



UNIVERSIDADE
DE SÃO PAULO

85 ANOS
1934 · 2019



Vacina Genética para prevenção de SARS-CoV-2

Aparecida Maria Fontes

aparecidamfontes@usp.br

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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

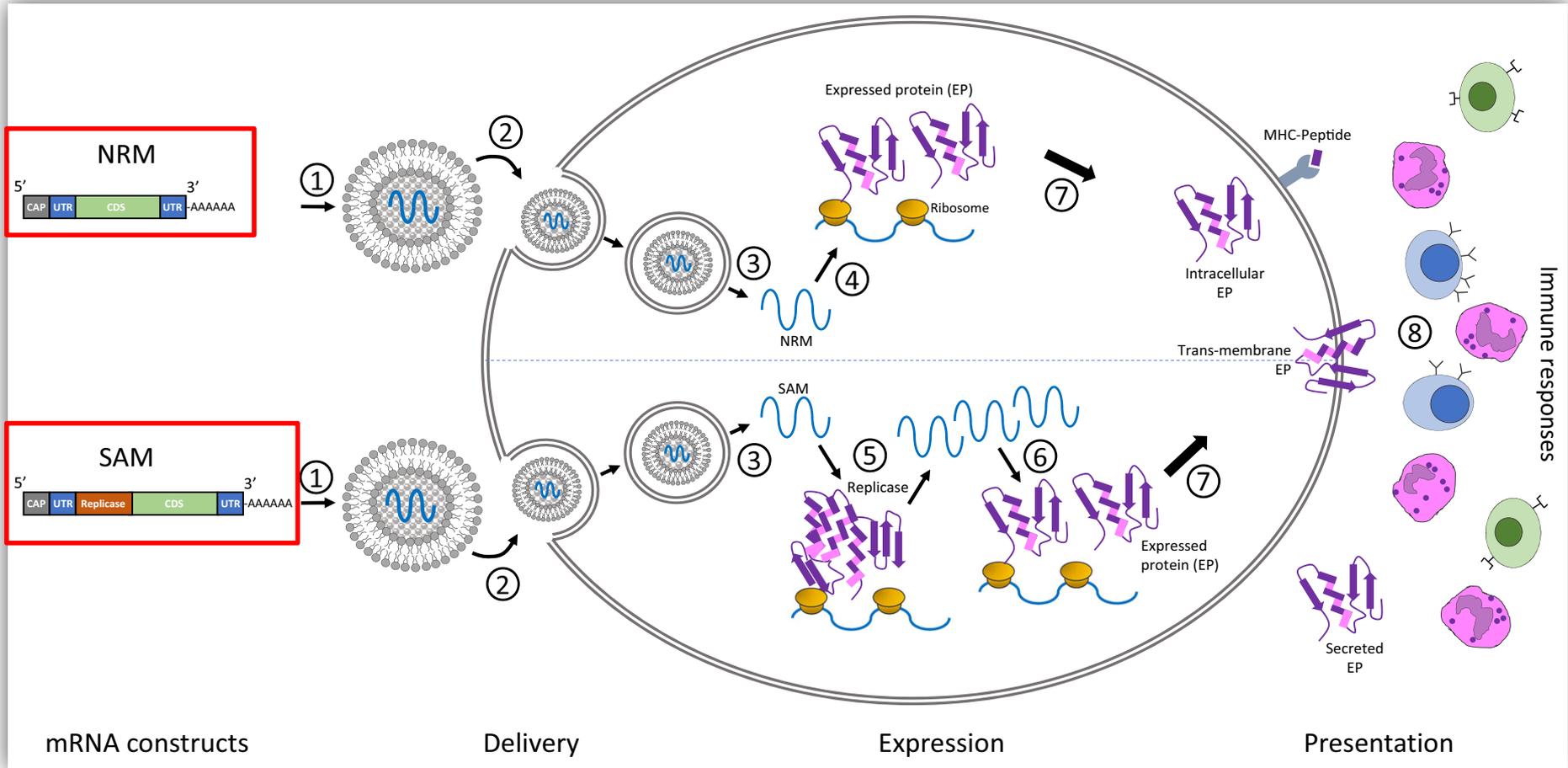
Pfizer

5. Vacina de mRNA que codifica proteína spike

6. Perspectivas: vacinas mRNA

1. Vacinação e Objetivos do desenvolvimento de vacinas

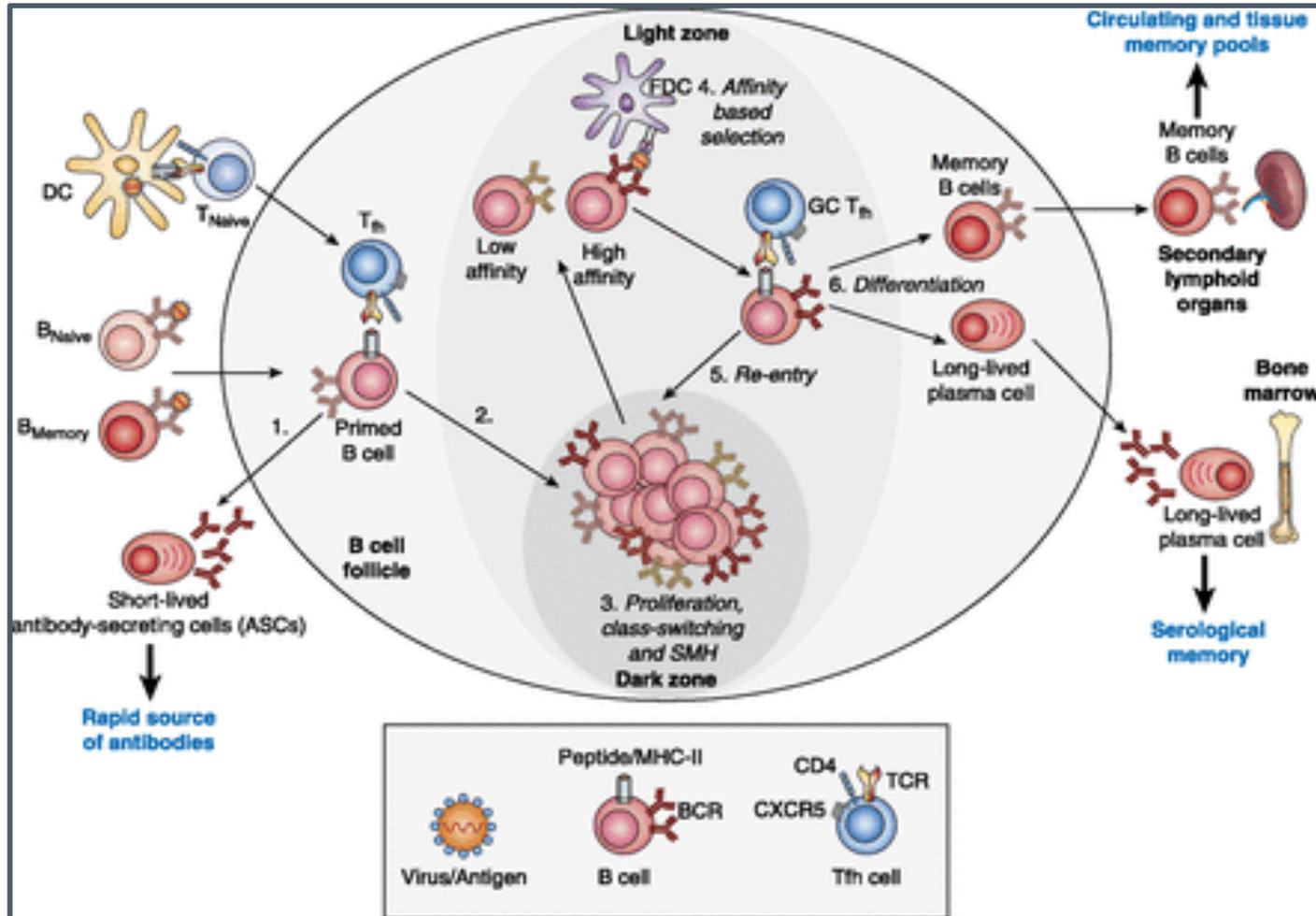
2 Tipos de vacina de mRNA:



1. Vacinação e Objetivos do desenvolvimento de vacinas

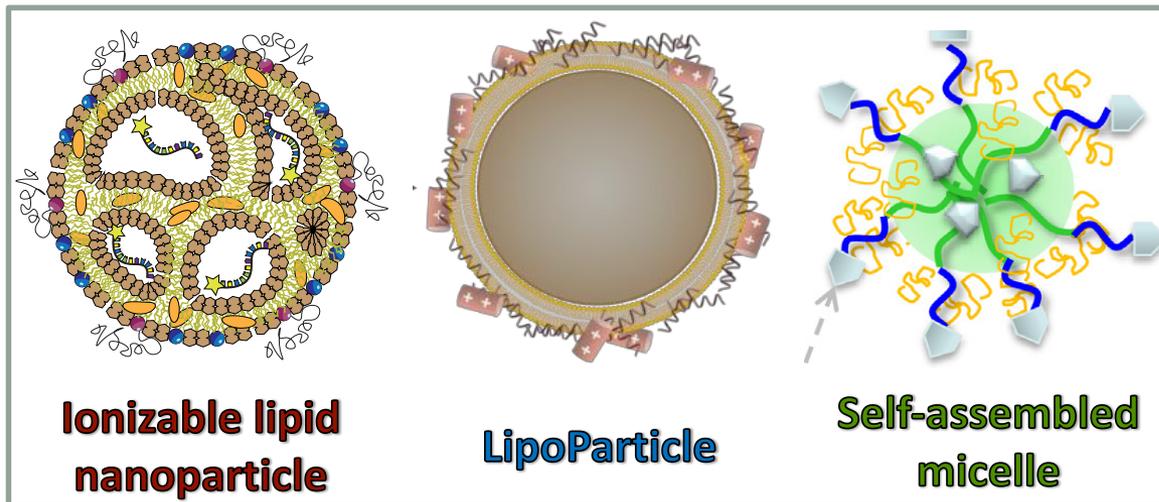
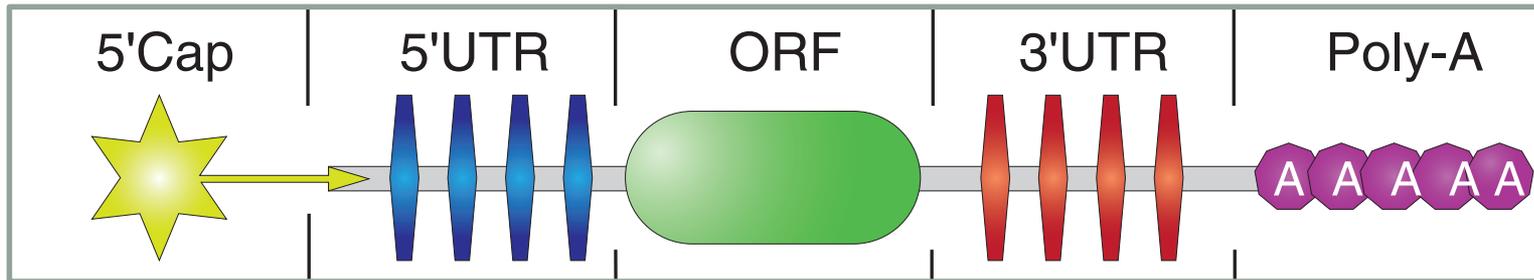
Como induzir a imunidade duradoura?

