Therapeutic Targeting of SARS-CoV-2

Group 3

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Coronaviruses and SARS-CoV-2

- **Coronaviruses**
  - Bats act as viral reservoirs[1]
  - Common traits[2]
    - Positive-sense, single-stranded RNA genome
    - Spherical shape
    - Structural and non-structural proteins
    - Genome organization

- **Recent bat-coronavirus outbreaks**[3]
  - SARS (2003)
  - MERS (2012)
  - SADS (2016)
  - ❖Covid-19 (2019)

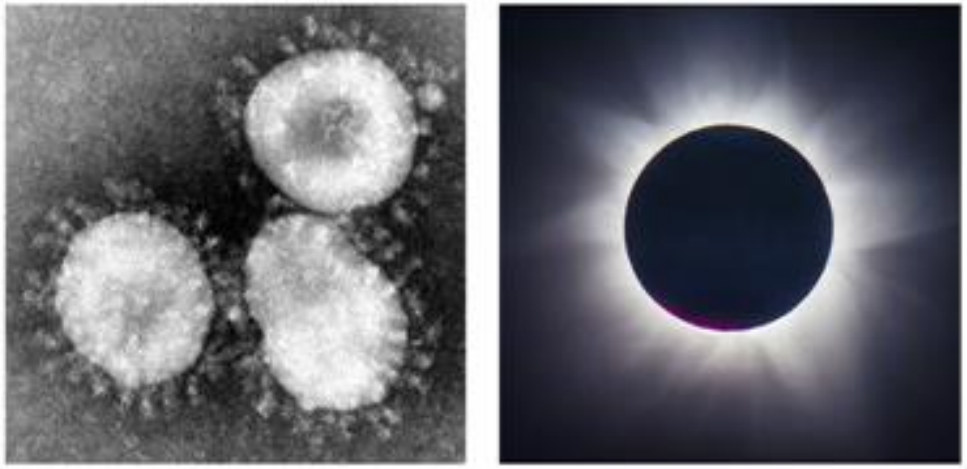


Image from Aronson, 2020

Coronavirus Genome

- Positive-sense, single-stranded RNA genome (27-32 kb)[3]

- **2/3 Non-structural proteins**[3]
  - ORF1a and ORF1b polyproteins

- **1/3 Structural proteins + accessory factors**[3]
  - Spike
  - Envelope
  - Membrane
  - Nucleocapsid

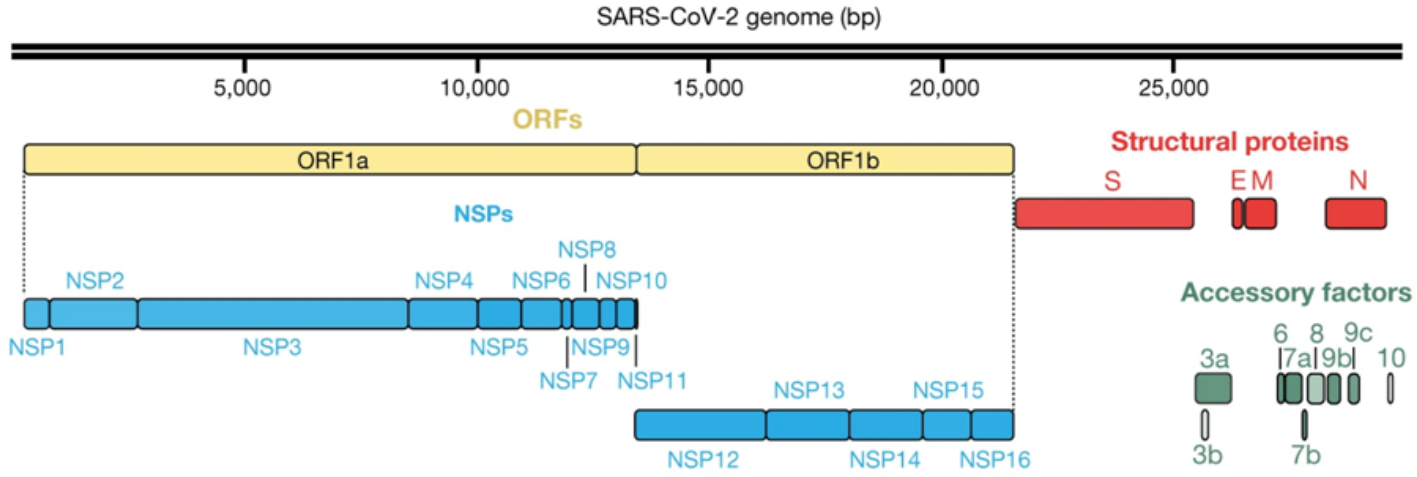


Image from Gordon et al., 2020

Coronavirus Structure

- Spherical[2]
- 125 nm diameter[2]
- **Four structural proteins**[2]
  - **Spike (S) -** Mediates attachment of the virus to host cells
  - **Envelope (E)**
  - **Membrane (M)**
  - **Nucleocapsid (N)**

