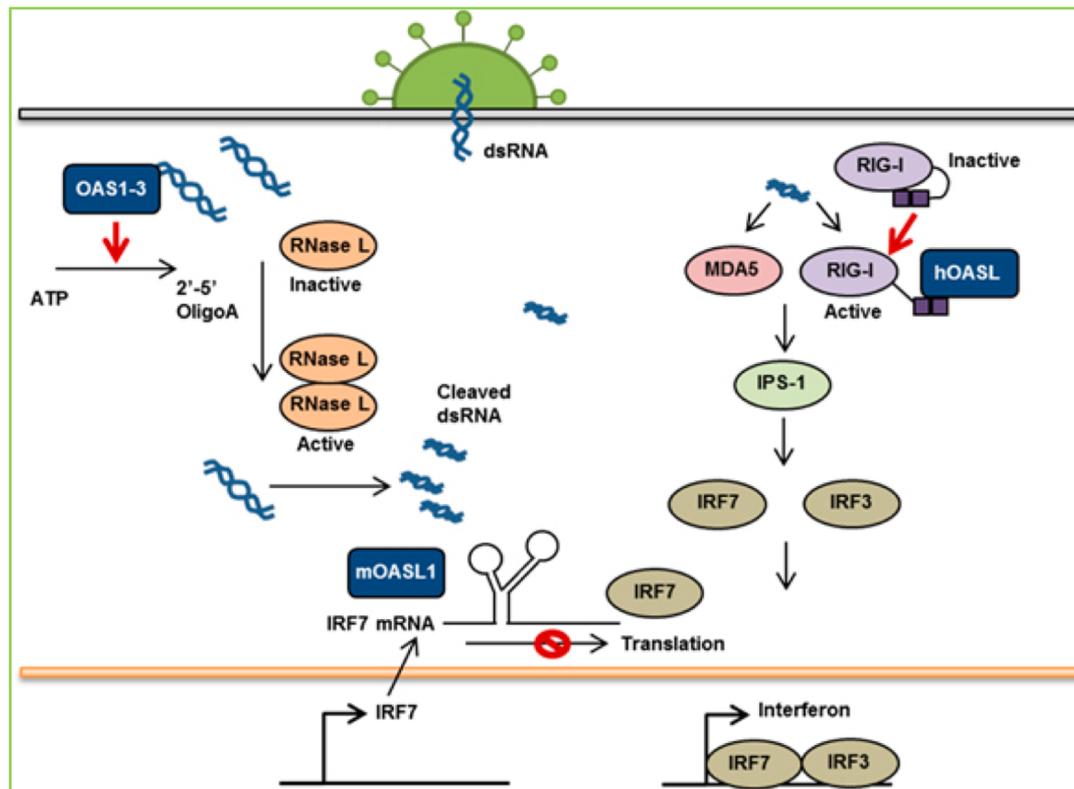
Se PKR for ativada, o fator eucariótico de iniciação da tradução (eIF-2) pode ser fosforilado e bloquear a tradução do mRNA.



## 2. Vacina de mRNA

### Imunogenicidade:

dsRNA pode atrair a ligação de OAS que pode ativar a enzima RNase L e levar a degradação do mRNA



## 2. Vacina de mRNA

### 5'UTR:

Otimização da região 5'UTR para aumentar a eficiência da tradução

Evitar a presença de AUG ou codon de iniciação não canônico (CUG) na região 5'UTR que podem diminuir a tradução da região ORF.

Evitar a presença de estruturas altamente estáveis na região 5'UTR que podem prevenir o recrutamento do ribossomo.

5'UTR de  $\alpha$ -globina e  $\beta$ -globina de *Xenopus laevis* ou *Homo sapiens* são muito utilizados.

## 2. Vacina de mRNA

### Otimização de códons:

Otimizar o conteúdo de C e G na região “*open reading frame*” (ORF) para aumentar a taxa de síntese durante a tradução do mRNA

Adicionar os codons presentes em maiores quantidades encontrados naturalmente na célula-alvo.

Adicionar codons com abundância de tRNA.

Evitar estruturas altamente estáveis na região ORF.

## 2. Vacina de mRNA

### Cauda poli-A:

Em geral, mRNA de células dendríticas derivadas de monócitos apresentam entre 120 e 150 nt, enquanto mRNAs de linfócitos T apresentam 300 nt em comprimento.

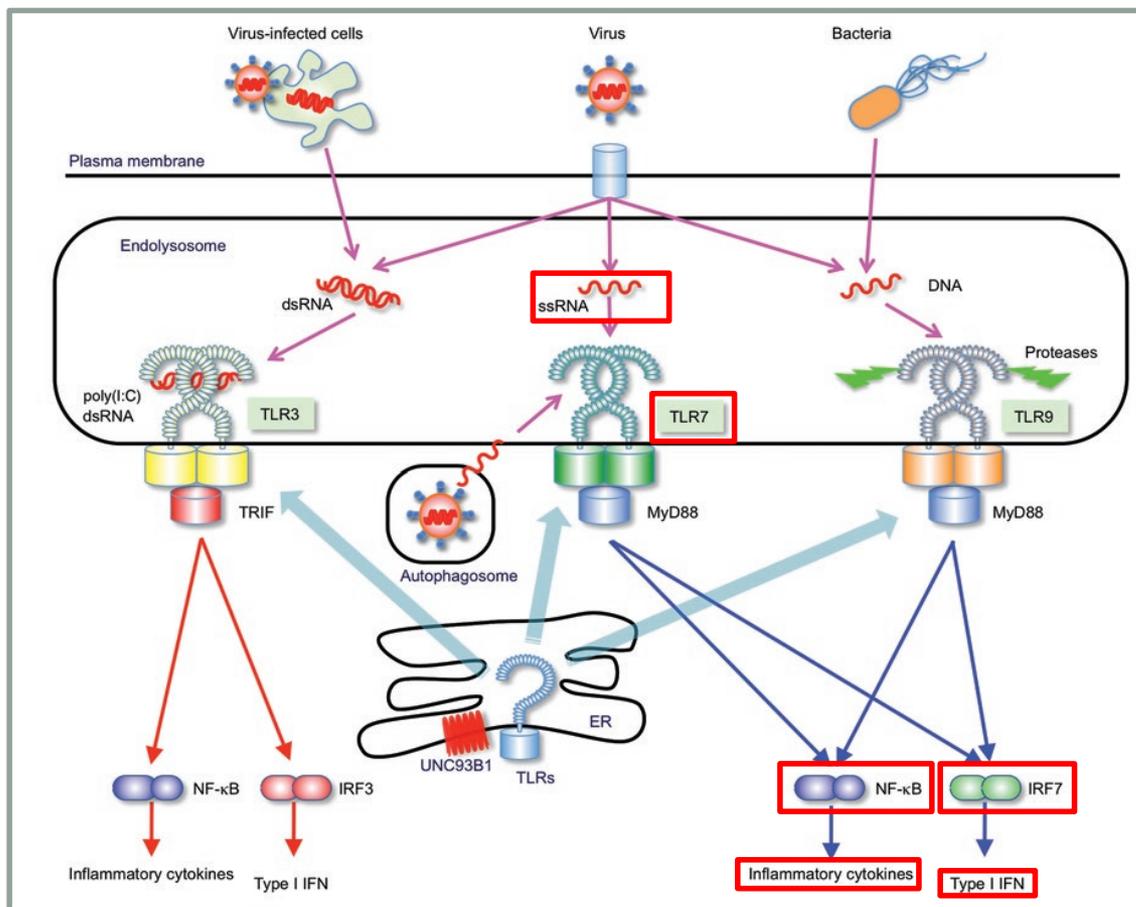
A proteína ligante de cauda poliA (PABP) pode interagir com a região 5' *cap* e promover uma estrutura que permitirá uma maior eficiência da tradução.

São necessários estudos adicionais para a compreensão da cinética do tamanho da cauda poli-A a nível de tradução do mRNA.

## 2. Vacina de mRNA

### Nucleosídeos modificados:

Receptores do tipo Toll reconhecem estruturas conservadas nos patógenos e ativam genes alvos da resposta inata e adaptativa.



Vírus de RNA de fita simples ou fita-dupla reconhecem os receptores do tipo *Toll*, TLR7 e TLR3, respectivamente.

TLR7 ativados induzem citocinas inflamatórias e interferon tipo I.

# 2. Vacina de mRNA

## Marco histórico: modificações químicas nos nucleosídeos de mRNA



**Suppression of RNA Recognition by Toll-like Receptors: The Impact of Nucleoside Modification and the Evolutionary Origin of RNA**

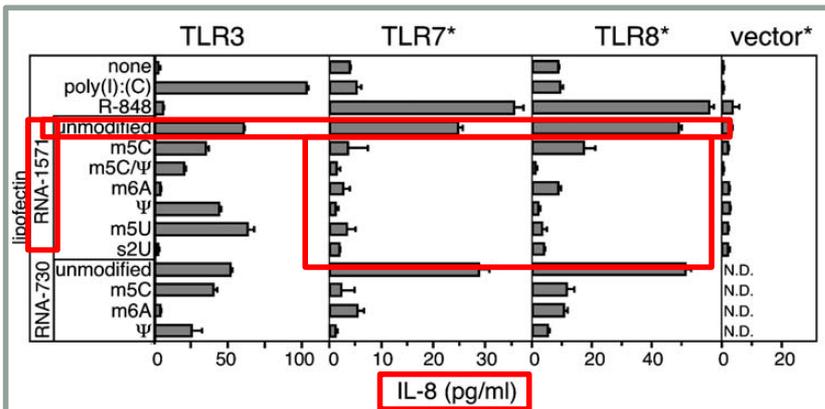
Immunity, Vol. 23, 165–175

Katalin Karikó,<sup>1,\*</sup> Michael Buckstein,<sup>2</sup> Houping Ni,<sup>2</sup> and Drew Weissman<sup>2</sup>

Drew Weissman e Katalin Karikó

**Table 1. TLR Recognition of Microbial Components**

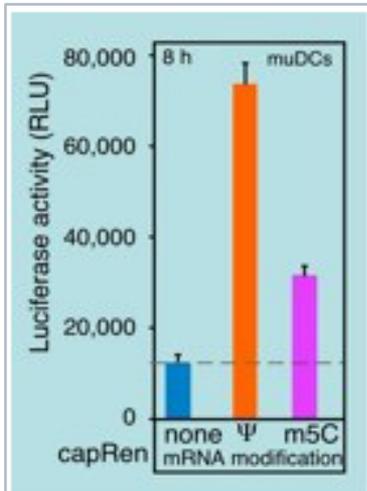
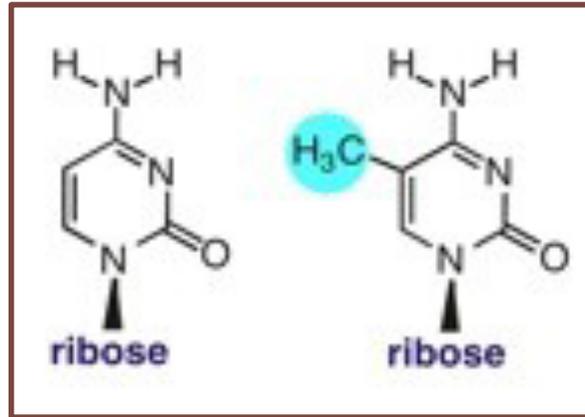
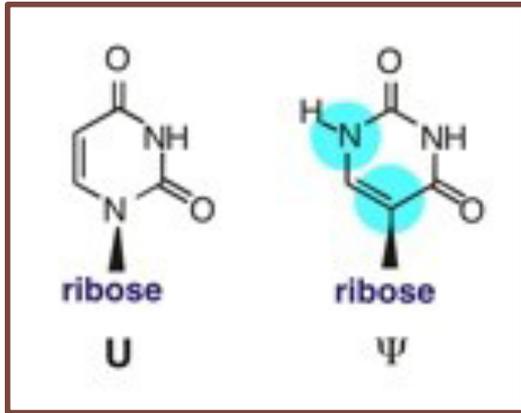
Microbial Components	Species	TLR Usage
Viruses		
DNA	Viruses	TLR9
dsRNA	Viruses	TLR3
ssRNA	RNA viruses	TLR7 and TLR8
Envelope proteins	RSV, MMTV	TLR4
Hemagglutinin protein	Measles virus	TLR2