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

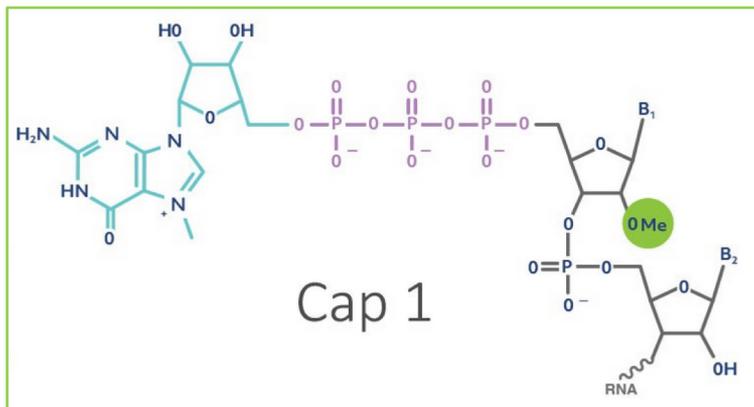
3' UTR

Imunogenicidade

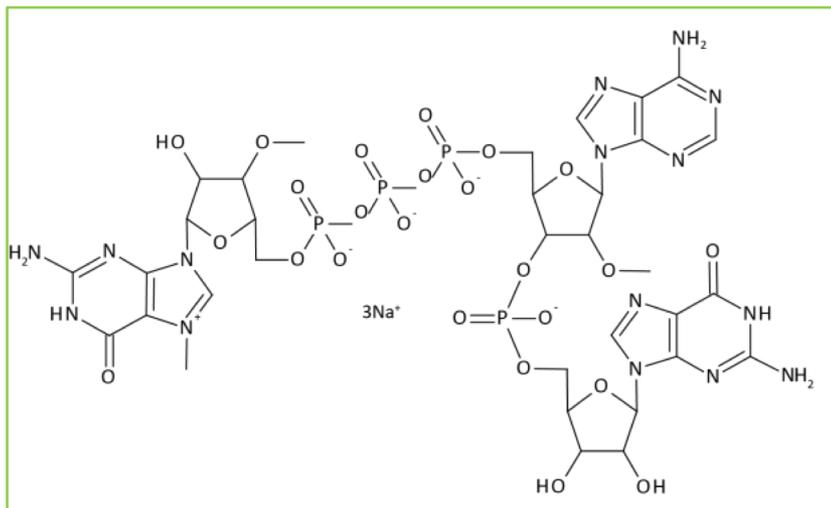
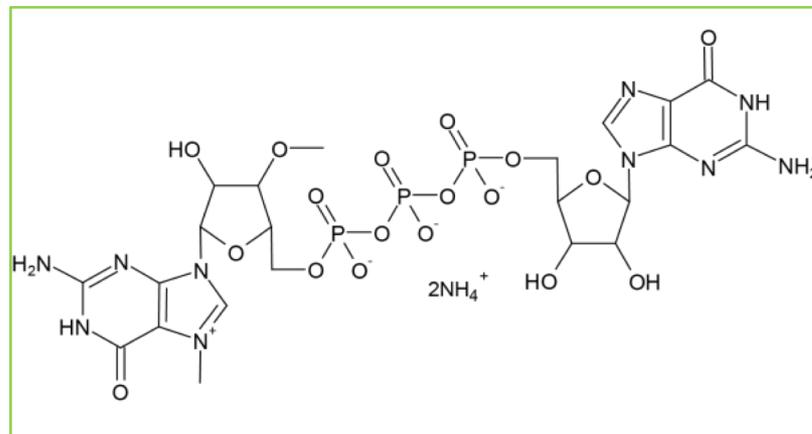
Nucleosídeos modificados

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.

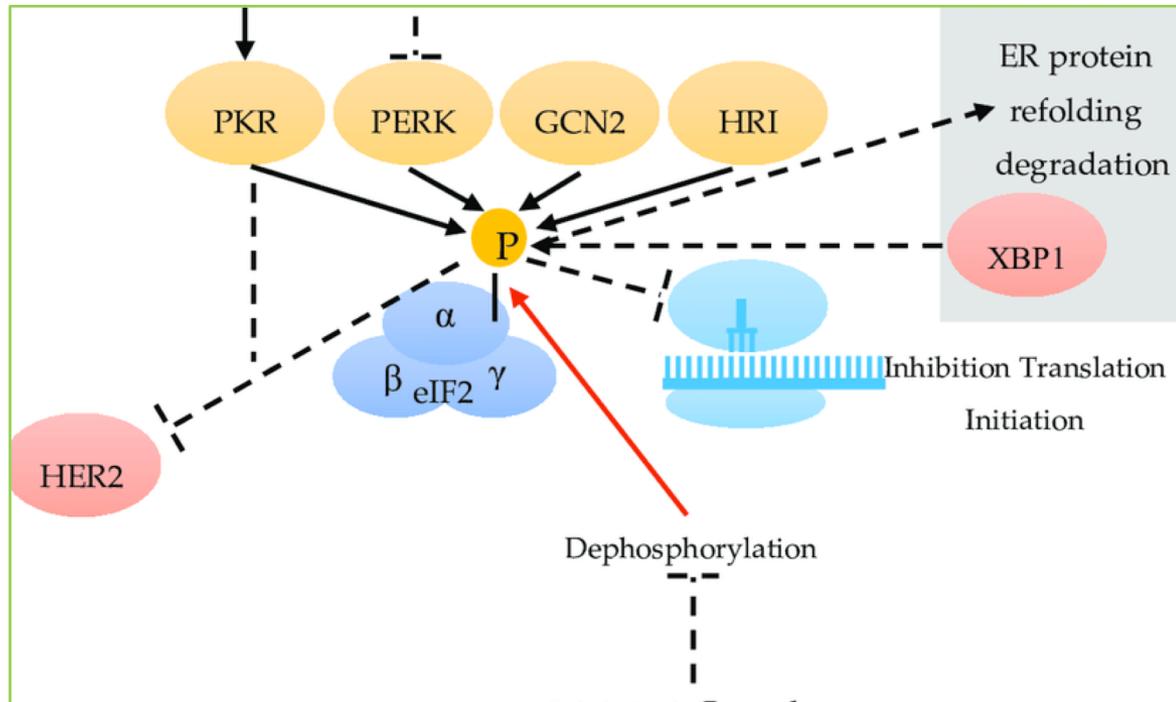
Receptores presentes no endossoma: TLR-7 e TLR-8 que ativam MyD88, interferon tipo-1 e a secreção de citocinas inflamatórias.

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:

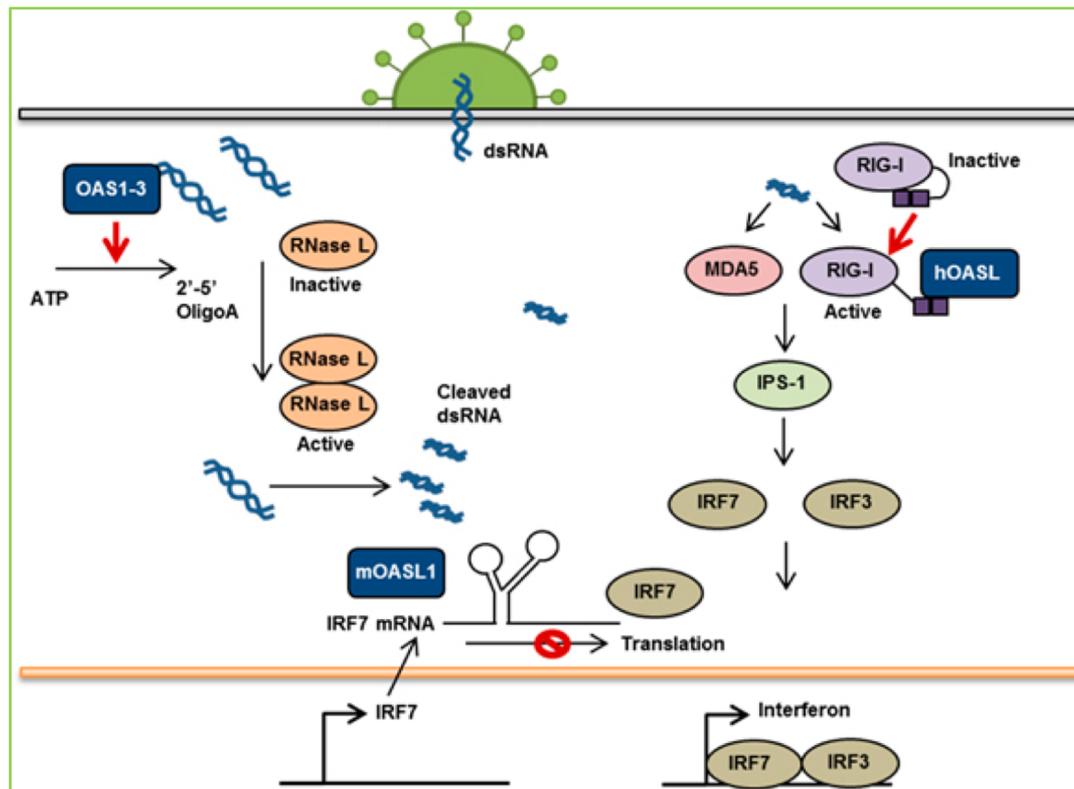
Se PKR for ativada, o fator eucariótico de iniciação da tradução (eIF-2) pode ser fosforilado e bloquear a tradução do mRNA.



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 α -globina e β -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.

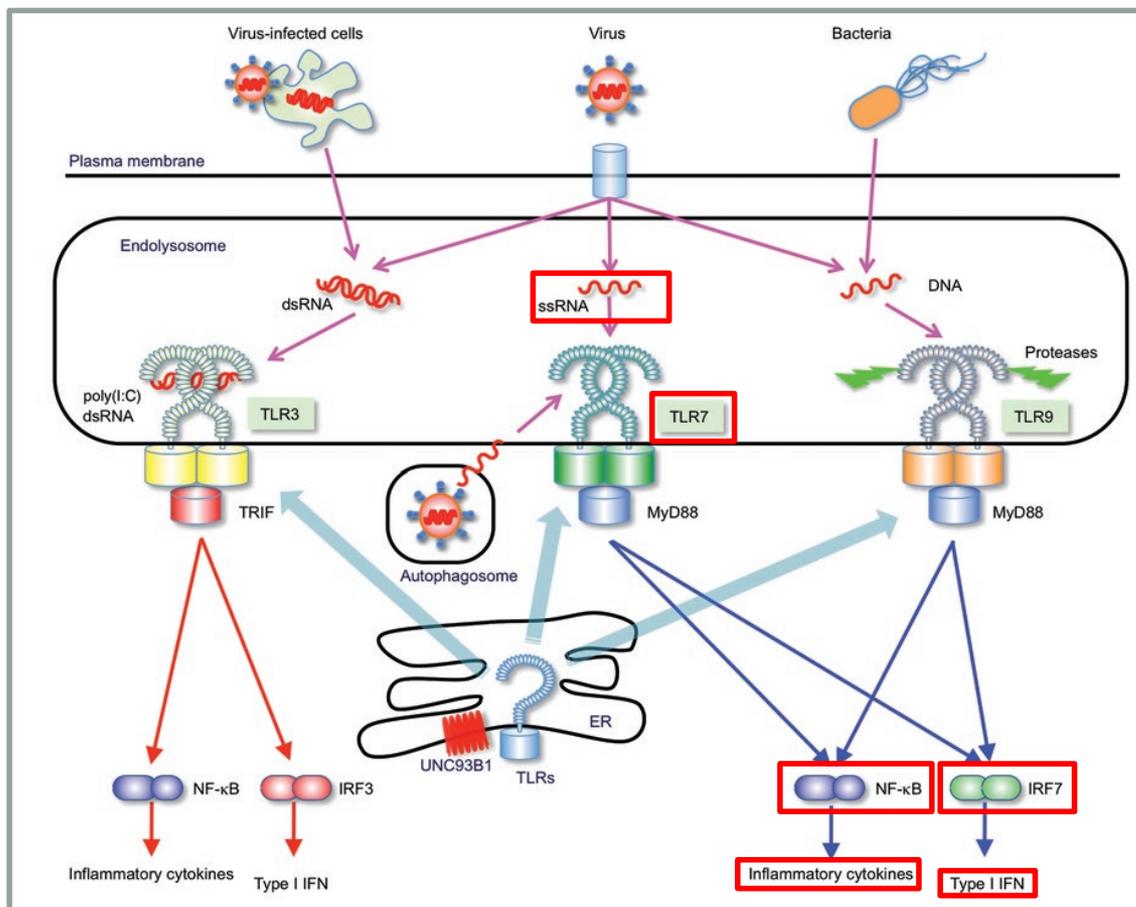
A proteína ligante de cauda poliA (PABP) pode interagir com a região 5' *cap* e promover uma estrutura que permitirá uma maior eficiência da tradução.