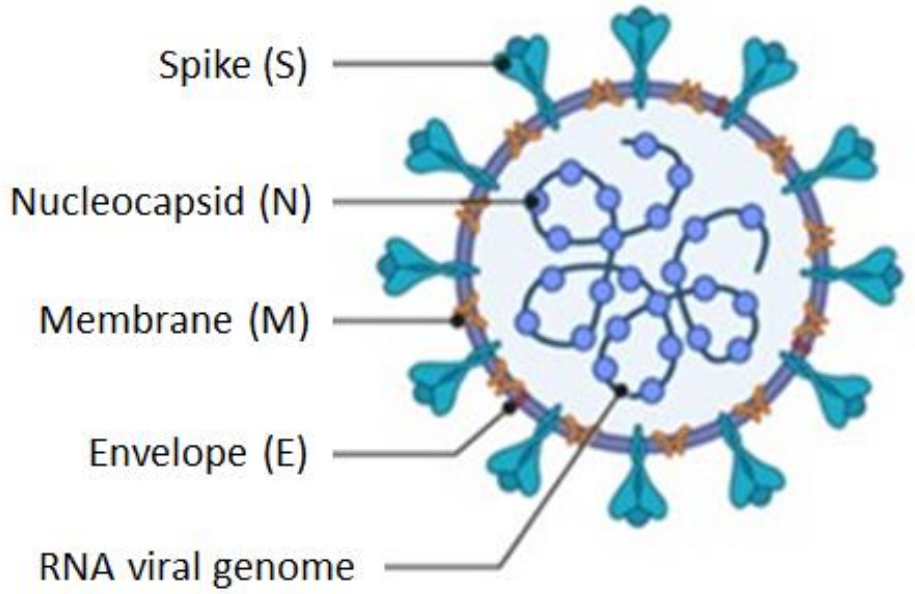


Image from King et al., 2020

SARS-CoV-2 Infection and the Spike Protein

- Binding of SARS-CoV-2 to host cell is mediated by **S protein** homotrimers[4]
- SARS-CoV-2 **S protein** contains two subunits: **S1** and **S2**[4]
- The receptor binding domain (RBD) of the **S1** subunit binds the **ACE2** receptor[4]
- The **S2** subunit anchors the virus to the host cell membrane[4]