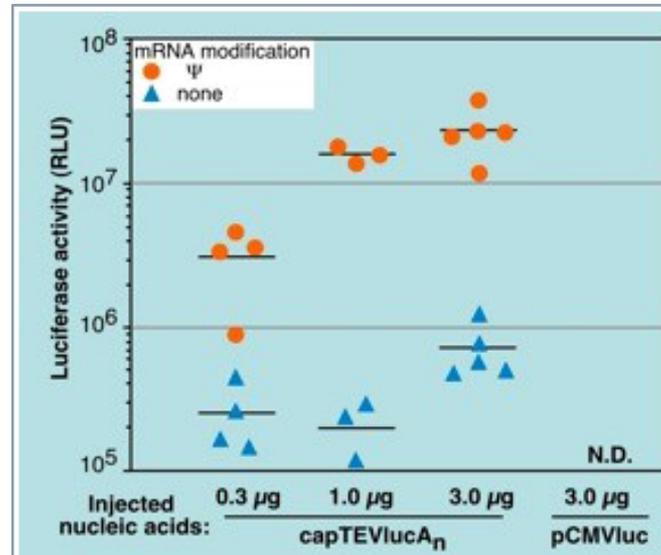
Células 293 expressando receptores do tipo *Toll* (TLR), simulando células do sistema imune que apresentam esses receptores, reconhecem e respondem a ácidos nucleicos. A ativação desses receptores levam a produção de interferon. A síntese de mRNA na presença de nucleotídeos quimicamente modificados modulam o potencial imunoestimulatório do RNA.

# 2. Vacina de mRNA

**Modificações:** pseudouridina e 5-metilcitosina



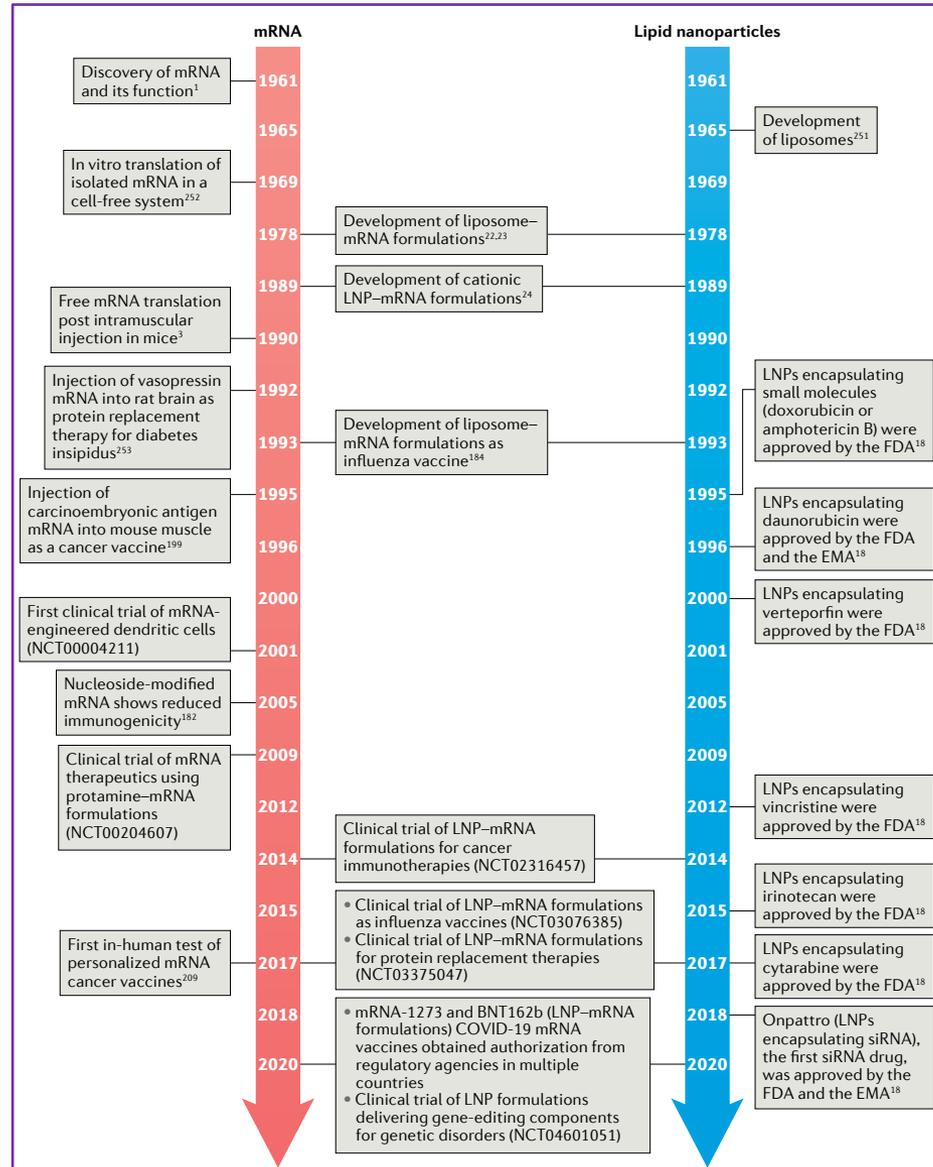
expressão *in vitro*



expressão *in vivo*

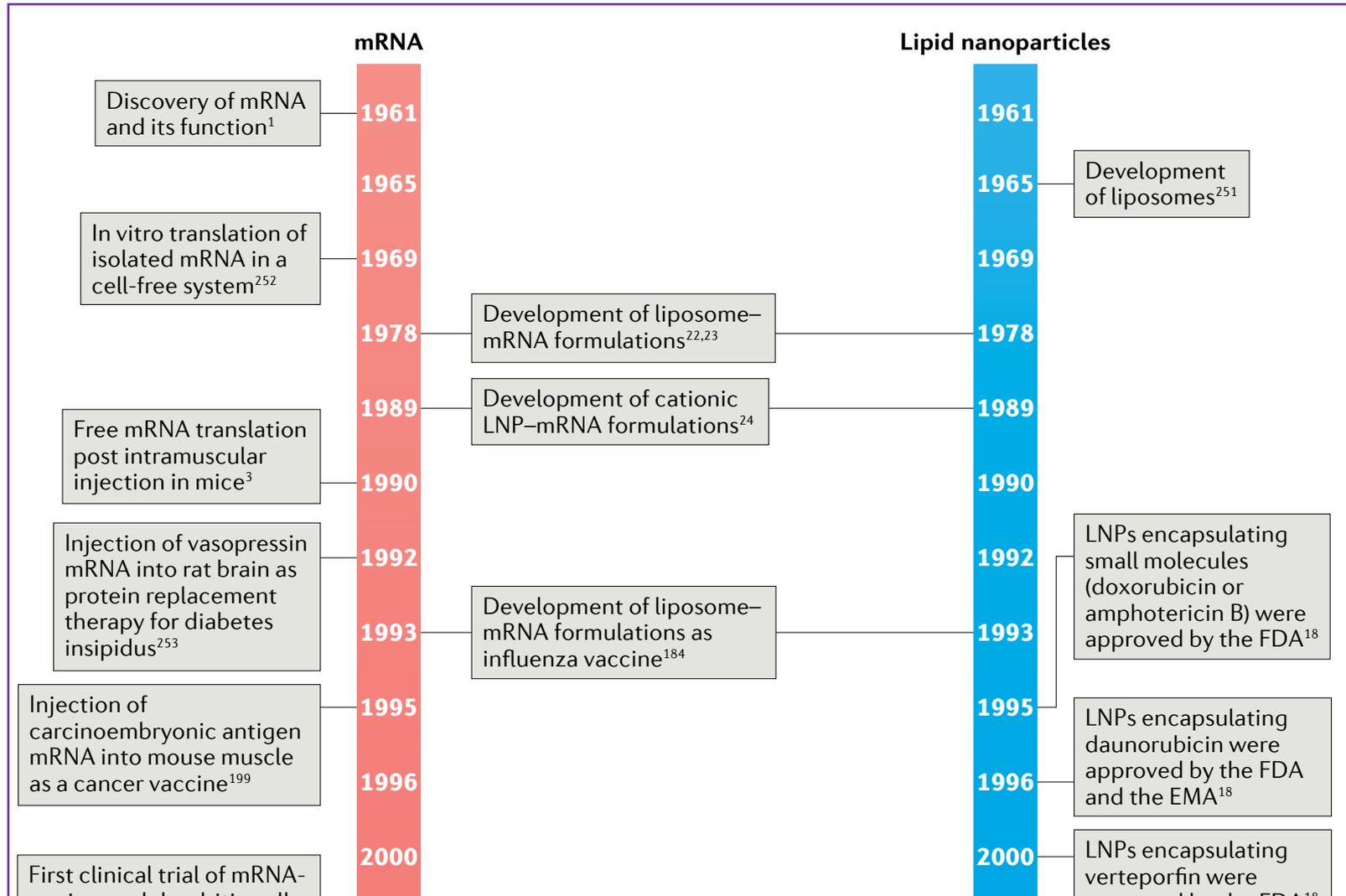
# 3. Sistema de entrega da molécula de mRNA