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

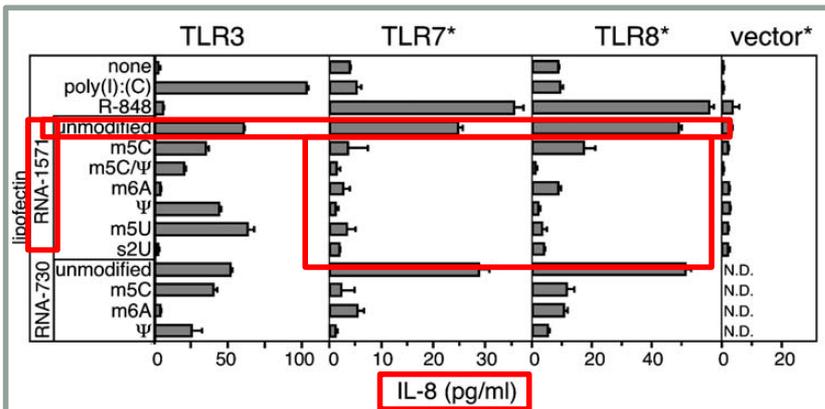
Immunity, Vol. 23, 165–175

Katalin Karikó,^{1,*} Michael Buckstein,² Houping Ni,² and Drew Weissman²

Drew Weissman e Katalin Karikó

Table 1. TLR Recognition of Microbial Components

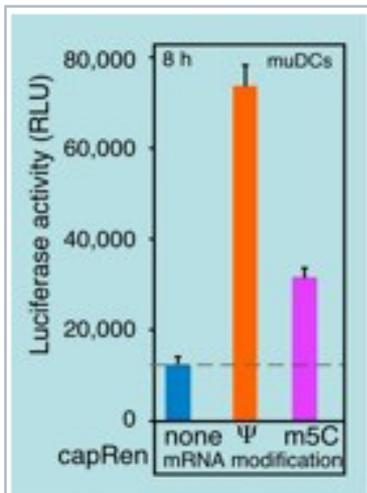
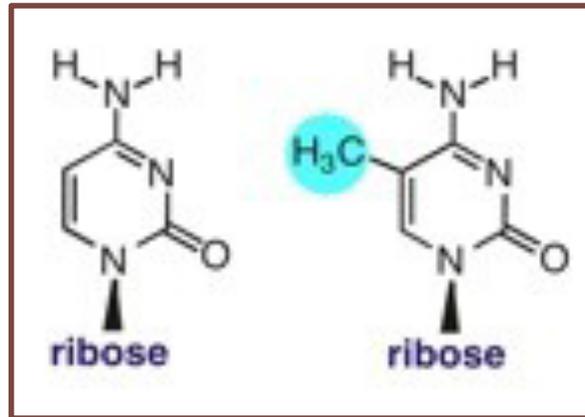
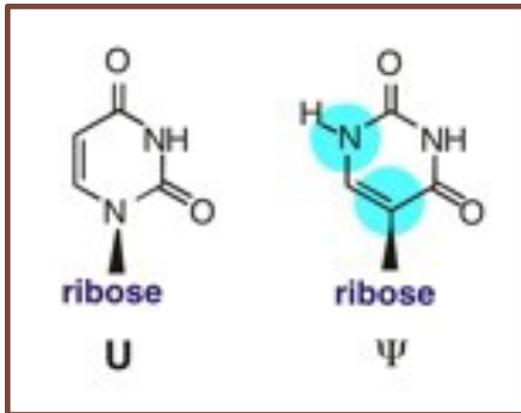
Microbial Components	Species	TLR Usage
Viruses		
DNA	Viruses	TLR9
dsRNA	Viruses	TLR3
ssRNA	RNA viruses	TLR7 and TLR8
Envelope proteins	RSV, MMTV	TLR4
Hemagglutinin protein	Measles virus	TLR2



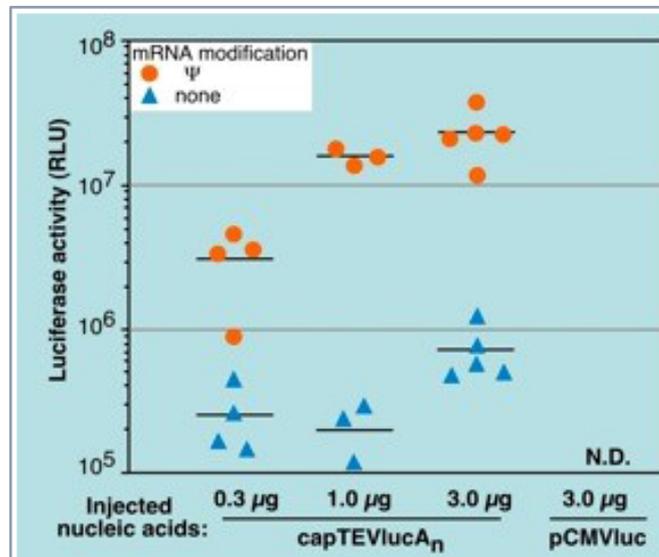
Células 293 expressando receptores do tipo *Toll* (TLR), simulando células do sistema imune que apresentam esses receptores, reconhecem e respondem a ácidos nucleicos. A ativação desses receptores levam a produção de interferon. A síntese de mRNA na presença de nucleotídeos quimicamente modificados modulam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

2. Vacina de mRNA

Modificações: pseudouridina e 5-metilcitidina



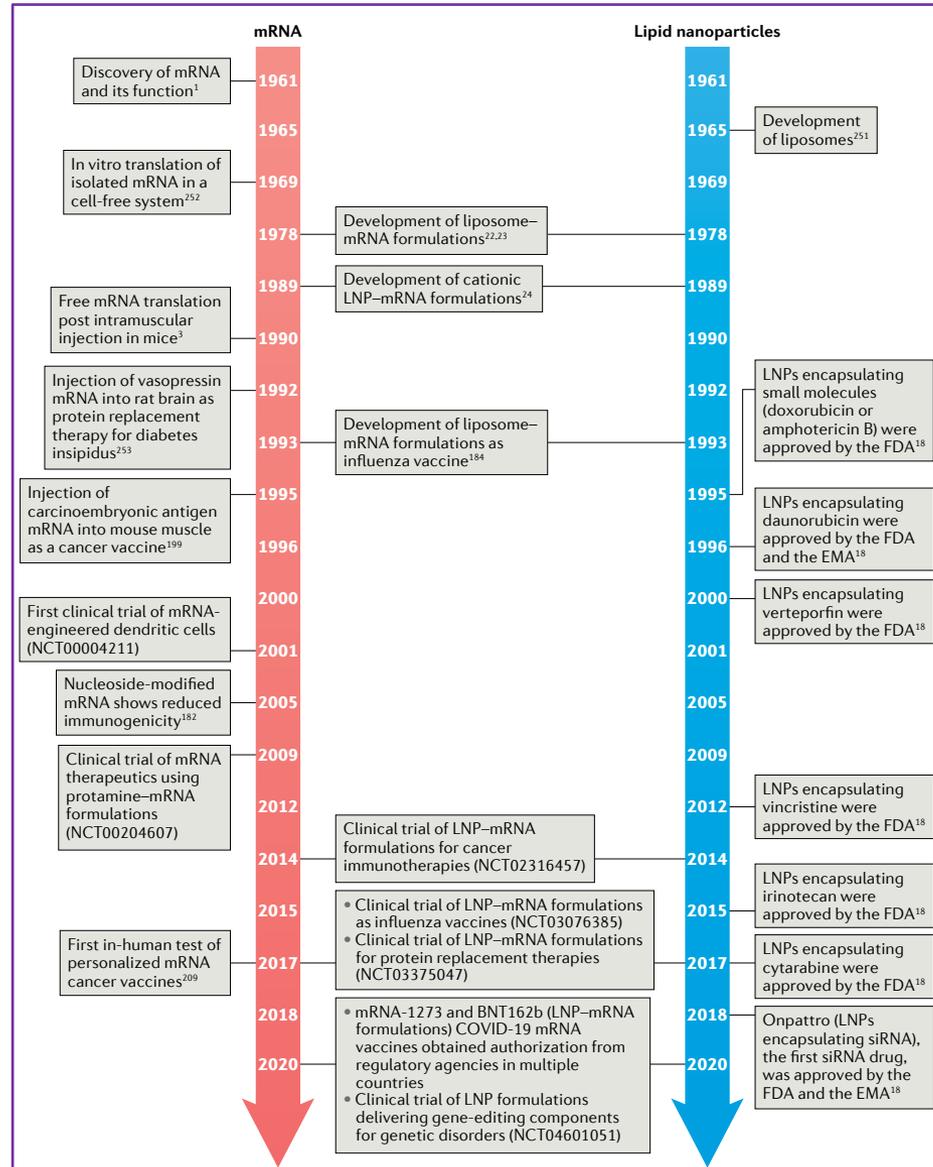
expressão *in vitro*



expressão *in vivo*

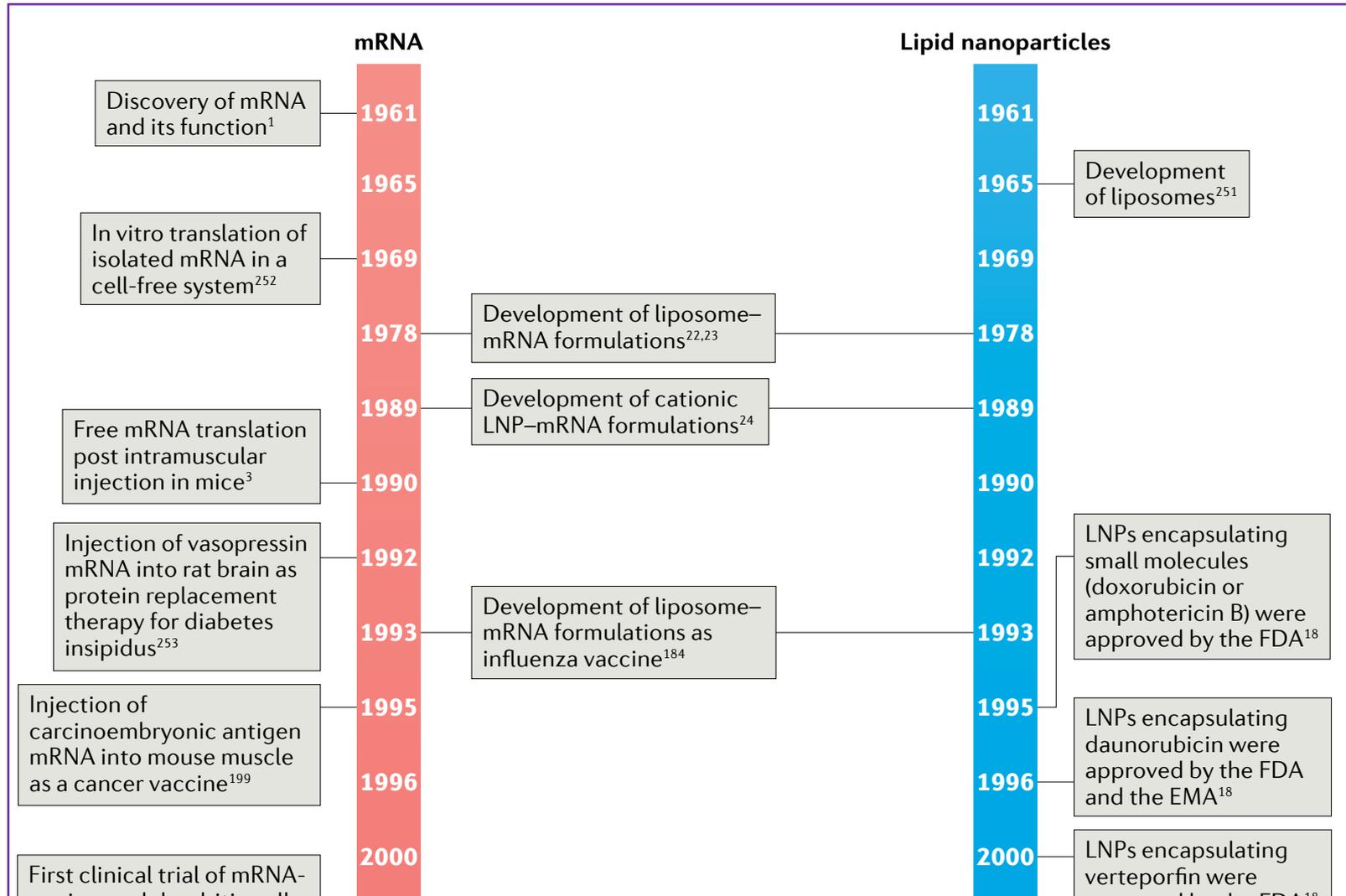
3. Sistema de entrega da molécula de mRNA