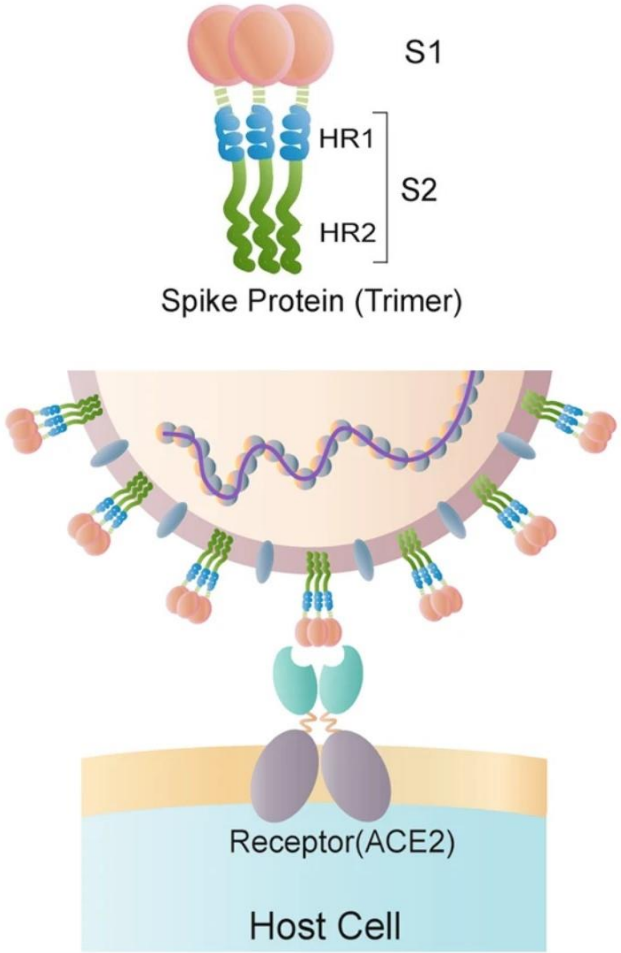


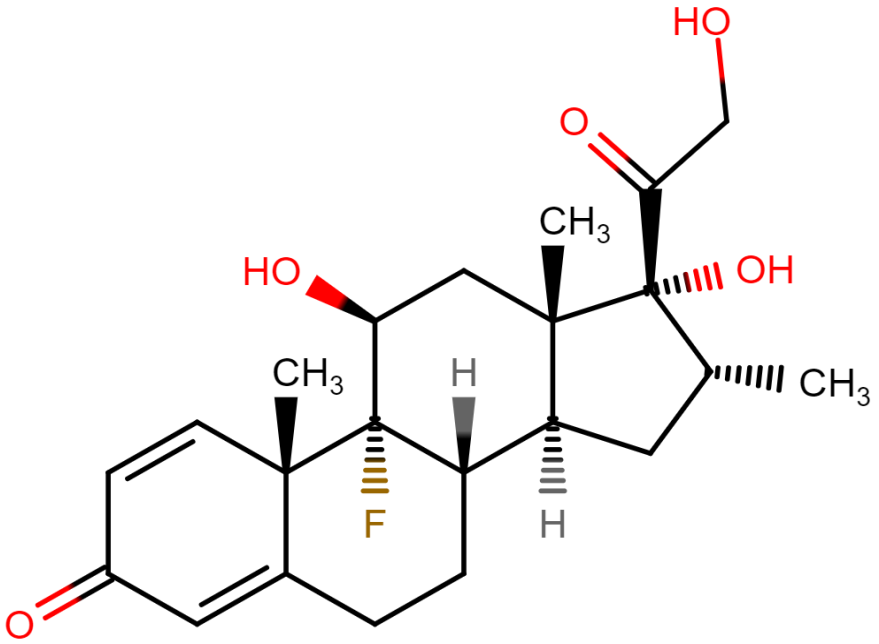
Image from Huang et al., 2020

Viral Therapies

- **Vaccines**[5,6]
  - long-term protection
  - Protection is not immediate

- **Need for alternative therapies**
  - Rapid protection against viral infection
  - Alleviate disease symptoms

- **Immunotherapeutics**[7]
- **Small molecules**[8]
  - e.g., Dexamethasone[9]



Dexamethasone

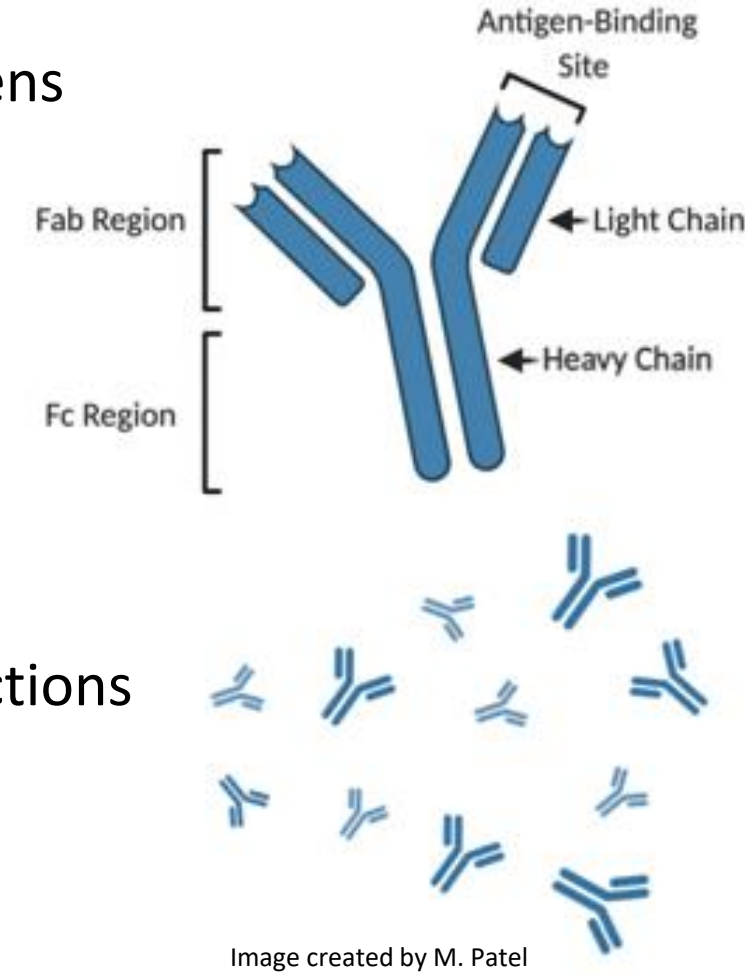
Image created by M. D'Ercole using Chem Space