## Nanopartícula lipídica:



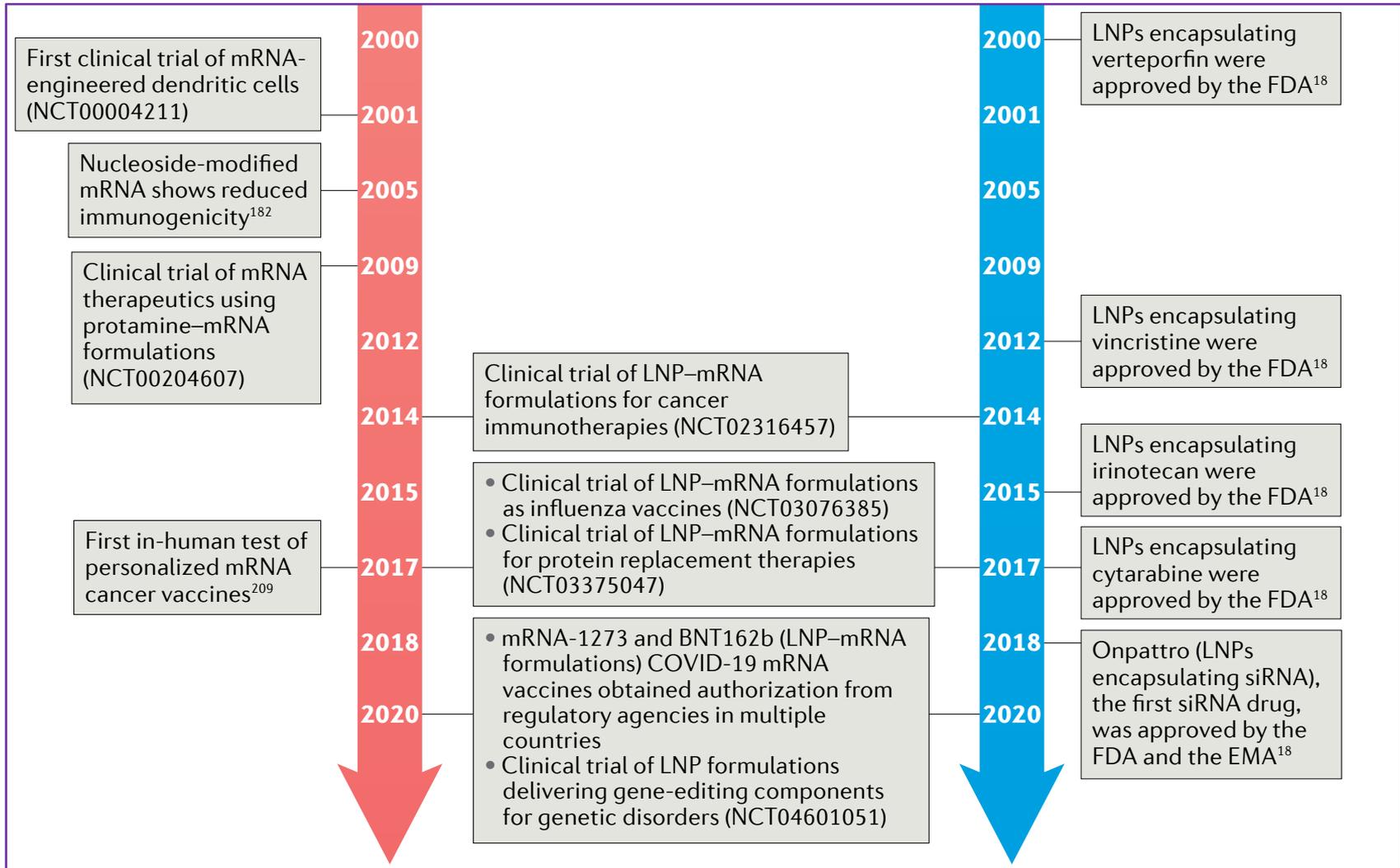
# 3. Sistema de entrega da molécula de mRNA

## Nanopartícula lipídica:



# 3. Sistema de entrega da molécula de mRNA

## Nanopartícula lipídica:



# 3. Sistema de entrega da molécula de mRNA

## Nanopartícula lipídica: vacinas de mRNA anti-COVID-19

### 4 moléculas lipídicas:

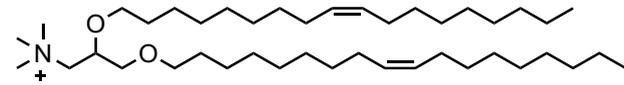
1. Lipídeo catiônico

2. Lipídeo ionizável

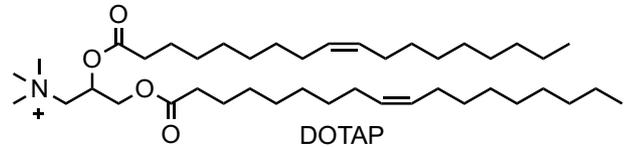
3. Fosfolipídeo

4. Colesterol

#### Cationic lipids

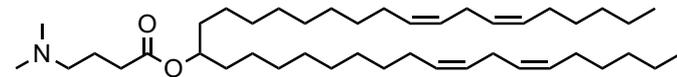


DOTMA

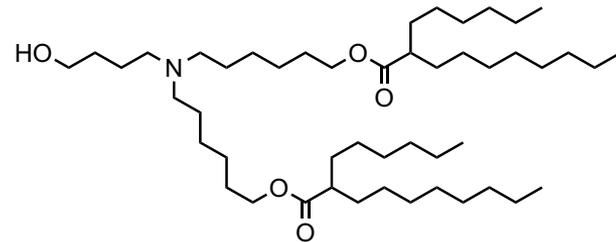


DOTAP

#### Ionizable lipids



DLin-MC3-DMA



ALC-0315

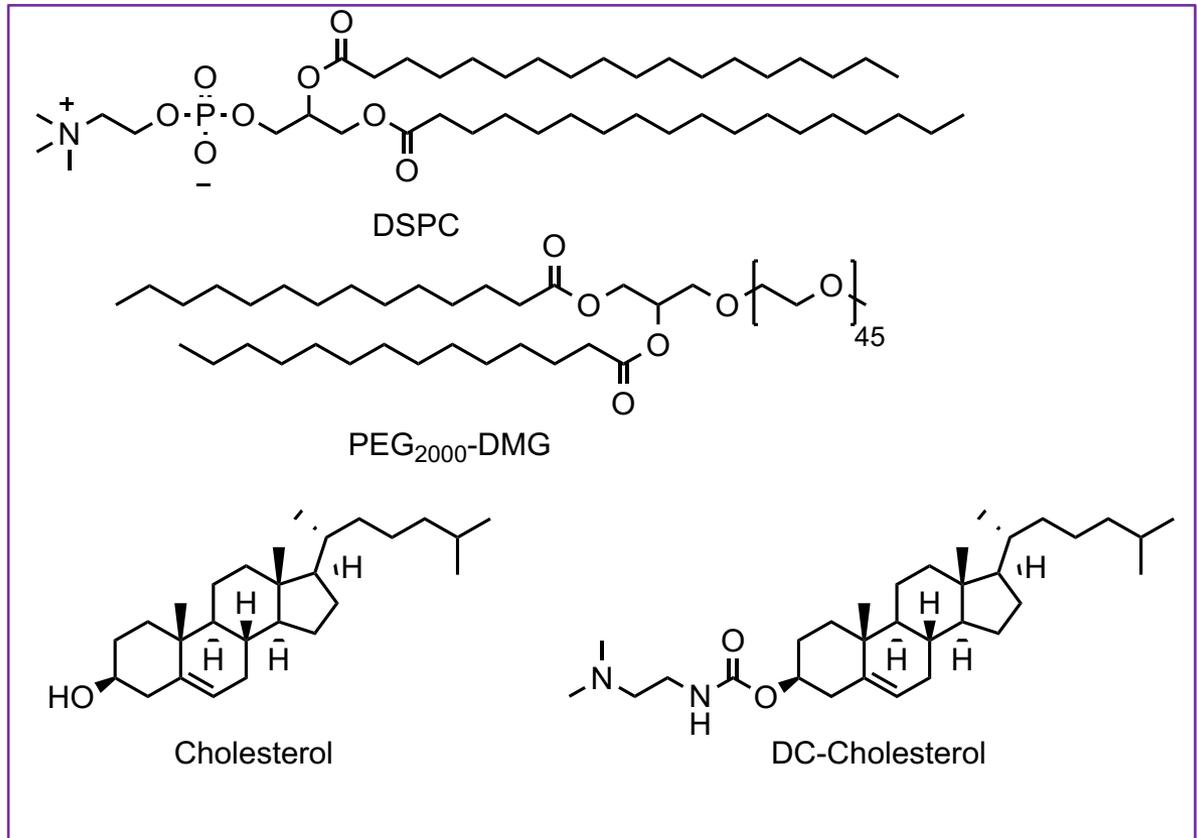
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## Nanopartícula lipídica: vacinas de mRNA anti-COVID-19

### 4 moléculas lipídicas:

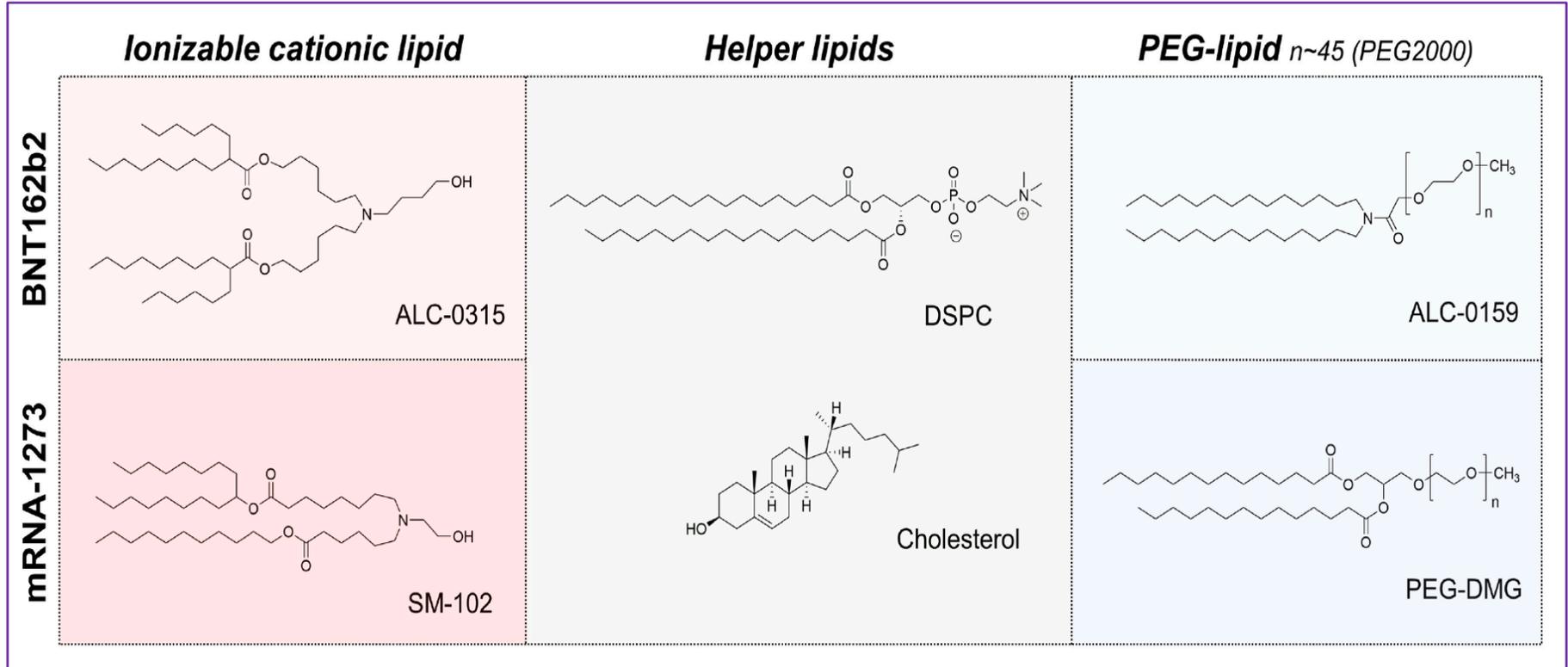
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#### 4. Colesterol