Nanopartícula lipídica:



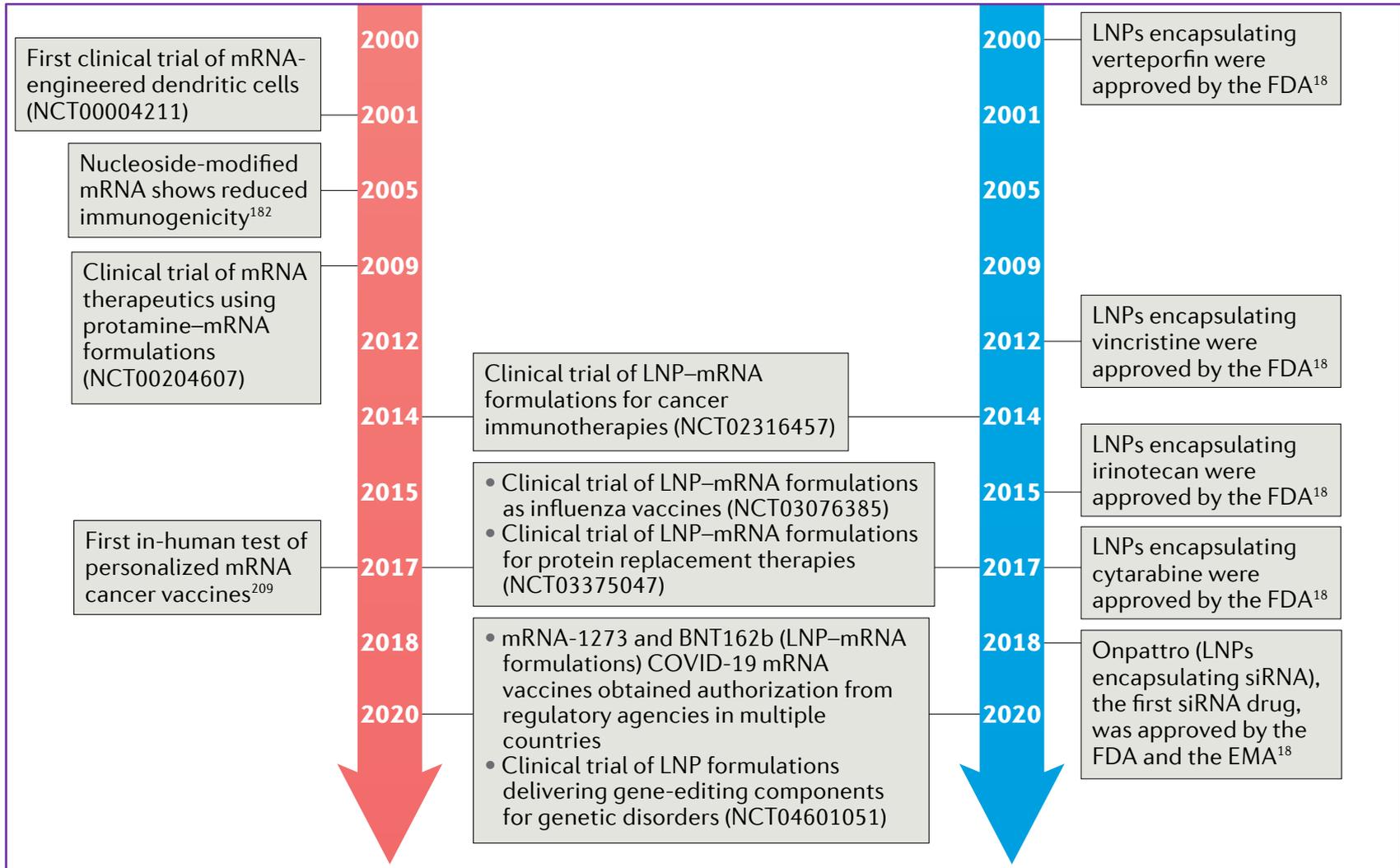
3. Sistema de entrega da molécula de mRNA

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:

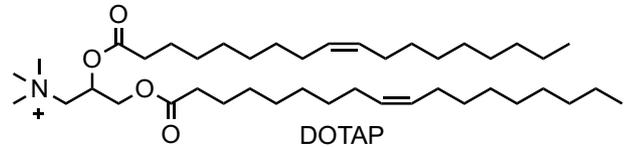
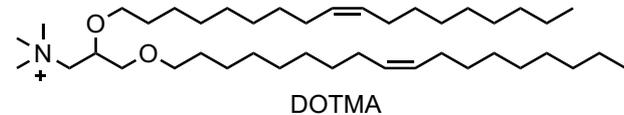
1. Lipídeo catiônico

2. Lipídeo ionizável

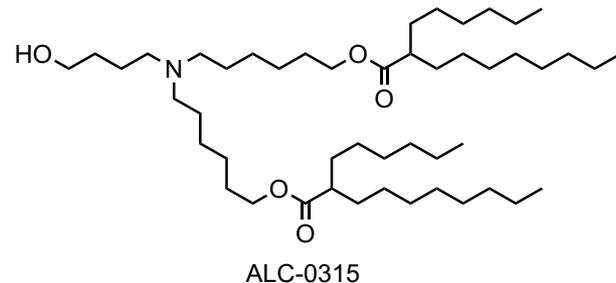
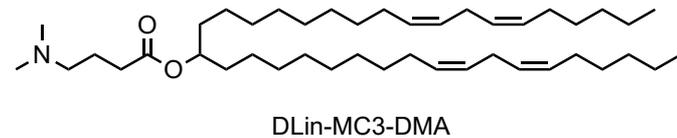
3. Fosfolipídeo

4. Colesterol

Cationic lipids



Ionizable lipids



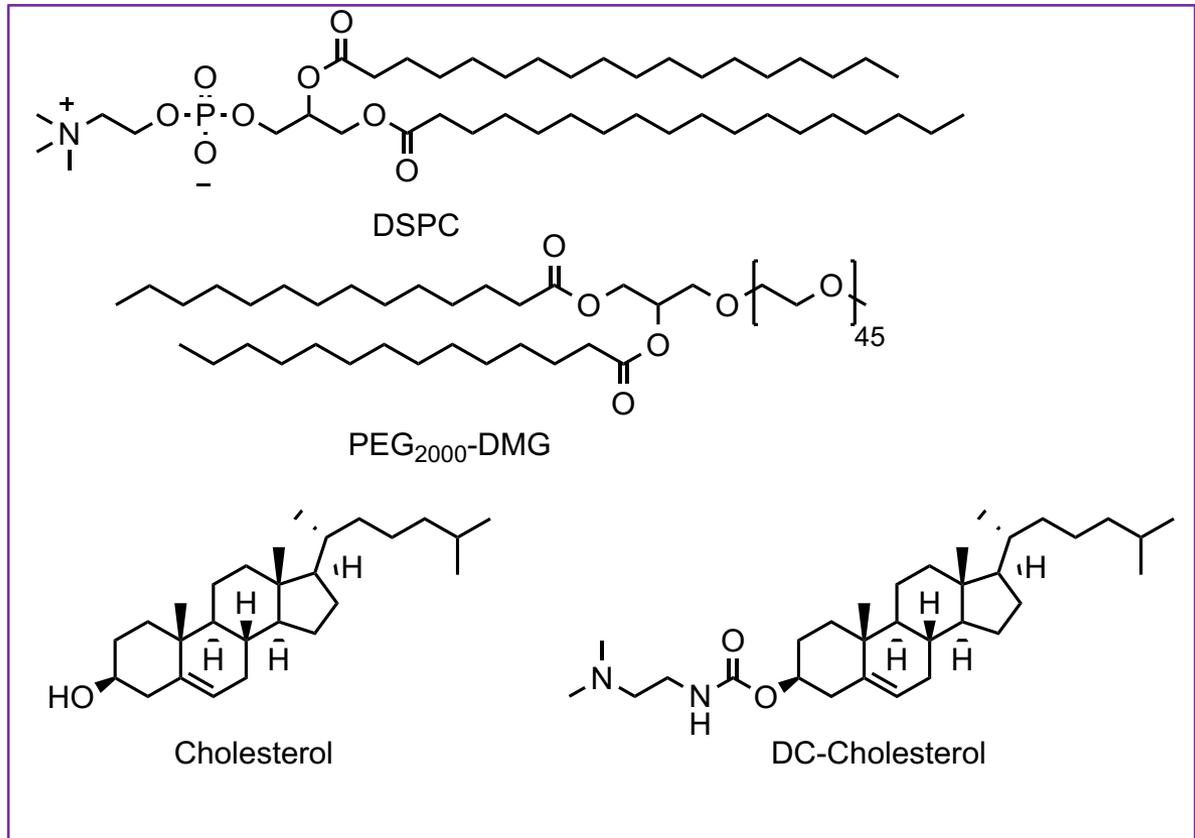
3. Sistema de entrega da molécula de mRNA

Nanopartícula lipídica: vacinas de mRNA anti-COVID-19

4 moléculas lipídicas:

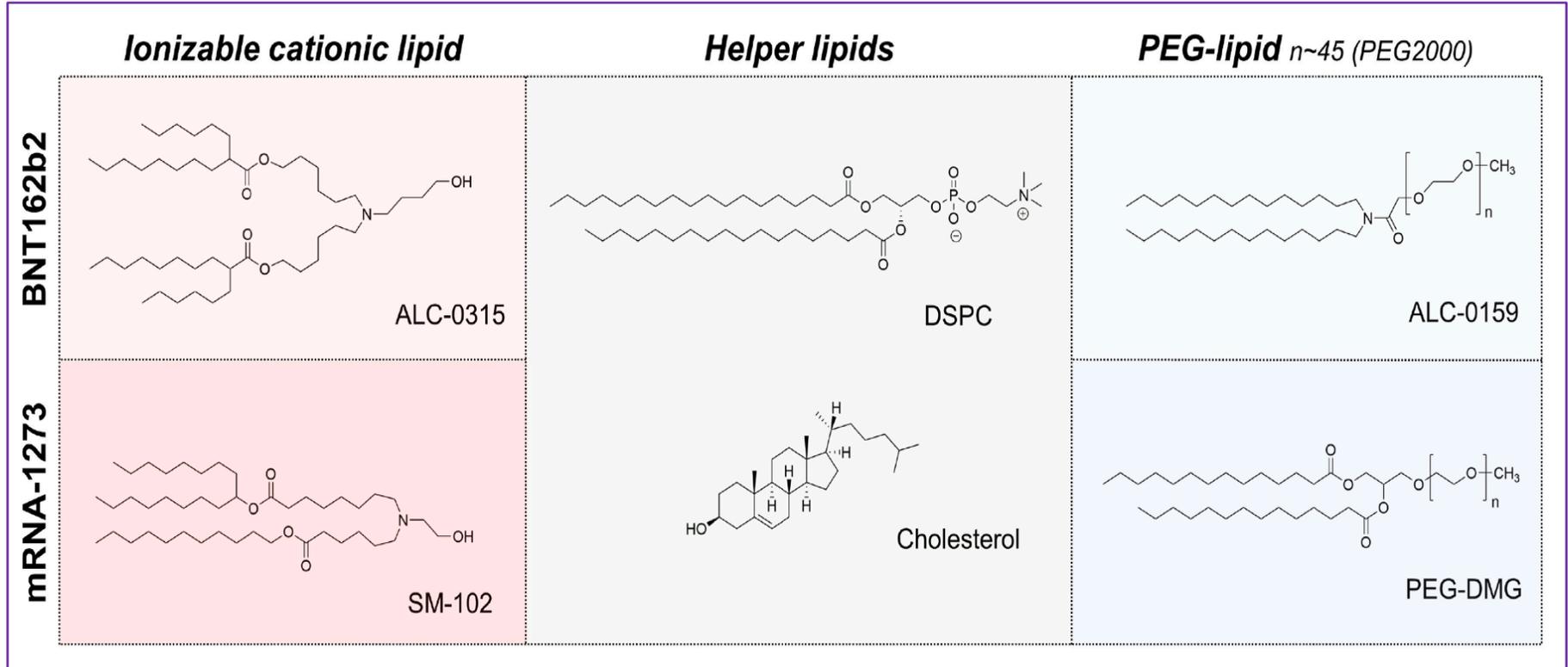
3. Fosfolipídeo

4. Colesterol



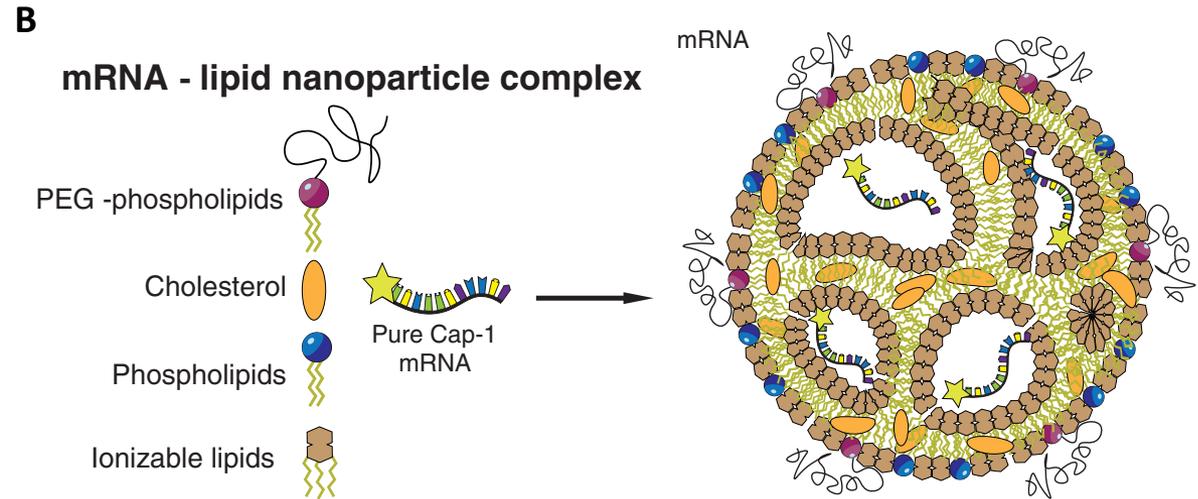
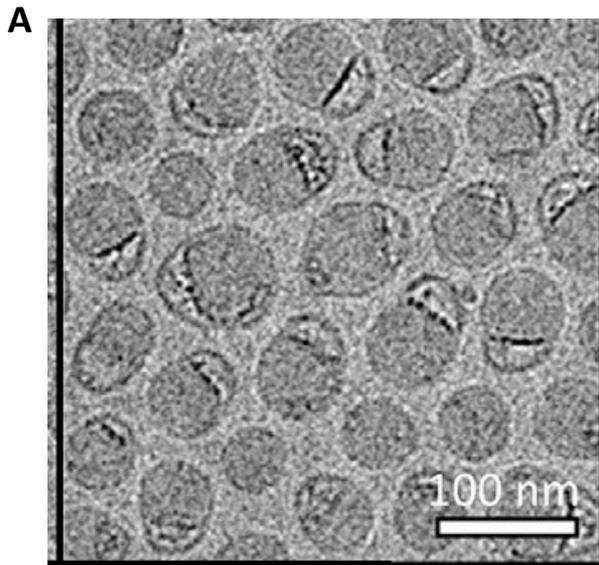
3. Sistema de entrega da molécula de mRNA

Nanopartícula lipídica: vacinas de mRNA anti-COVID-19



3. Sistema de entrega da molécula de mRNA

Nanopartícula lipídica: vacinas de mRNA anti-COVID-19

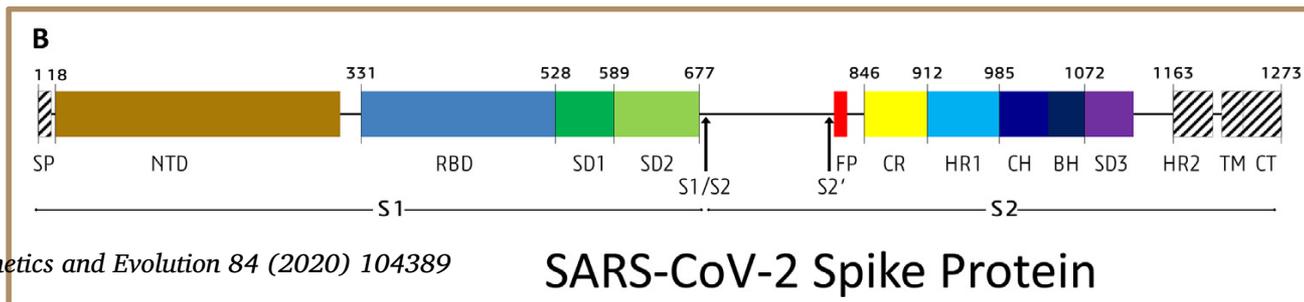
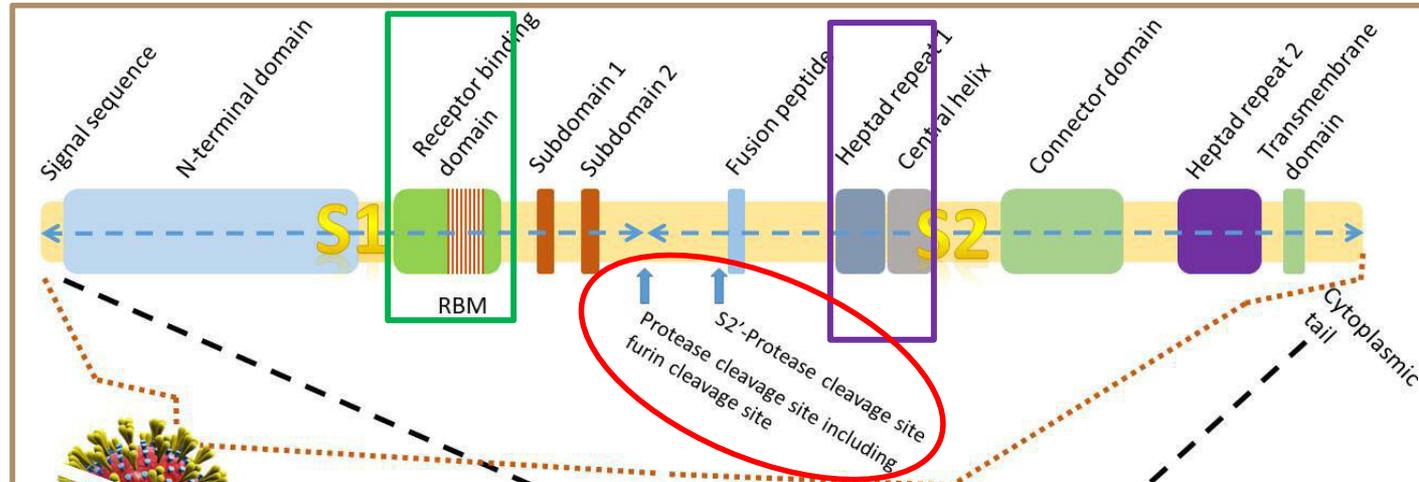


4. mRNA and Protein Spike

Gene of the month

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

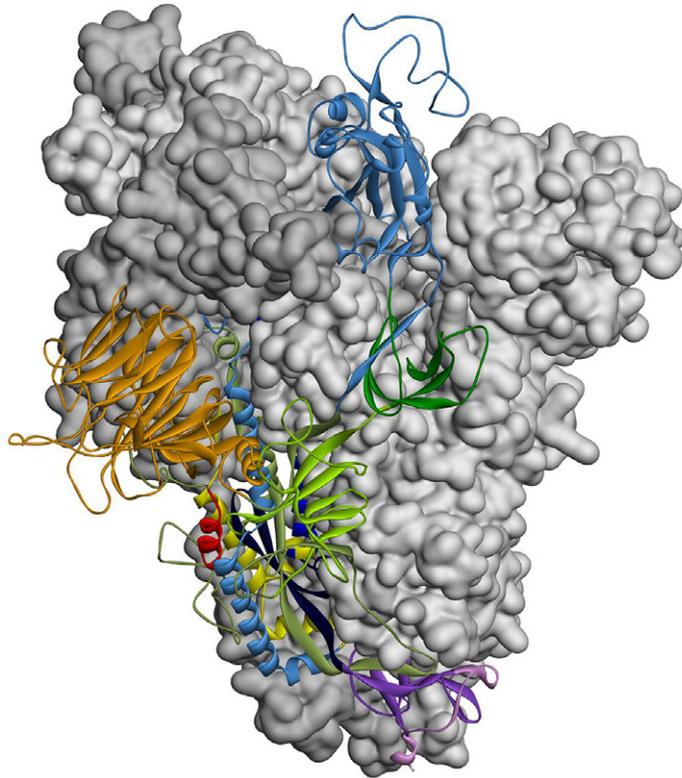
Pillay TS. *J Clin Pathol* 2020;**73**:366–369.