Monoclonal Antibodies (mAbs)

- **Purpose:** The use of antibodies to target specific antigens

- **Targets:**[10]
  - Cell Surface Antigens
  - Plasma Proteins

- **Therapeutic Uses:**
  - Cancer therapy, Autoimmune Diseases & Viral Infections



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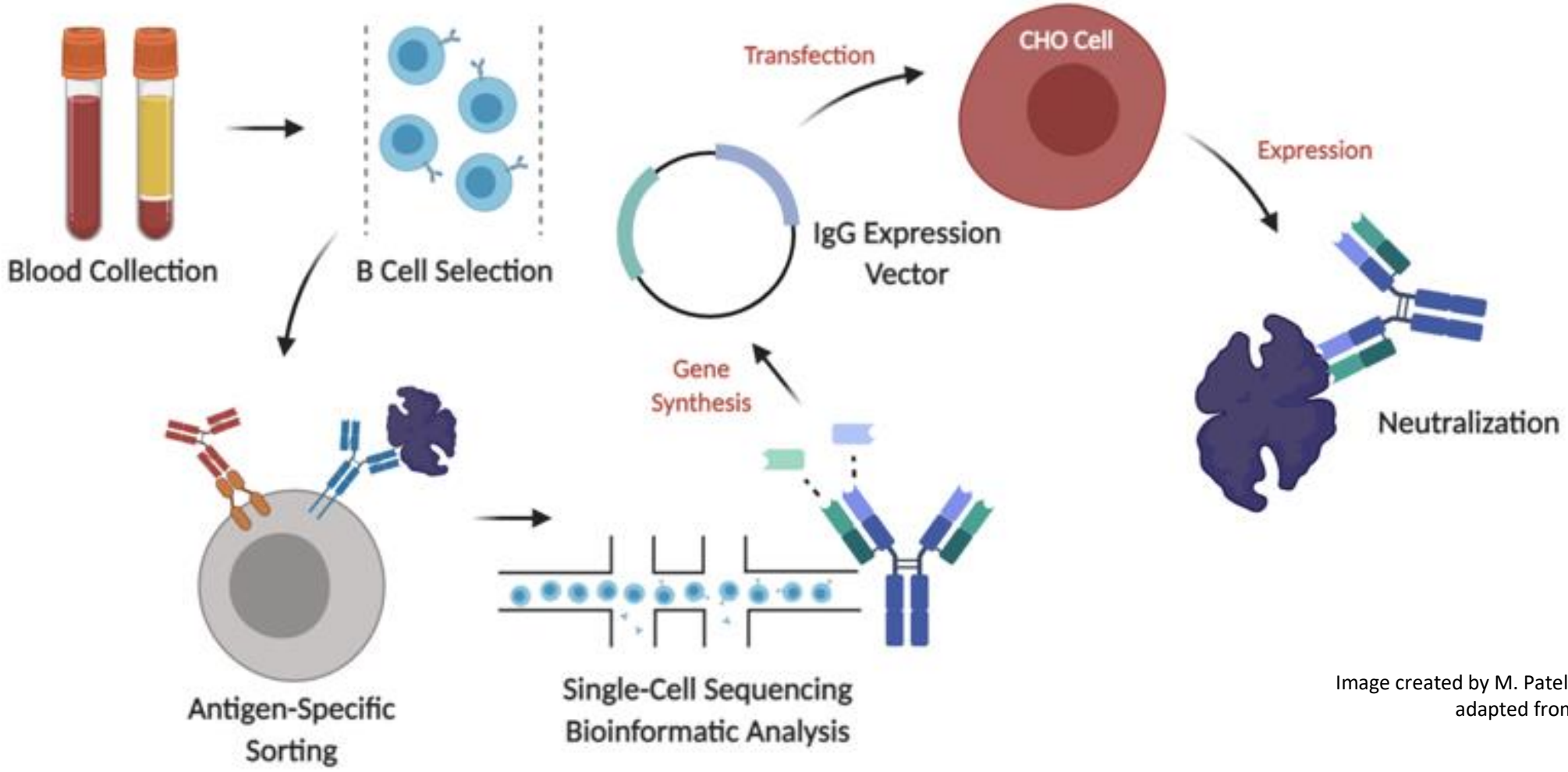
Overview of Human mAbs (-umab) Production