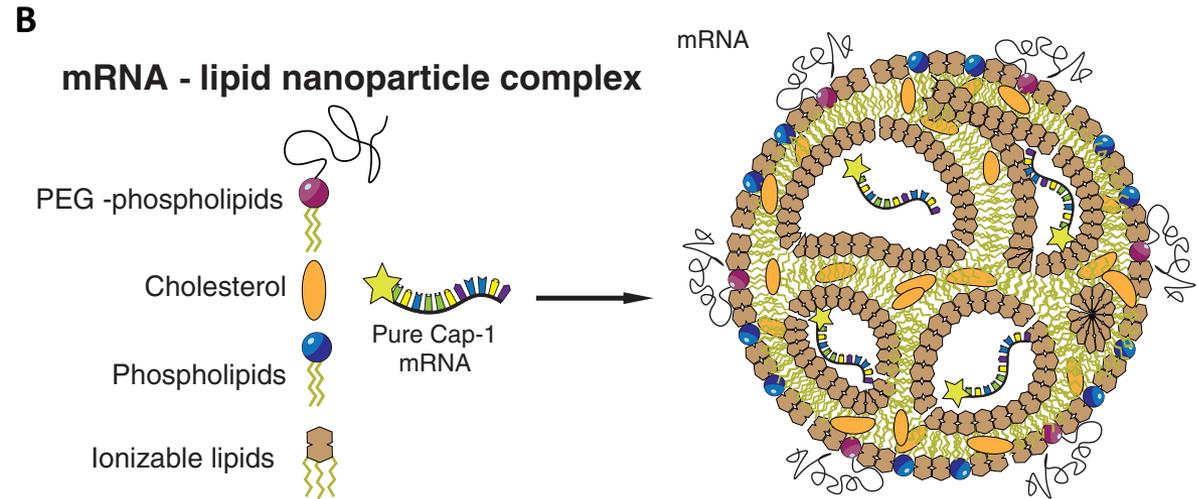
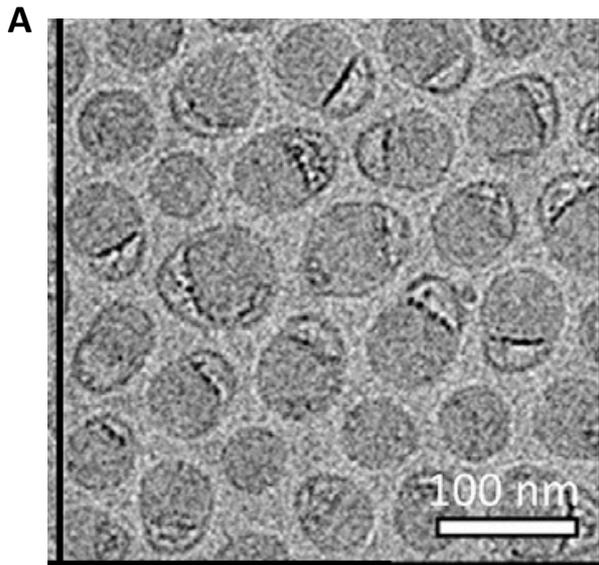
# 3. Sistema de entrega da molécula de mRNA

## Nanopartícula lipídica: vacinas de mRNA anti-COVID-19



# 3. Sistema de entrega da molécula de mRNA

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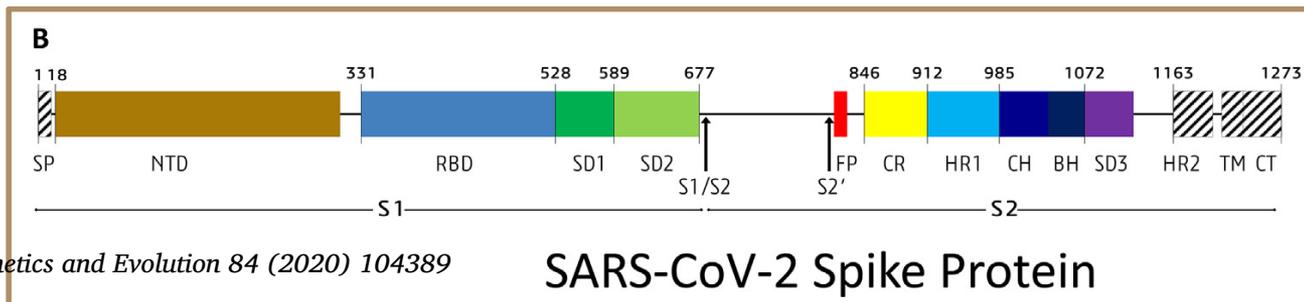
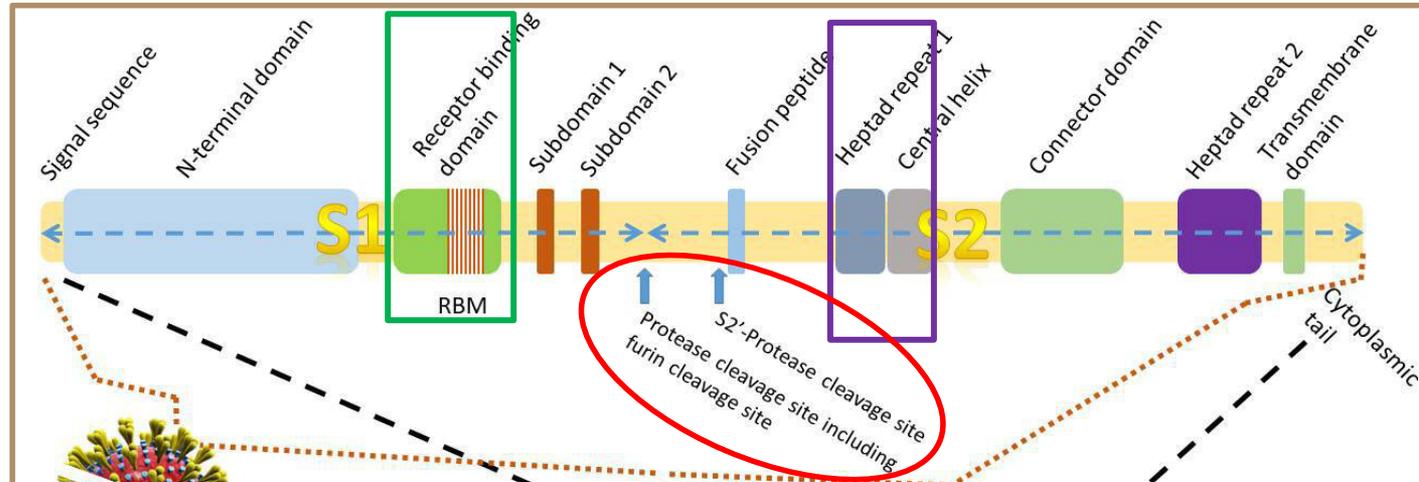


# 4. mRNA and Protein Spike

Gene of the month

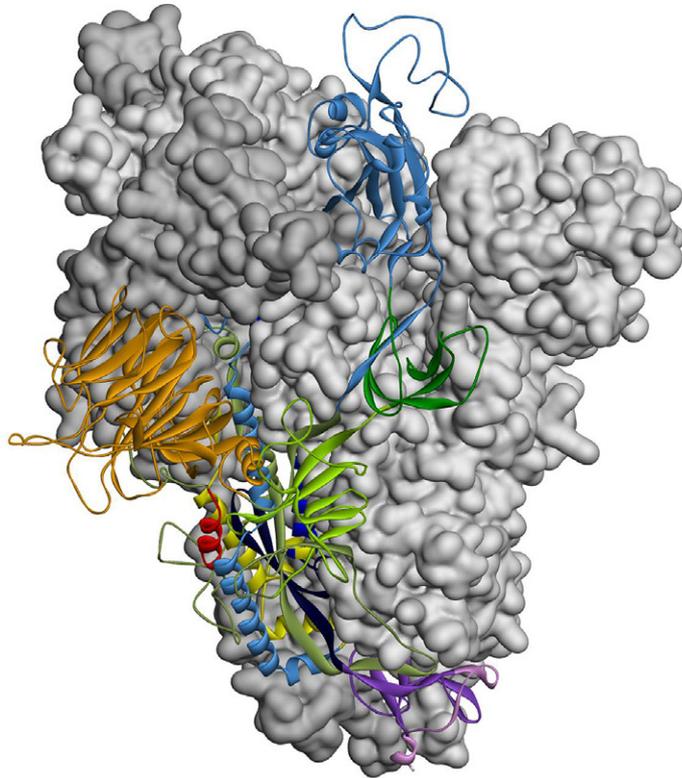
Gene of the month: the 2019-nCoV/SARS-CoV-2 novel coronavirus spike protein

Pillay TS. *J Clin Pathol* 2020;**73**:366–369.

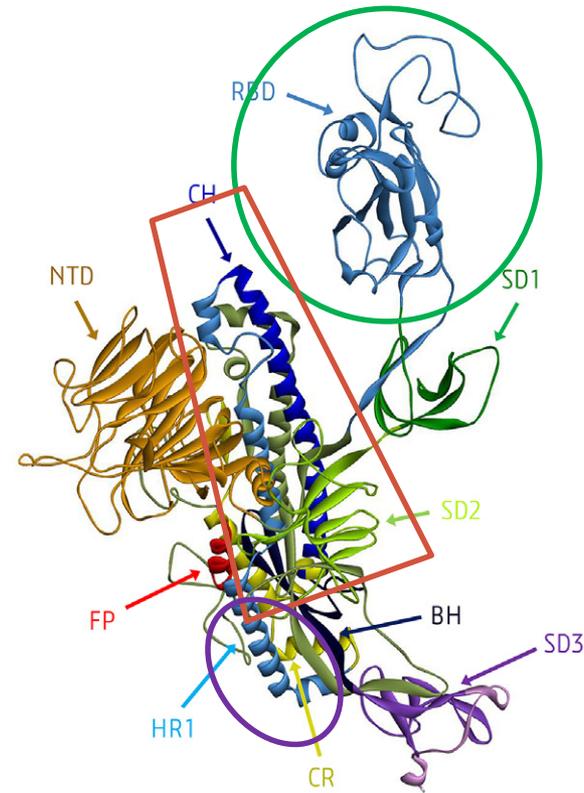


Proteína transmembranaral do tipo I, semelhante a proteína ENV do HIV-1, apresenta dois domínios **S1** e **S2** e dois sítios de clivagem no domínio S2.