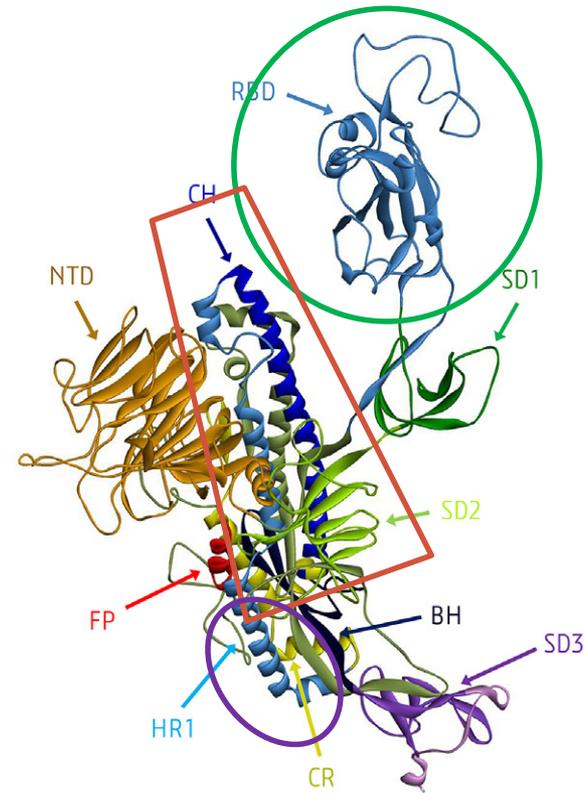
Infection, Genetics and Evolution 84 (2020) 104389

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 AGAAGCTCAATTACCCCTGCATACACATAATTTCTTACACGGTGGTGTATTTACCCGTGAC
41 K V F R S S V L H S T Q D L F L P P F S
121 AAAGTTTTCAGATCCTCAGTTTACATTCACCTCAGAGCTTGTCTTACCTTCTTTCTTTCC
121 N N A T N V I K V C E F Q F C N D P F
61 N V T W F H A I H V S G T N G T K R F D
181 AATGTTACTTGGTCCATGCTATCATGTCTCTGGGACCAATGGTACTAAGAGGTTTGTAT
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 I K V C E F Q F C N D P F
361 AATAACGCTACTAATGTTGTTTAAAGTCTGTGAATTTCAATTTGTAATGATCCATTT
141 L G V Y T H H K N N K S W M E S E F R V Y
421 TTGGGTGTTTTATTACCACAAAACAACAAAAGTTGGATGGAAGTGAGTTCAGAGTTTTAT
161 S S A N N C T F E Y V S Q P F T L M D L E
481 TCTAGTGGCAATAATGCCTTTGAATATGTCTCTCAGCCCTTTCTTATGGACCTTGAA
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 TCGGCTTTAGAACCATTGGTAGATTTGCAATAGGTATTAACACTCAGTAGTTTTCAAAT
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 GGTGCTCAGCTTATTTAGGGTTATCTCAACCTAGGACTTTTCTAATTAATAATTAAT
841 N G C T I T D A V D C A L D P L S E T F 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 CAACCAACAGAAATCTATTGTTAGATTTCCCTAATATACAACTGTGCGCCTTTGGTGAA
341 V F N A C T R F A S V Y A W N R K R I S N
1021 GTTTTTAACGCCACAGATTTGCATCTGTTTTATGCTTGGACAGGAAGAGAATCAGCAAC
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 GGAGTGTCTTACTAAATTAATGATCTCTGCTTTACTAATGTCTACAGGATTCATTT
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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1381 CTCAAAACCTTTGAGAGACATTTCAACTGAAATCTTACGGCCGATGACACACCTTGT
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1441 AATGGTGTGAAGGTTTTAATTTGTTACTTTCCCTTACAATCATATGTTTTCCAACCCACT
501 N G V G Y Q P Y R V T V V L S F E L L H A
1501 AATGGTGTGGTTACCAACCATCAGAGTAGTAGTCTTTCTTTGAACTTCAATCATGCA
521 P A T V C G P K K S T N L V K K N C T C A V N
1561 CCAGCAACTGTTTGTGGACATAAAAAGTCTACTAATTTGGTTAAAACAAATGTGTCAAT
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 ACACCTGAGATCTTGACATTAACACATGTTCTTTGGTGGTCACTGTTTATAACACCA
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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 L 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 AATGTTTTCAAACACGTGAGCGTGTAAATAGGGCTGAACATGTCAACACATAT
661 E C D I P I G A G I C G S Y Q Q T N S
1981 GAGTGTGACATACCCTTGGTGCAGGTATATGCGASATYQACTCAGACTAATTTCT
681 P R R A R S V A S Q S I I A Y T M S L G
2041 CCTCGGGCGGCAAGTGTAGTGTAGTCAATCCATCATTCCTCAGACTATGTCACTTGGT
701 A E N S V A Y S N N S I A I P T N F T I
2101 CGAGAAAATCAGTTGCTTACTCTAATAACTCTATTGGCCATACCCCAADTTTACTATT
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 TACATTTGGTGTATTCAACTGAATGCAGCAATCTTTTGTGCAATATGGCAGTTTTTGT
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 GTTTTGGCAAGTCAACAATAATTTCAAAAACCCCAATTAAGATTTTGGTGGTTTT
801 N F S Q I L P D P S K P S K R S F I E D
2401 AATTTTCAAAATATTACAGATCCATCAAAACCAAGCAAGAGTCAATTTTGAAGT
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 CTTGGTATATTGCTGTAGAGACCTATTTGTGCACAAAAGTTTACCGCCTTACTGTT
861 L P P L L T D E M I A Q Y T S A L L A G
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881 T I T S G W T F G A G A A L Q I P F A M
2641 ACAATCACTCTGTTGGACCTTGGTGCAGGTGCTGCATTAACAATACATTTGCTATG
901 Q M A Y R F N G I G V T Q N V L Y E N G
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2761 AAATGATGGCAACCAATTTAATGCTATTGGCAAAATTCAGACTCACTTTCTTCC
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2821 ACAGCAAGTGCACCTGGAAAACCTCAAGATGTGGTCAACCAAAATGCACAAGCTTTAAAC
961 T L V K Q L S N F G A I S S V L N D I
2881 ACGTGTGTTAAACAACTCCCAATTTGGTCAATTTCAAGTGTNTTAAATGATATC
981 L S R L E K V A E V Q I D R L I T G R
2941 CTTTCAAGCTTGAAGAAGTGAAGTCAAAATGATAGGTGTGATCAGCCAGCAGA
1001 L Q S L Q L 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
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3181 GTCTTCTTGCATGCACTTATGTCCCTGCACAAAGAAAGAACTTCAAACTGCTCCTGCC
1081 I C H D G K A H F P R E G V F V S N G T
3241 AITGTGATGATGAAAGACACACTTCCCTGGAAGGTGCTTTGTTTCAAATGGCACA
1101 H W F V T Q R N F Y E P Q I I T D N T
3301 CACTGGTTTGTACACAAAGGAATTTTATGAACCAAAATCATTTACTACAGACACACA
1121 F V S G N C D V V I G I V N N T V Y D P
3361 TTTGTGCTGTAACCTGTGATTTGTAATAGGAATGTCAACACACAGTTTATGCTCT
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 AAAGAAATGACCCCTCAATGAGGTTGCCAAGAAATTAATGAATCTCTCATCGATCTC
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1201 Q E L G K Y E Q Y I K W P W Y I W L G F
3601 CAAGAAGTGGAAAGTATGAGCAGTATATAAATGGCCATGGTACATTTGGCTAGGTTTT
1221 I A G L I A I V M V T I M L C M T S C
3661 ATAGCTGGCTGATTGCCATAGTAAAGTGGACAAATTTGCTTTGCTATGACCAAGTTC
1241 C S C L K G C C S C G S C C K F D E D
3721 TGTAGTTGCTCAAGGCTGTTGTTCTTGGTATGCTGCAAAATTTGATGAAGACAG
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])



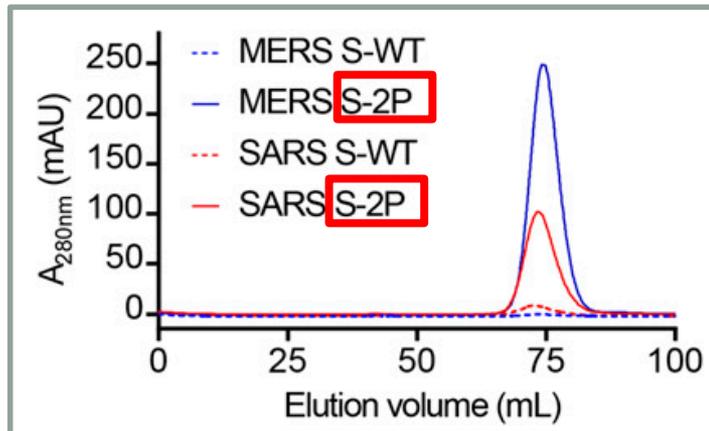
Pfizer e BioNTech



Moderna e Pfizer

4. mRNA and Protein Spike

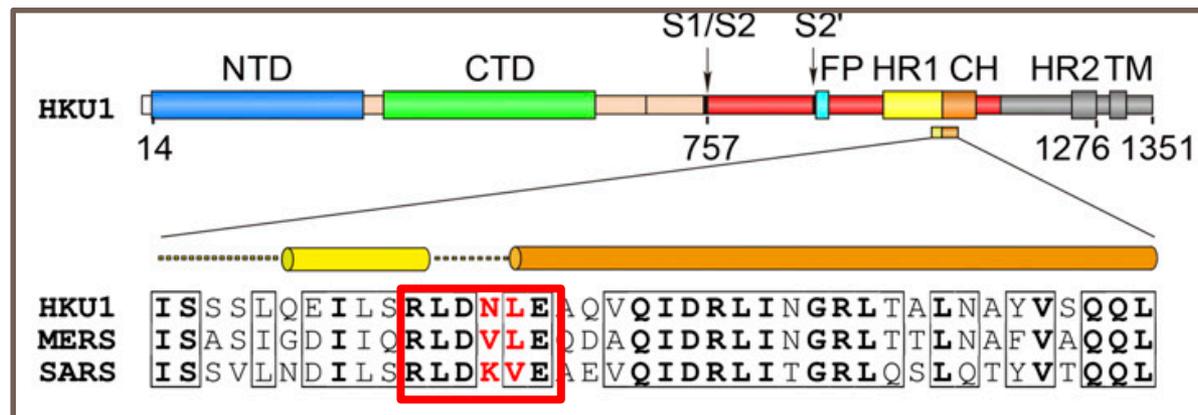
Características outras spikes: Pallessen *et al* 2017



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

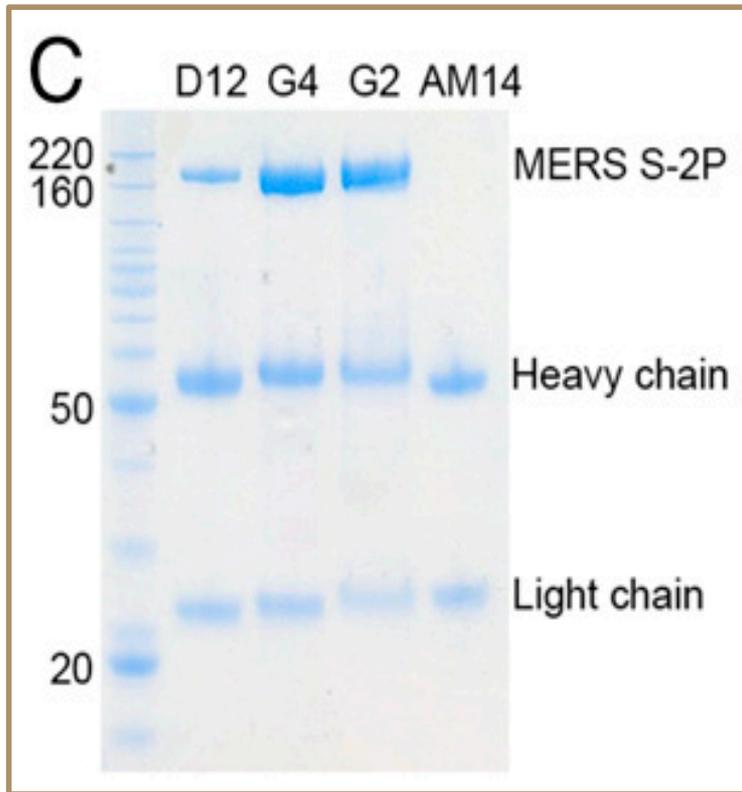
Jesper Pallesen^{a,1}, Nianshuang Wang^{b,1,2}, Kizzmekia S. Corbett^{c,1}, Daniel Wrapp^b, Robert N. Kirchdoerfer^a, Hannah L. Turner^a, Christopher A. Cottrell^a, Michelle M. Becker^d, Lingshu Wang^e, Wei Shi^e, Wing-Pui Kong^e, Erica L. Andres^d, Arminja N. Kettenbach^{b,f}, Mark R. Denison^{d,g},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



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



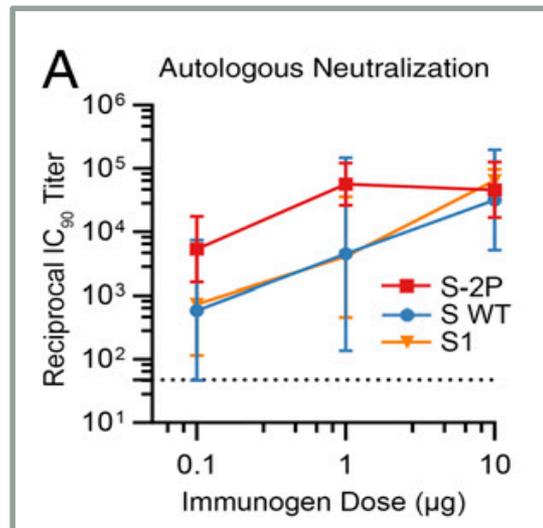
Análise em SDS/PAGE da proteína MERS-S-2P expressa em células 293-T mostrando que mantém a capacidade de reconhecer diferentes anticorpos. AM14 – anticorpo controle.