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Advantages & Disadvantages to mAbs

Advantages[11,12]	Disadvantages[11,12]
• High-specificity for a single epitope of an antigen	• Mono-specificity limits their applications
• Renewably generate once suitable hybridoma or CHO cells are developed	• Minor changes in antigen epitope structure affects the function of mAbs
• mAbs are homogenous & highly consistent	• Production is expensive
• Immediately treat an existing SARS-CoV-2 infection	• Protection is short-term (Weeks to Months)

Cross-Neutralization of SARS-CoV

- One focus of therapeutic efforts: blocking infection capacity of virus using specific human mAb[7]
  - **Neutralizing Antibodies**
- One specific neutralizing mAb is a potential candidate for further testing as a therapeutic agent[7,13]
  - Capable of neutralizing SARS-CoV-2 and several SARS-CoV pseudoviruses
  - Identified from B cells of a SARS-CoV patient

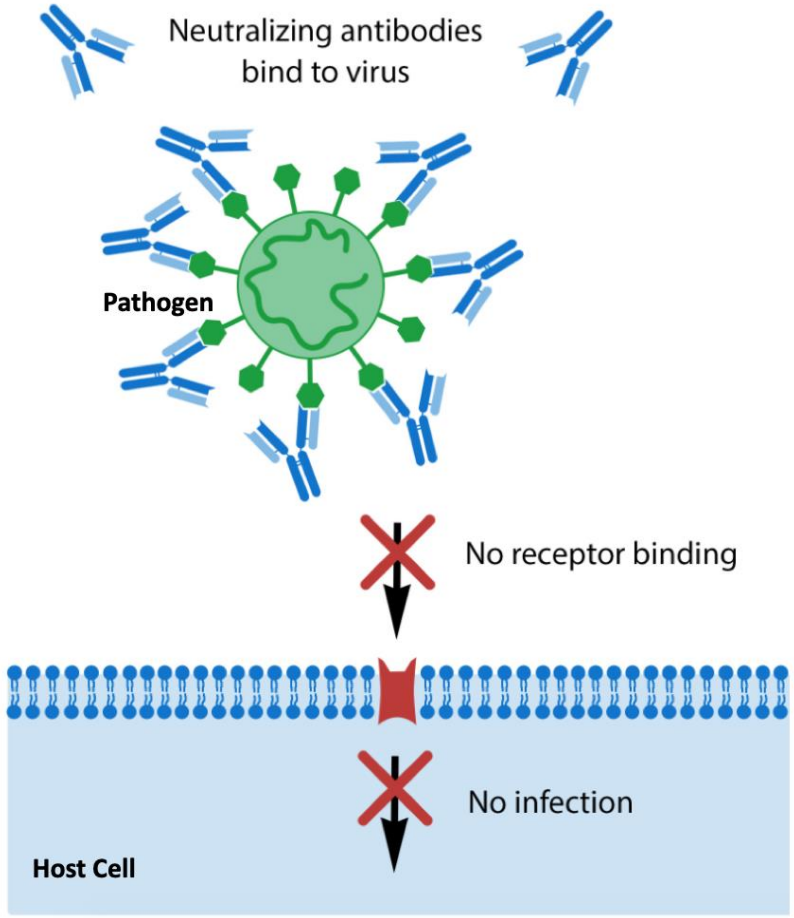


Image created by A. Nagi using BioRender, adapted from Ringe et al., 2013

Neutralizing Monoclonal Antibodies

- **S glycoprotein** of SARS-CoV-2 is the main focus for therapeutic agents[7]
- The neutralizing antibody in focus: **S309**
  - specific IgG antibody, which can bind to immobilized **SARS-CoV-2 S^B domain** and **S glycoprotein** with significant affinities[7]
- One or more IgG-specific mechanisms might be involved[7,14]:
  - **Cross-linking** of S-glycoprotein trimer
  - **Steric hindrance**
  - **Aggregation of virions**[15]