# 4. mRNA and Protein Spike



Homology Model of  
SARS-CoV-2 Spike Protein Trimer



Ribbon diagram of  
SARS-CoV-2 Spike Protein Monomer

# 4. mRNA and Protein Spike

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1 M F V F L V L L P L V S S Q C V N L T T
1 ATGTTTGTTTTTCTGTTTTATGTGCCACTAGTCTCTAGTCAGTGTGTTAATCTTACAACC
21 R T Q L P P A Y T N S F T R G V Y Y P D
61 AGAAGCTCAATTACCOCCTGCATACACATAATTTCTTACACGGTGGTGTATTTACCCGTGAC
41 K V F R S S V L H S T Q D L F L P P F S
121 AAAGTTTTCAGATCCTCAGTTTACATTCACCTCAGAGCTTGTCTTACCTTCTCTTTTCC
161 N V T W F H A I H V S G T N G T K R F D
181 AATGTTACTTGGTCCATGCTATACATGCTCTGGGACCAATGGTACTAAGAGGTTTGTAT
81 N P V L P F N D G Y F A S T E K S N I
241 AACCTGTCTACCAATTAATGATGGTGTATTTTGTCTCCACTGAGAAGTCTAACATA
101 I R G W I F G T T L D S K T Q S L L V I
301 ATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGACCCAGTCCCTACTTATTTGTT
121 N N A T N V V I K V C E F Q F C N D P F
361 AATAACGCTACTAATGTTGTTTAAAGTCTGTGAATTTCAATTTGTAATGATCCATTT
141 L G V Y T H K N N K S W M E S E F R V Y
421 TTGGGTGTTTTATACCACAAAACAACAAAAGTTGGATGGAAGTGAGTTCAGAGTTTTAT
161 S S A N N C T F E Y V S Q P F T L M D L E
481 TCTAGTGGCAATAATGCCTTTGAATATGCTCTCAGCCCTTTCTTATGGACCTTGAA
181 G K Q G N F K N L R E F V F K N I D G Y
541 GGAAAACAGGGTAAATTCAAAATCTTAGGGAATTTGTGTTTAAAGAAATTTGATGGTTAT
201 F K I Y S K H T P I N L V R D L P Q G G F
601 TTTAAAATATATTCAAGCACGCCCTATTAATTTAGTGGCTGATCTCCCTCAGGTTTT
221 S A L E P L V D L P I G I N I T R F Q T
661 TCGCCTTTAGAACCATTGCTAGATTTGCAATAGTATTAACACTCACTAGTTTTCAAACT
241 L L A L H R S Y L T P G D S S G W T A
721 TTACTTGGTTTACATAGAGATTTAGACTCCTGGTGTATCTTCTCAGGTTGGACAGCT
261 G A A A Y Y V G Y L P Q P R T F L L K Y N
781 GGTGCTCAGCTTATTTAGTGGTGTATCTCAACCTAGGACTTTTCTAATAAATATAAT
841 N G C T I T D A V D C A L D P L S E T K
841 GAAAATGGAACCATTACAGATGCTGTAGACTGTGCACCTTGACCCCTCTCAGAAAACAAAG
301 C T L K S F T V E K G I Y Q T S N F R V
901 TGTACGTTGAAATCCTTCACTGTAGAAAAGGAATCTATCAAACTTCAACTTTAGAGTC
321 Q P T E S I V R F P N I T N L C P F G E
961 CAACCAACAGAACTATTGTTAGATTTCCCTAATATACAACTGTGCCCCTTTGGTGAA
341 V F N A C T R F A S V Y A W N R K R I S N
1021 GTTTTTAAGCCACAGATTTGCATCTGTTTTATGCTTGGACAGGAAGAGAATCAGCAAC
361 C V A D Y S V L Y N S A S F S T P K C Y
1081 TGTGTTGCTGATTTCTGCTCCTATATAATCCCGATCAATTTTCCACTTTTTAAGTGTTAT
381 G V S P T K L N D L C F T N V Y A D S F
1141 GGAGTGTCTTACTAAATTAATGATCTCTGCTTTACTAATGCTCATGTCAGATTCATTT
401 V I R G D E V R Q I A P G Q T G K T L A D
1201 GTAATTAGAGGTCATGAAGTCAGACAAATCGCTCAGCGGCAACTGGAAGATTCGCTGAT
421 Y N Y K L P D D F T G C V I A W N S N
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1321 CTTGATCTAAGGTTGGTGGTAAATATAATTTACCTGTATAGATGTTTAGGAAGTCTAAT
461 L K P F E R D I S T E I Y Q A G S T P C
1381 CTCAAAACCTTTGAGAGACATTTCAACTGAAATCTTACGGCCGATGACACACCTTGT
481 N G V E G E N C Y F E L Q S Y G E Q P T
1441 AATGGTGTGAAGTTTTAATTTGTTACTTTCCCTTTACAATCATATGTTTTCCAACCCACT
501 N G V G Y Q P Y R V T V V L S F E L L H A
1501 AATGGTGTGGTTACCAACCATACAGAGTAGTAGTCTTTCTTTTGAACCTTCAATGCA
521 P A T V C G P K K N S T N L V K K C N C A V
1561 CCAGCAACTGTTTGTGGACATAAAAAGTCTACTAATTTGGTTAAAACAAATGTGTCAAAT
541 F N F N G L T G T G V L T E S N K K F L
1621 TTCACCTCAATGTTTACAGCCACAGGTCTTCTACTGAGTCTAACAAAAGTTTCTC
561 P F Q F G R D I A D T D A V R D P Q
1681 CCTTTCCAACAATTTGGCAGAGACATGCTGACACTACTGATGCTGTCCGATGATCCACAG
581 T L E I L D I T P C S F G V G V I T P
1741 ACACTTGAGATCTTGACATTAACACATGCTCTTTGGTGGTCACTGTTTATAACACCA
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601 G T N T S N Q V A V L Y Q D>GV N C T E V
1801 GGAACAATACTTCTAACCAGGTGCTGTCTTTATCAGATGTTAACTGCACAGAAGTC
621 P V A I H A D Q A L T P T W R V Y S T G S
1861 CCTGTTGCTATTCATGCAGATCACTTACTCTACTTGGCGTGTATTTCTACAGGTTCT
641 N V F O T R A G C L I G A E H V N N S Y
1921 AATGTTTTCAACACGCTGAGCGTGTAAATAGGGCTGAACATGCTCAACACATAT
661 E C D I P I G A G I C G S Y T Q T Q T N S
1981 GAGTGTGACATACCCATGGTGCAGGTATATGCGASATYQACTCAGACTAATCTT
681 P R R A R S V A S Q S I I A Y T M S L G
2041 CCTCGGGCGGCAAGTGTAGTGTAGTCAATCCATCATTCCTCAGACTATGTCAGTTGGT
701 A E N S V A Y S N N S I A I P T N F T I
2101 GCAGAAAATCAGTTGCTTACTCTAATAACTCTATTGGCCATACCCCAADTTTACTATT
721 S V T T E I L P V S M T K T S V D C T M
2161 AGTGTACCACAGAAATCTACCAGTGTCTATGACCAAGACATCAGTAGATTTGACATG
741 Y I C G D S T E C S N L L L Q Y G S F C
2221 TACATTTGTTGGTATTCAACTGAATGCAGCAATCTTTTGTGCAATATGGCAGTTTTTGT
761 T Q L N R A L T G I A V E Q D K N T Q E
2281 ACACAATTAACCCGTCTTAACTGGAATAGTGTGAAACAAGCAAAAACCCCAAGAA
781 V F A Q V K Q I Y K T P P I K D F G G F
2341 GTTTTGCACAAGTCAACAAATTTTCAAAAACCCCAATTAAGATTTTGGTGGTTTT
801 N F S Q I L P D P S K P S K R S F I E D
2401 AATTTTCAAAATATACAGATCCATCAAAACCAAGCAAGAGTCAATTTATGAAGT
821 L L F N K V T L A D A G F I K Q Y G D C
2461 CTACTTTCAACAAGTGACACTGCAGCTGCTGGCTTCATCAACAATATGGTATTGC
841 L G D I A A R D L I C A Q K F N G L T V
2521 CTTGGTATATTTGCTGCTAGAGACCTATTTGTGCACAAAAGTTTACCGCCTTACTGTT
861 L P P L L T D E M I A Q Y T S A L L A G
2581 TTGCCACCTTGTCTCAGATGAATGATGCTCAATACACTCTGCAGCTGTAGCGGGT
881 T I T S G W T F G A G A A L Q I P F A M
2641 ACAATCACTCTGTTGGACCTTTGGTGCAGGTGCTGCATTAACAATACATTTGCTATG
901 Q M A Y R F N G I G V T Q N V L Y E N G
2701 CAATGGCTTATAGTTTTAATGTTATGGAGTTACACAGAATGTTCTCTATGAGAACCAA
921 K L I A N Q F N S A I G K I Q D S L S S
2761 AAATGATGGCAACCAATTTAATGCTATTGGCAAAATTCAGACTCACTTTCTTCC
941 T A S A L G K L Q D V V N Q N A Q A L N
2821 ACAGCAAGTGCACCTGGAAAACCTCAAGATGTTGGTCAACCAAAAATGCACAAGCTTTAAAC
961 T L V K Q L S N F G A I S S V L L N D I
2881 ACGTGTGTTAAACAACTTCCCAATTTGGTCAATTTCAAGTGTNTTAAATGATATC
981 L S R L E K V A E V Q I D R L I T G R
2941 CTTTCAAGCTTGAARAGTGAAGTCAAAATGATAGTTGATCAGCCAGCAGA
1001 L Q S L Q V T T Q Q L I R A A E I R A
3001 CTTCAAAGTTTGACACATATGTACTCAACAATTAATAGAGCTGCAGAAATCAGAGCT
1021 S A N L A A T K M S E C V L G Q S K R V
3061 D T C G T A A T T C G C T A C T A A A A T G C A G A G T G T A C T T G G A C A T C A A A A G A G T T
1041 D F C G K G Y H L M S F P Q S A P H G V
3121 GATTTTTGTGGAAGGGCTATCATCTTATGCTCTCCCTCAGTCAGCACCTCATGGTGA
1061 V F L H V T Y V P A Q E K N F T T A P A
3181 GTCTTCTTGCATGCACTTATGCTCCCTGCACAAAGAAAGAACTTCAAACTGCTCCTGCC
1081 I C H D G K A H F P R E G V F V S N G T
3241 ATTGTGATGATGAAAGACACACTTCCCTGTAAGGTGCTTTGTTTCAAATGGCACA
1101 H W F V T Q R N F Y E P Q I I T D N T
3301 CACTGGTTTGTACACAAAGGAATTTTATGAACCAAAATCATTACTACAGACACACA
1121 F V S G N C D V V I G I V N N T V Y D P
3361 TTTGTGCTGGTAACTGTGATTTGTAATAGGAATGTCAACACACAGTTTATGCTCT
1141 L Q P E L D S F K E L D K Y F K N H T
3421 TTGCAACCTGAATTAGACTCATTCAAGGAGGATGATATAATATTTAAGAAATCATACA
1161 S P D V D L G D I S G I N A S V N I Q
3481 TCACCAAGTTTGAATAGTGCATCTCTGGCAATATGCTTCAAGTTGTAACATTTCAA
1181 K E I D R L N E V A K N L N E S L I D L
3541 AAAGAAATGACCCCTCAATGAGGTTGCCAAGAAATTAATGAATCTCTCATGATCTC
```