4. mRNA and Protein Spike

Spike: imunogenicidade - outros coronavirus

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

Jesper Pallesen^{a,1}, Nianshuang Wang^{b,1,2}, Kizzmekia S. Corbett^{c,1}, Daniel Wrapp^b, Robert N. Kirchdoerfer³, Hannah L. Turner³, Christopher A. Cottrell³, Michelle M. Becker^d, Lingshu Wang^e, Wei Shi^e, Wing-Pui Kong^e, Erica L. Andres^d, Arminja N. Kettenbach^{b,4}, Mark R. Denison^{d,9}, James D. Chappell^d, Barney S. Graham^c, Andrew B. Ward^{a,2}, and Jason S. McLellan^{b,2}

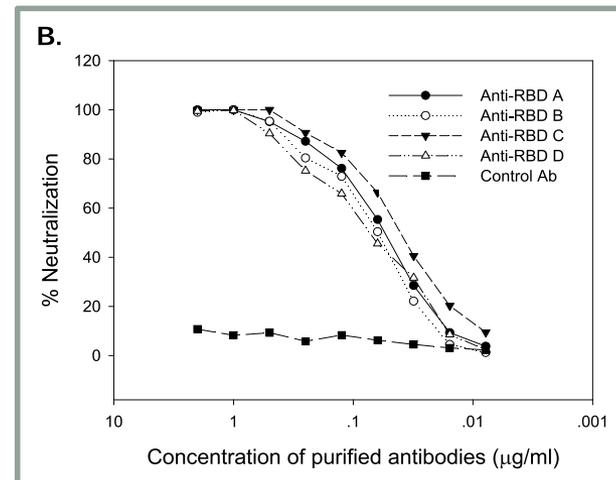


Spike 2P [MERS]
atividade neutralizante
no soro

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^{1†}, Lifeng Liu^{2†}, Lanying Du³, Chao Zhang¹, Shibo Jiang³, Taisheng Li^{2*}, Yuxian He^{1*}