```
1201 Q E L G K Y E Q Y I K W P W Y I W L G F
3601 CAAGAAGTGGAAAGTATGAGCAGTATATAAATGGCCATGGTACATTTGGTGTAGTTTT
1221 I A G L I A I V M V T I M L C M T S C
3661 ATAGCTGGCTGATTGCCATAGTAAAGTGGTGAACAAATTTGCTTGTATGACCAAGTTC
1241 C S C L K G C C S C G S C C K F D E D
3721 TGTAGTTGCTCAAGGCTGTTGTTCTTGGTATGCTGCTCAAAATTTGATGAAGACAG
1261 S E P V L K G V K L H Y T *
3781 TCTGAGCCAGTCTCAAGGAGTCAAAATACATACACATAA
```

Domínio CH  
(central helix)

Domínio RBD

Destaques em: 1) variantes brasileiras de Fov/21 (domínio RBD K417T); 2) variante D614>G - mais comum e 3) sítio de furina (rosa).

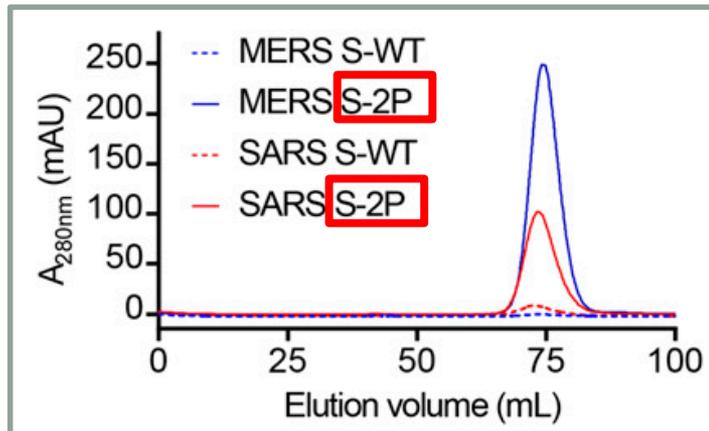
Há duas vacinas da Pfizer: BNT162b1 (apenas domínio RDB) e BNT162b2 (similar a Moderna [completa e 2P])

  
Pfizer e BioNTech

  
Moderna e Pfizer

# 4. mRNA and Protein Spike

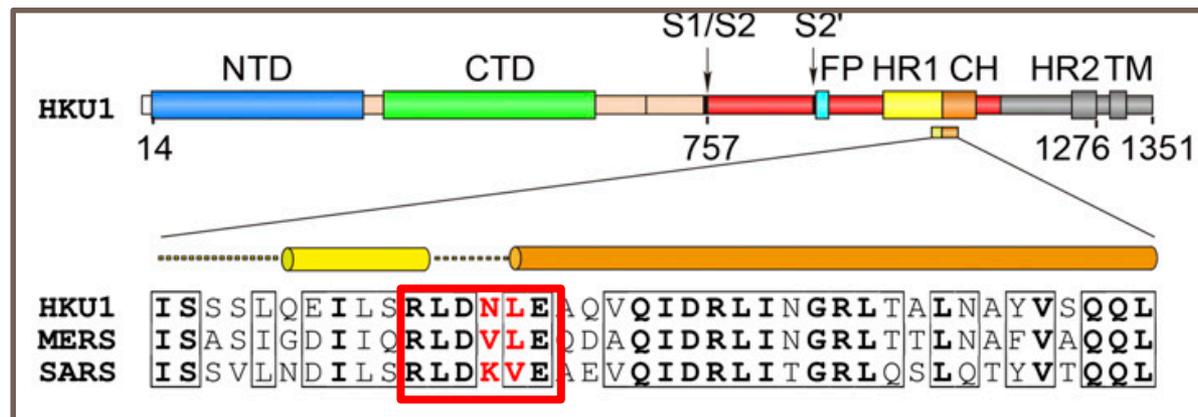
## Características outras spikes: Pallesen *et al* 2017



Perfil gel-filtração a partir de 1 litro de produção transiente. Com relação ao sítio assinalado: duas proteínas spikes são mutantes (2P) e duas selvagens.

### Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen PNAS E7348-E7357

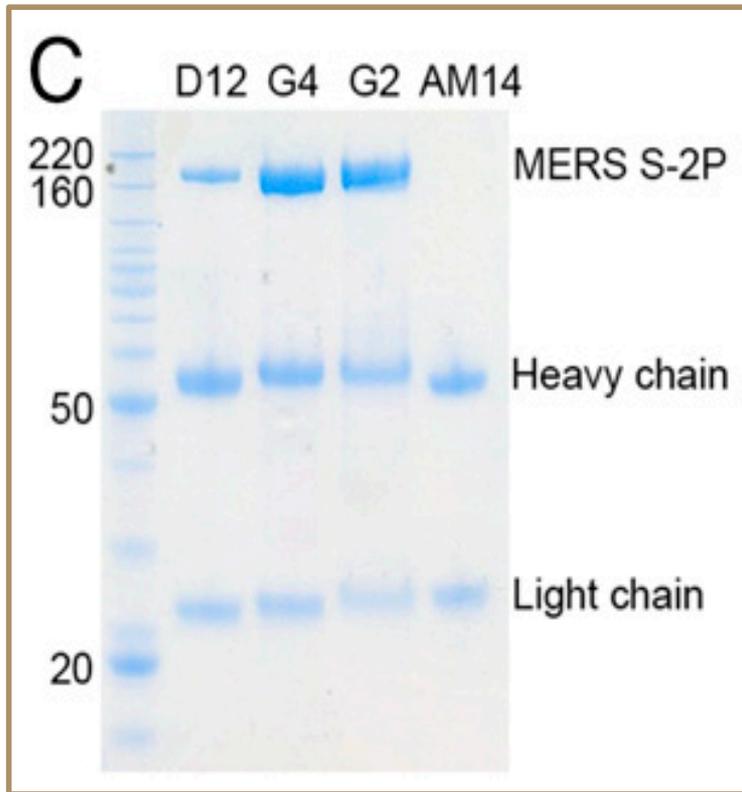
Jesper Pallesen<sup>a,1</sup>, Nianshuang Wang<sup>b,1,2</sup>, Kizzmekia S. Corbett<sup>c,1</sup>, Daniel Wrapp<sup>b</sup>, Robert N. Kirchdoerfer<sup>a</sup>, Hannah L. Turner<sup>a</sup>, Christopher A. Cottrell<sup>a</sup>, Michelle M. Becker<sup>d</sup>, Lingshu Wang<sup>e</sup>, Wei Shi<sup>e</sup>, Wing-Pui Kong<sup>e</sup>, Erica L. Andres<sup>d</sup>, Arminja N. Kettenbach<sup>b,f</sup>, Mark R. Denison<sup>d,g</sup>, James D. Chappell<sup>d</sup>, Barney S. Graham<sup>c</sup>, Andrew B. Ward<sup>a,2</sup>, and Jason S. McLellan<sup>b,2</sup>



## 4. mRNA and Protein Spike

### Características *spike* híbrida: Pallessen *et al* 2017

All four proteins were expressed with a C-terminal T4 fibrin trimerization domain



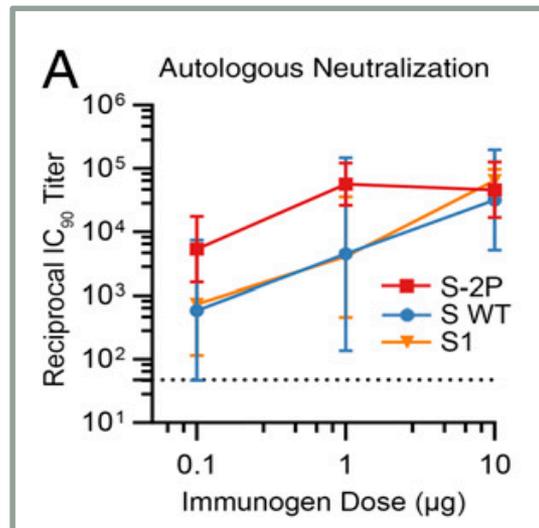
Análise em SDS/PAGE da proteína MERS-S-2P expressa em células 293-T mostrando que mantém a capacidade de reconhecer diferentes anticorpos. AM14 – anticorpo controle.

# 4. mRNA and Protein Spike

## Spike: imunogenicidade - outros coronavirus

### Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen

Jesper Pallesen<sup>a,1</sup>, Nianshuang Wang<sup>b,1,2</sup>, Kizzmekia S. Corbett<sup>c,1</sup>, Daniel Wrapp<sup>b</sup>, Robert N. Kirchdoerfer<sup>3</sup>, Hannah L. Turner<sup>3</sup>, Christopher A. Cottrell<sup>a</sup>, Michelle M. Becker<sup>d</sup>, Lingshu Wang<sup>e</sup>, Wei Shi<sup>e</sup>, Wing-Pui Kong<sup>e</sup>, Erica L. Andres<sup>d</sup>, Arminja N. Kettenbach<sup>b,1</sup>, Mark R. Denison<sup>d,9</sup>, James D. Chappell<sup>d</sup>, Barney S. Graham<sup>c</sup>, Andrew B. Ward<sup>a,2</sup>, and Jason S. McLellan<sup>b,2</sup>

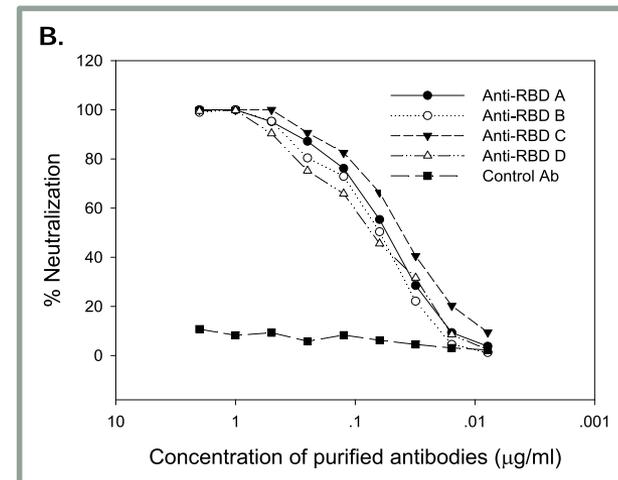


Spike 2P [MERS]  
atividade neutralizante  
no soro

Pallesen et al 2017 PNAS E7349

### Potent and persistent antibody responses against the receptor-binding domain of SARS-CoV spike protein in recovered patients

Zhiliang Cao<sup>1†</sup>, Lifeng Liu<sup>2†</sup>, Lanying Du<sup>3</sup>, Chao Zhang<sup>1</sup>, Shibo Jiang<sup>3</sup>, Taisheng Li<sup>2\*</sup>, Yuxian He<sup>1\*</sup>



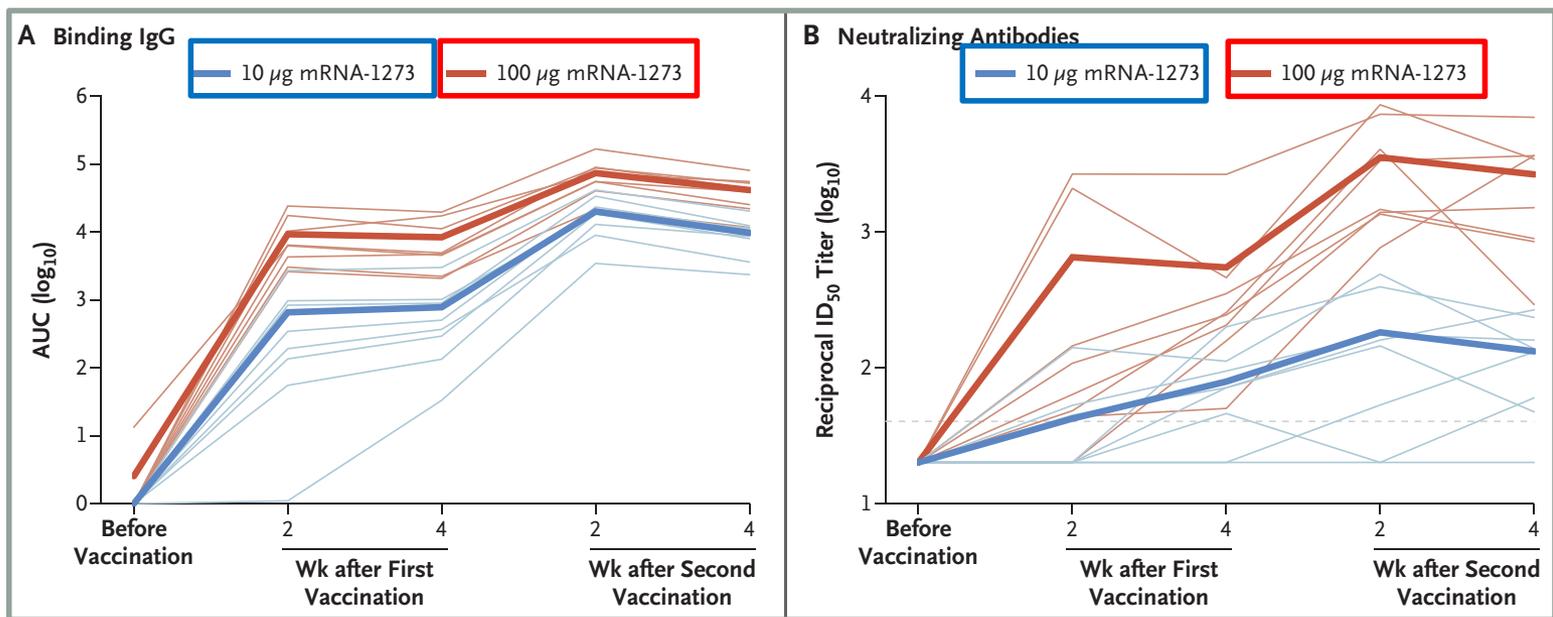
Neutralização da infecção por  
pseudovírus SARS-CoV-1  
utilizando anticorpo  
específico domínio RBD

Cao et al 2010 Virology J. 7:299

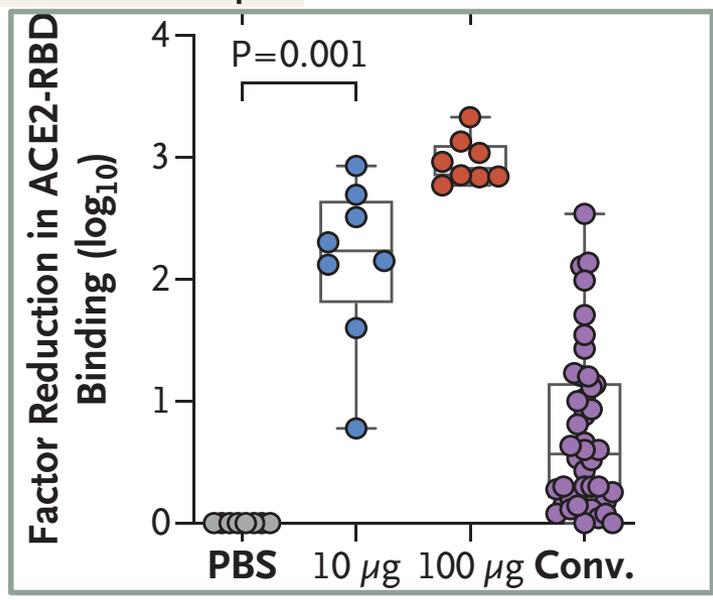
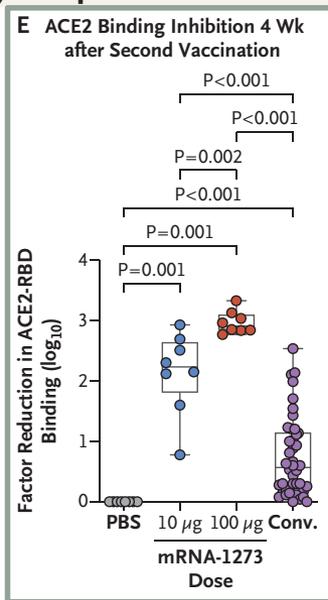
# 5. mRNA-1273 – MODERNA – SARS-CoV-2 S-SP

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# 5. mRNA-1273 – MODERNA – Resposta humoral



**Figure 1.** Antibody Responses after mRNA-1273 Vaccination in Rhesus Macaques.





# 5. mRNA-1273 – MODERNA – SARS-CoV-2 S-SP

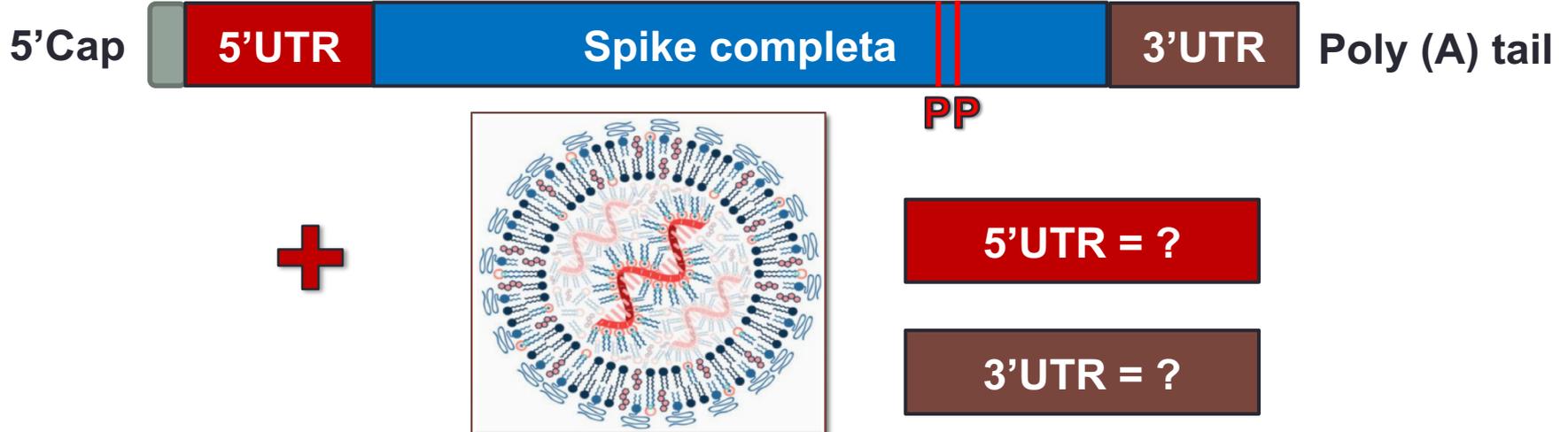
## RESULTS

First, we evaluated temporal SARS-CoV-2 S-2P-specific antibody responses after vaccination. IgG binding to the conformationally defined prefusion S-2P protein<sup>25,26</sup> was increased over baseline in a dose-dependent manner after two vaccine

25. Pallesen J, Wang N, Corbett KS, et al. Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. Proc Natl Acad Sci U S A 2017; 114:E7348-E7357.

26. Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020;367:1260-3.

## Como é a moléculas mRNA da vacina anti-SARS-CoV-2 da Moderna



# 5. mRNA-1273 – MODERNA – SARS-CoV-2 S-SP

*The NEW ENGLAND JOURNAL of MEDICINE*

ORIGINAL ARTICLE

## Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine

L.R. Baden, H.M. El Sahly, B. Essink, K. Kotloff, S. Frey, R. Novak, D. Diemert, S.A. Spector, N. Roupael, C.B. Creech, J. McGettigan, S. Khetan, N. Segall, J. Solis, A. Brosz, C. Fierro, H. Schwartz, K. Neuzil, L. Corey, P. Gilbert, H. Janes, D. Follmann, M. Marovich, J. Mascola, L. Polakowski, J. Ledgerwood, B.S. Graham, H. Bennett, R. Pajon, C. Knightly, B. Leav, W. Deng, H. Zhou, S. Han, M. Ivarsson, J. Miller, and T. Zaks, for the COVE Study Group\*

### **RESULTS**

The trial enrolled 30,420 volunteers who were randomly assigned in a 1:1 ratio to receive either vaccine or placebo (15,210 participants in each group). More than

## 5. mRNA-1273 – MODERNA – SARS-CoV-2 S-SP

### Conclusão:

mRNA com 3822 nt contendo a região codificadora completa, isto é, 1273 aas da proteína *spike* e duas mutações *missense* nas posições – K986 > P e V987 > P.

### Perguntas:

Quais os tamanhos das regiões 5' UTR e 3' UTR e suas respectivas sequências?

Qual o tamanho da cauda poli A?

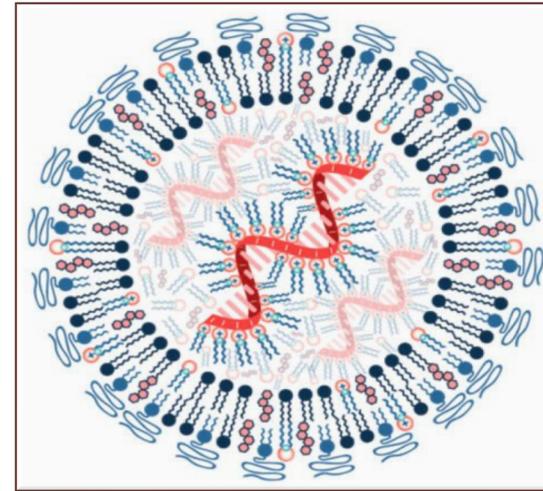
# 6. mRNA vaccine **BNT162b2**

## Construção da molécula de mRNA: similar à Moderna



+

**Nanopartícula lipídica**  
**4 moléculas lipídicas**  
**(50:10:38.5:1.5)**



### Article

## BNT162b vaccines protect rhesus macaques from SARS-CoV-2

Nature | Vol 592 | 8 April 2021 | **283**

<https://doi.org/10.1038/s41586-021-03275-y>

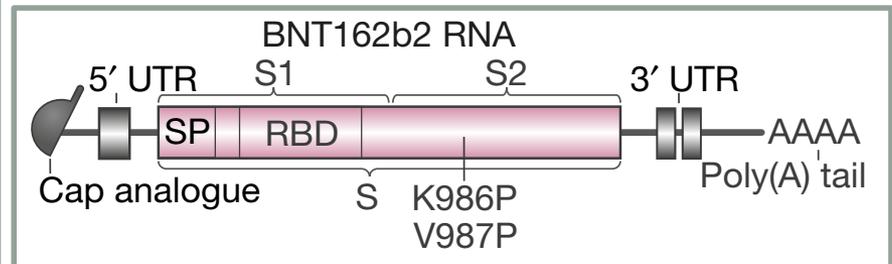
Received: 1 September 2020

Accepted: 20 January 2021

Published online: 1 February 2021

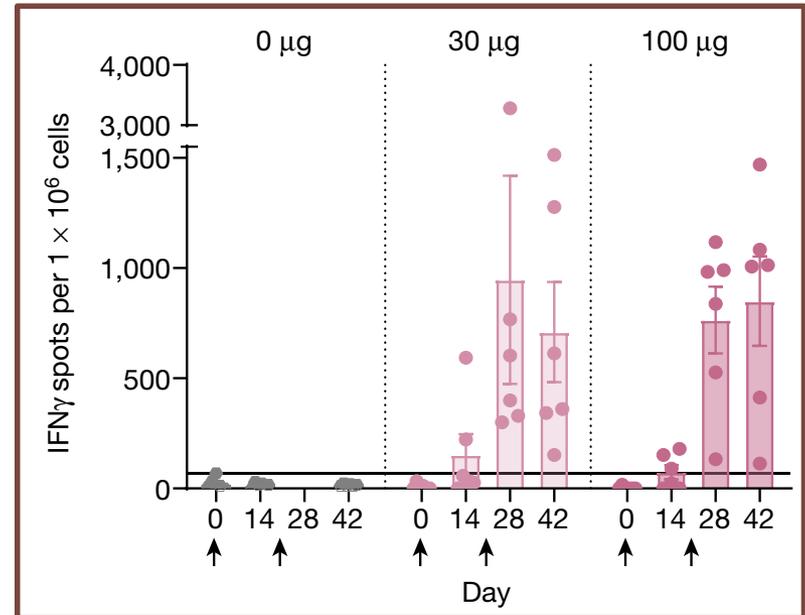
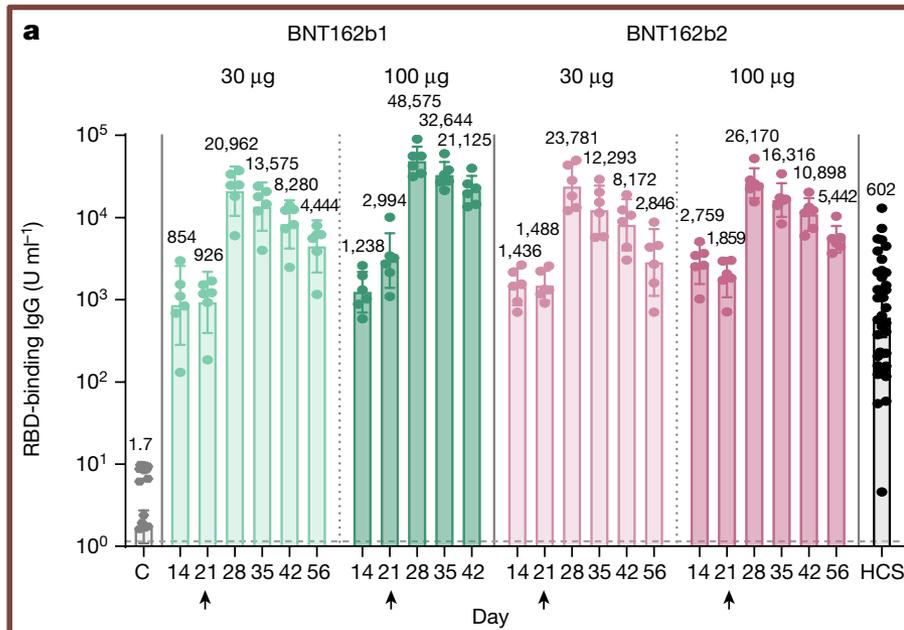
Check for updates