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

```
1 M F V F L V L L L P L V S S Q C V N L T T 601 G T N T S N Q V A V L Y Q D V N C T E V 1201 Q E L G K Y E Q Y I K W P W Y I W L G F
21 R T Q L P P A Y T N S F T R G V Y Y P D 1801 G G A C A A A A C T T C T A A C C A G G T T G C T T T T A T C A G G A T G T T A A C T G C A C A G A A G T C 3601 C A A G A A C T T G G A A G T A T G A G C A G T A T A T A A A A T G G C C A T G G T A C A T T T G G T A G G T T T
61 A G A A C T C A A T A C C C C T G C A T A C A A T T C T T C A C A C G T G T T T A T T A C C C T G A C 1861 C C T G T G C T A T T C A T G C A G A T C A A C T A C T C C T A C T T G G C G T G T T A T T C T A C A G G T T C 1221 I A G L I A I V M V T I M L C C M T S C
41 K V F R S S V L H S T Q D L F L P F F S 641 N V F Q T R A G C C L I G A E H V N N S Y 3661 A T A G C T G G C T T G A T G C C A T A G T A A T G G T G A C A A T T A T G C T T T G C T G T A T G A C C A G T T C
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1681 C C T T T C C A A C A A T T T G C A G A G A C A T T G C T G A C A C T A C T G A T G C T G C C G T G A T C C A C A G
1181 K E I D R L N E V A K N L N E S L I D L
581 T L E I L D I T P C S F G G V S V I T P 3541 A A A G A A A T T A C C G C C T C A A T G A G G T T G C C A A G A A T T T A A A T G A A T C T C A T C G A T C T C
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5. mRNA-1273 – MODERNA – Resposta humoral

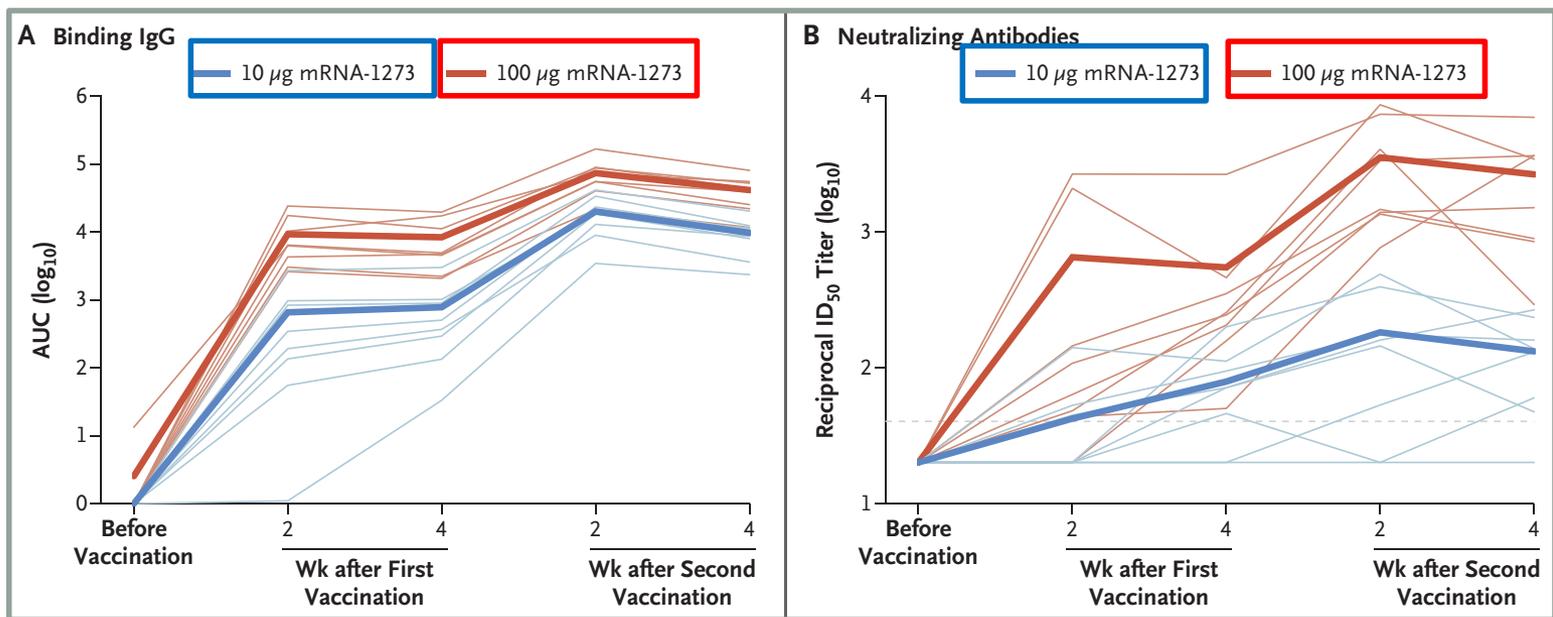
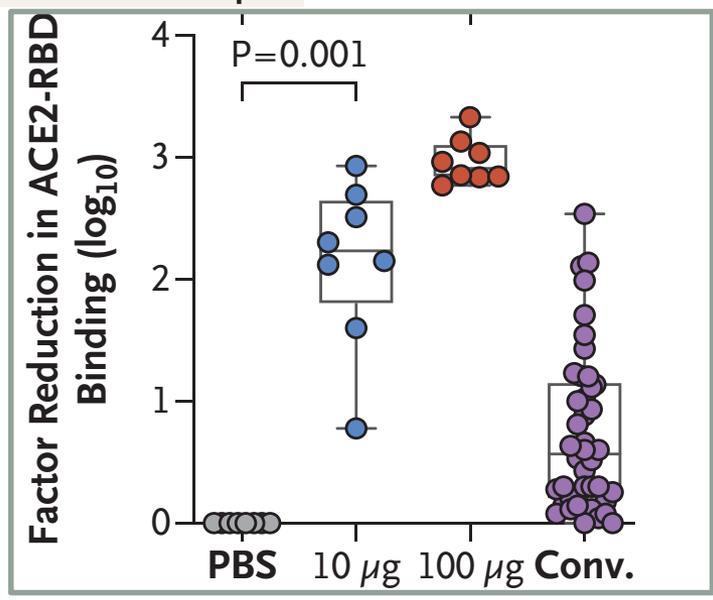
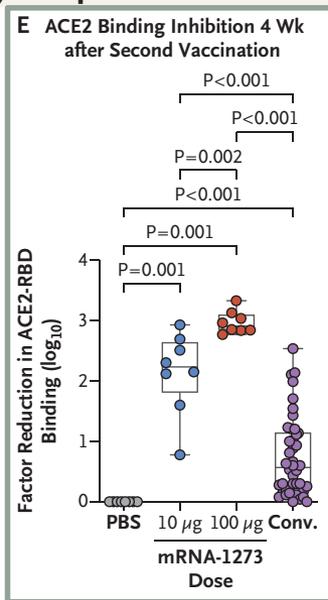


Figure 1. Antibody Responses after mRNA-1273 Vaccination in Rhesus Macaques.



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

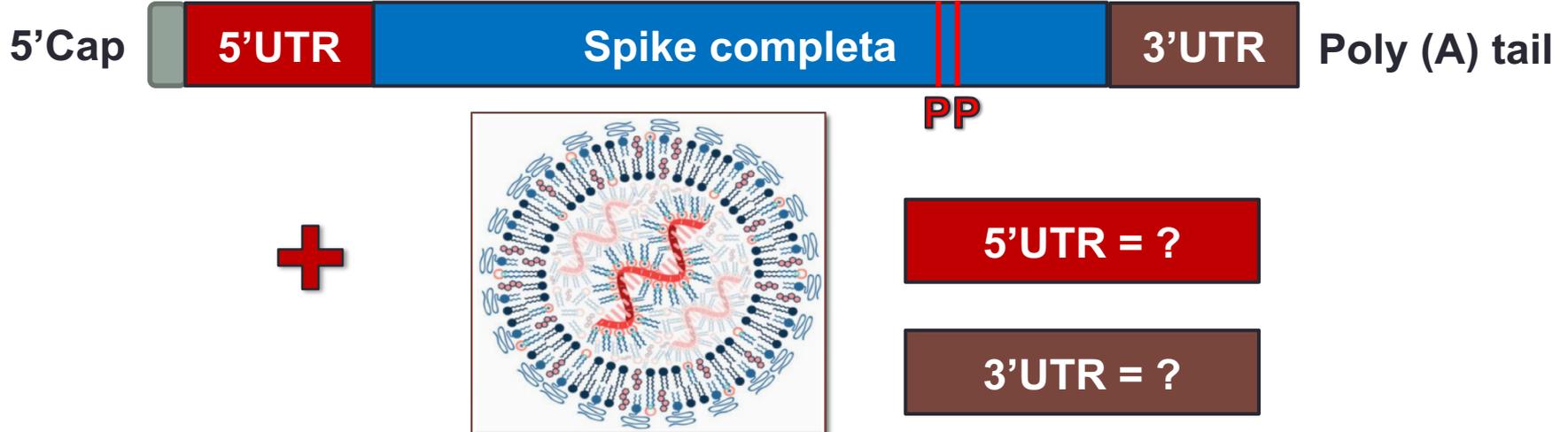
RESULTS

First, we evaluated temporal SARS-CoV-2 S-2P-specific antibody responses after vaccination. IgG binding to the conformationally defined prefusion S-2P protein^{25,26} 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?

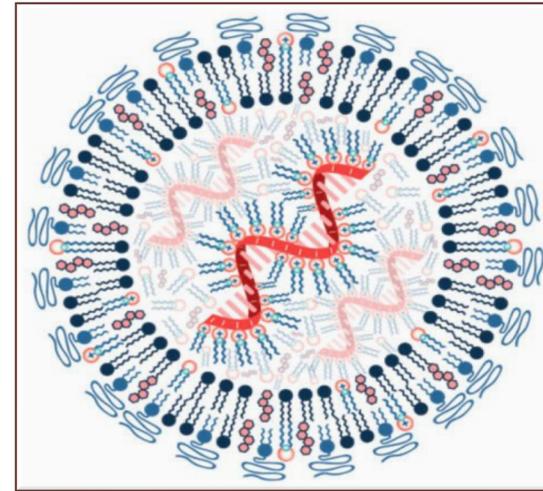
6. mRNA vaccine **BNT162b2**

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>

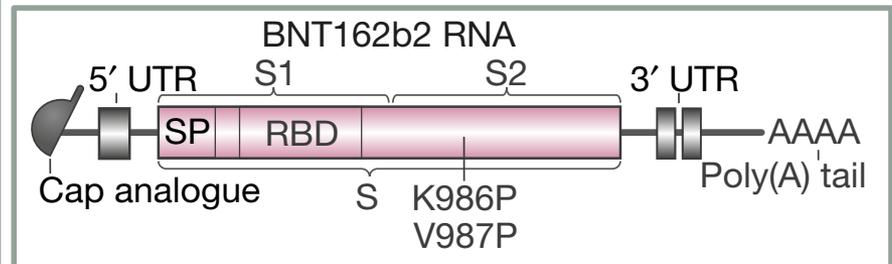
Received: 1 September 2020

Accepted: 20 January 2021

Published online: 1 February 2021

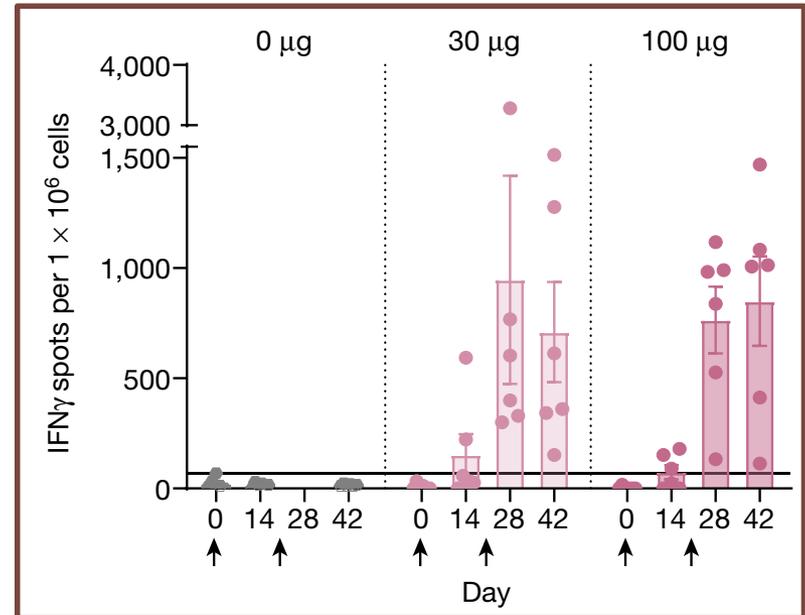
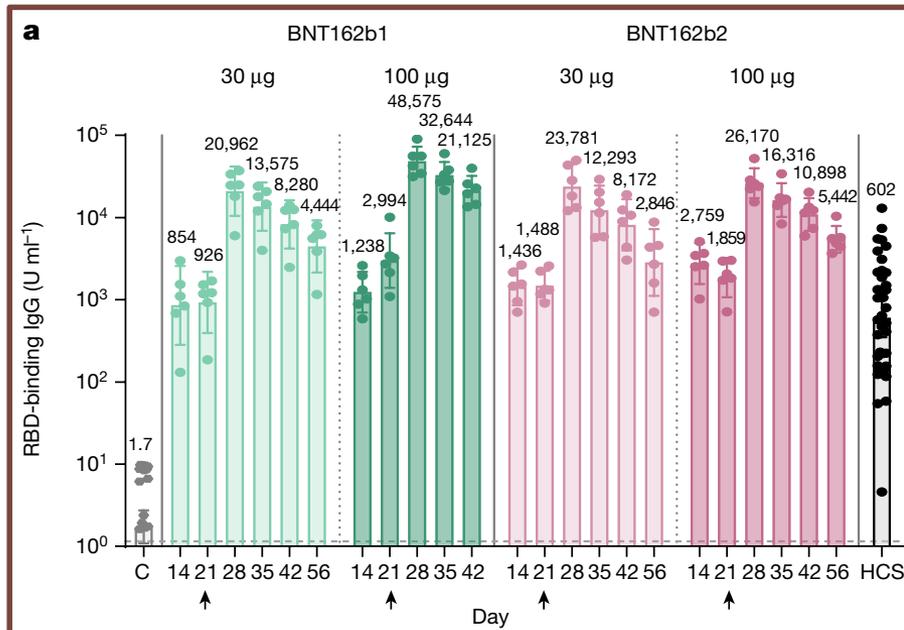
Check for updates

Annette B. Vogel^{1,2,3}, Isis Kanevsky^{2,3,4}, Ye Che^{3,5}, Kena A. Swanson², Alexander Muik¹, Mathias Vormehr¹, Lena M. Kranz², Kerstin C. Walzer¹, Stephanie Hein¹, Alptekin Güler¹, Jakob Loschko¹, Mohan S. Maddur², Ayuko Ota-Setlik², Kristin Tompkins², Journey Cole⁴, Bonny G. Lui², Thomas Ziegenhals¹, Arianne Plaschke², David Eisel¹, Sarah C. Dany¹, Stephanie Fesser¹, Stephanie Erbar¹, Ferdia Bates¹, Diana Schneider¹, Bernadette Jesionek¹, Bianca Sänger¹, Ann-Kathrin Wallisch¹, Yvonne Feuchter¹, Hanna Junginger¹, Stefanie A. Krumm¹, André P. Heinen¹, Petra Adams-Quack¹, Julia Schlereth¹, Stefan Schille¹, Christoph Kröner¹, Ramón de la Caridad Güimil García¹, Thomas Hiller¹, Leyla Fischer¹, Rani S. Sellers², Shambhunath Choudhary², Olga Gonzalez², Fulvia Vascotto², Matthew R. Gutman², Jane A. Fontenot², Shannan Hall-Ursone², Kathleen Brasky², Matthew C. Griffor², Seungil Han², Andreas A. H. Su¹, Joshua A. Lees², Nicole L. Nedoma², Ellene H. Mashalidis², Parag V. Sahasrabudhe², Charles Y. Tan², Danka Pavliakova², Guy Singh², Camila Fontes-Garfias², Michael Pride², Ingrid L. Scully², Tara Ciolino², Jennifer Obregon², Michal Gazi², Ricardo Carrion Jr¹, Kendra J. Alfson², Warren V. Kalina², Deepak Kaushal², Pei-Yong Shi², Thorsten Klamp¹, Corinna Rosenbaum¹, Andreas N. Kuhn¹, Özlem Türeci¹, Philip R. Dormitzer², Kathrin U. Jansen² & Ugur Sahin^{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}



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- γ .

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

6. mRNA vaccine **BNT162b2**

The NEW ENGLAND JOURNAL *of* MEDICINE

ESTABLISHED IN 1812

DECEMBER 31, 2020

VOL. 383 NO. 27

Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine

Fernando P. Polack, M.D., Stephen J. Thomas, M.D., Nicholas Kitchin, M.D., Judith Absalon, M.D., Alejandra Gurtman, M.D., Stephen Lockhart, D.M., John L. Perez, M.D., Gonzalo Pérez Marc, M.D., Edson D. Moreira, M.D., Cristiano Zerbini, M.D., Ruth Bailey, B.Sc., Kena A. Swanson, Ph.D., Satrajit Roychoudhury, Ph.D., Kenneth Koury, Ph.D., Ping Li, Ph.D., Warren V. Kalina, Ph.D., David Cooper, Ph.D., Robert W. Frenc, Jr., M.D., Laura L. Hammitt, M.D., Özlem Türeci, M.D., Haylene Nell, M.D., Axel Schaefer, M.D., Serhat Ünal, M.D., Dina B. Tresnan, D.V.M., Ph.D., Susan Mather, M.D., Philip R. Dormitzer, M.D., Ph.D., Uğur Şahin, M.D., Kathrin U. Jansen, Ph.D., and William C. Gruber, M.D., for the C4591001 Clinical Trial Group*

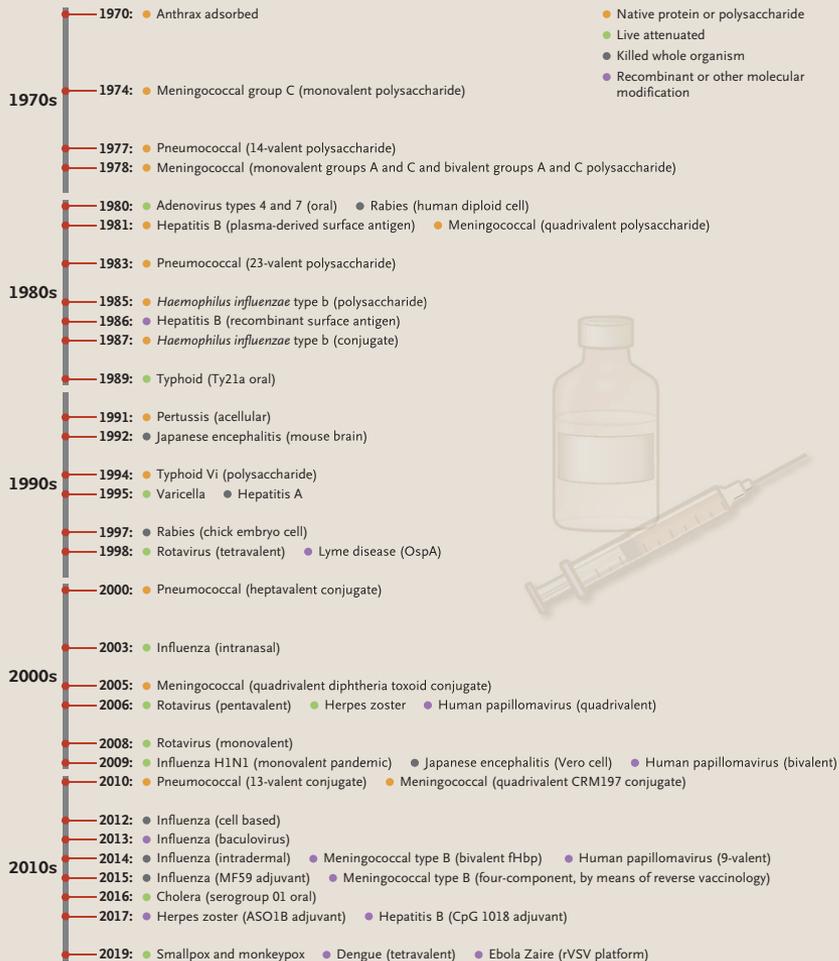
RESULTS

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



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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.