Annette B. Vogel<sup>1,2,3</sup>, Isis Kanevsky<sup>2,3,4</sup>, Ye Che<sup>3,5</sup>, Kena A. Swanson<sup>2</sup>, Alexander Muik<sup>1</sup>, Mathias Vormehr<sup>1</sup>, Lena M. Kranz<sup>2</sup>, Kerstin C. Walzer<sup>1</sup>, Stephanie Hein<sup>1</sup>, Alptekin Güler<sup>1</sup>, Jakob Loschko<sup>1</sup>, Mohan S. Maddur<sup>2</sup>, Ayuko Ota-Setlik<sup>2</sup>, Kristin Tompkins<sup>2</sup>, Journey Cole<sup>4</sup>, Bonny G. Lui<sup>2</sup>, Thomas Ziegenhals<sup>1</sup>, Arianne Plaschke<sup>2</sup>, David Eisel<sup>1</sup>, Sarah C. Dany<sup>1</sup>, Stephanie Fesser<sup>1</sup>, Stephanie Erbar<sup>1</sup>, Ferdia Bates<sup>1</sup>, Diana Schneider<sup>1</sup>, Bernadette Jesionek<sup>1</sup>, Bianca Sanger<sup>1</sup>, Ann-Kathrin Wallisch<sup>1</sup>, Yvonne Feuchter<sup>1</sup>, Hanna Junginger<sup>1</sup>, Stefanie A. Krumm<sup>1</sup>, Andre P. Heinen<sup>1</sup>, Petra Adams-Quack<sup>1</sup>, Julia Schlereth<sup>1</sup>, Stefan Schille<sup>1</sup>, Christoph Kroner<sup>1</sup>, Ramon de la Caridad Guimil Garcia<sup>1</sup>, Thomas Hiller<sup>1</sup>, Leyla Fischer<sup>1</sup>, Rani S. Sellers<sup>2</sup>, Shambhunath Choudhary<sup>2</sup>, Olga Gonzalez<sup>2</sup>, Fulvia Vascotto<sup>2</sup>, Matthew R. Gutman<sup>2</sup>, Jane A. Fontenot<sup>2</sup>, Shannan Hall-Ursone<sup>2</sup>, Kathleen Brasky<sup>2</sup>, Matthew C. Griffor<sup>2</sup>, Seungil Han<sup>2</sup>, Andreas A. H. Su<sup>1</sup>, Joshua A. Lees<sup>2</sup>, Nicole L. Nedoma<sup>2</sup>, Ellene H. Mashalidis<sup>2</sup>, Parag V. Sahasrabudhe<sup>2</sup>, Charles Y. Tan<sup>2</sup>, Danka Pavliakova<sup>2</sup>, Guy Singh<sup>2</sup>, Camila Fontes-Garfias<sup>2</sup>, Michael Pride<sup>2</sup>, Ingrid L. Scully<sup>2</sup>, Tara Ciolino<sup>2</sup>, Jennifer Obregon<sup>2</sup>, Michal Gazi<sup>2</sup>, Ricardo Carrion Jr<sup>1</sup>, Kendra J. Alfson<sup>2</sup>, Warren V. Kalina<sup>2</sup>, Deepak Kaushal<sup>2</sup>, Pei-Yong Shi<sup>2</sup>, Thorsten Klamp<sup>1</sup>, Corinna Rosenbaum<sup>1</sup>, Andreas N. Kuhn<sup>1</sup>, Ozlem Tureci<sup>1</sup>, Philip R. Dormitzer<sup>2</sup>, Kathrin U. Jansen<sup>2</sup> & Ugur Sahin<sup>1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80,81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100</sup>



# 6. mRNA vaccine BNT162b2

## Avaliação da imunogenicidade em macaco:



Concentração do anticorpo IgG em diferentes teipos após a segunda dose da vacina em duas diferentes concentrações.

Elevada fraçãp de células T-CD4+ e T-CD8+ por meio do nível de produção de IFN- $\gamma$ .

**\*\* A seta no eixo X indica o dia da segunda dose**

## 6. mRNA vaccine **BNT162b2**

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## Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine

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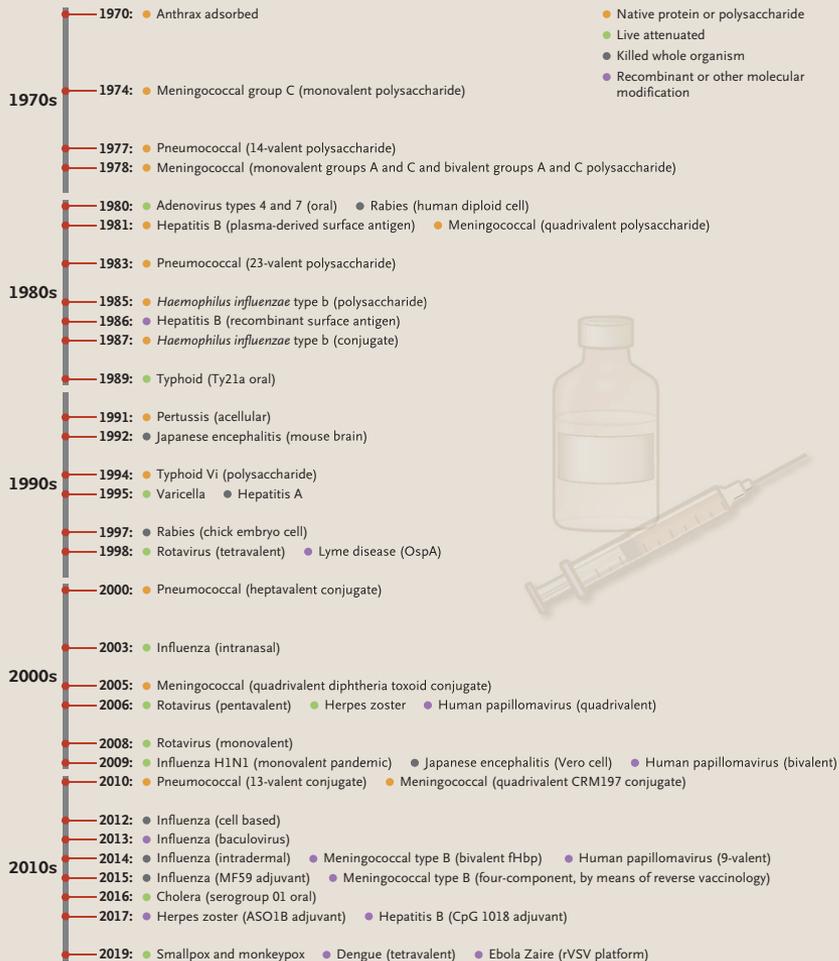
### **RESULTS**

A total of 43,548 participants underwent randomization, of whom 43,448 received injections: 21,720 with BNT162b2 and 21,728 with placebo. There were 8 cases of



# The NEW ENGLAND JOURNAL of MEDICINE

Perspective  
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A resposta a COVID-19 foi o melhor exemplo de como novas vacinas podem agora serem desenvolvidas.

Em menos de 1 ano, o primeiro ensaio clínico estava completo e foram as primeiras vacinas autorizadas para o uso emergencial.

Inicia-se um novo desafio em outras áreas da medicina. Ex. vacinas terapêuticas anti-tumorais.