Molecular surface representation of SARS-CoV-2 S-glycoprotein

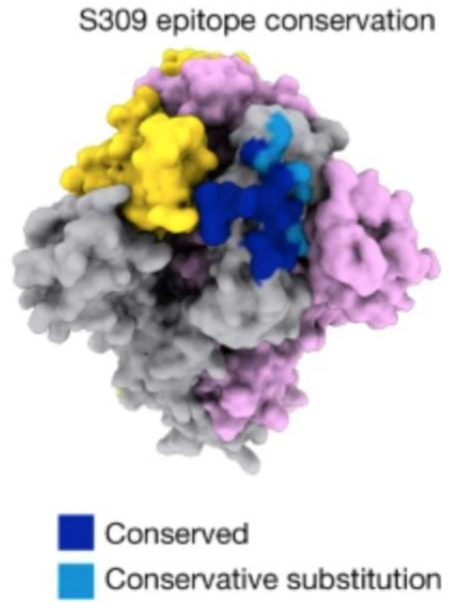


Image adapted from Pinto et al., 2020

Small Molecules as Therapeutics

- Organic compounds with low molecular weights
- **Benefits[16]:**
  - Can test multiple variants with high throughput screening methods
  - Cheap to manufacture and administered orally

- Candidates for **SARS-CoV-2**:
  1. Nucleoside analog **RdRp** inhibitors[8]
  2. **Mpro** inhibitors[8]
  3. Blocking **S protein-ACE-2** binding[8]
  4. **Corticosteroids** for reduced inflammation[17]

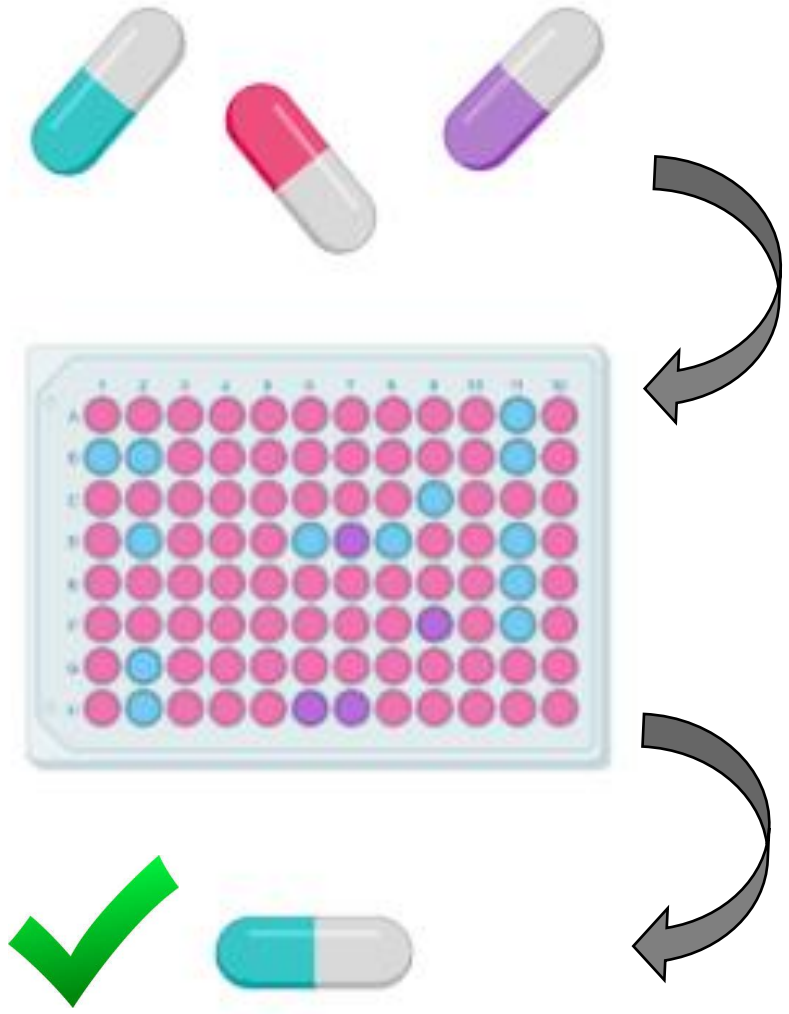


Image made using BioRender

1. Nucleoside Analogs as RdRp Inhibitors

- RdRp is essential for SARS-CoV-2 genome replication and production of viral proteins[2]

- **How it Works[18]:**
  - Nucleoside analogs (e.g., **Remdesivir & Sofosbuvir**) bind to nsp12 region of RdRp

- **Limitations[19]:**
  - Conversion from prodrug to active form produces toxic metabolites
  - Not cell-specific in delivery

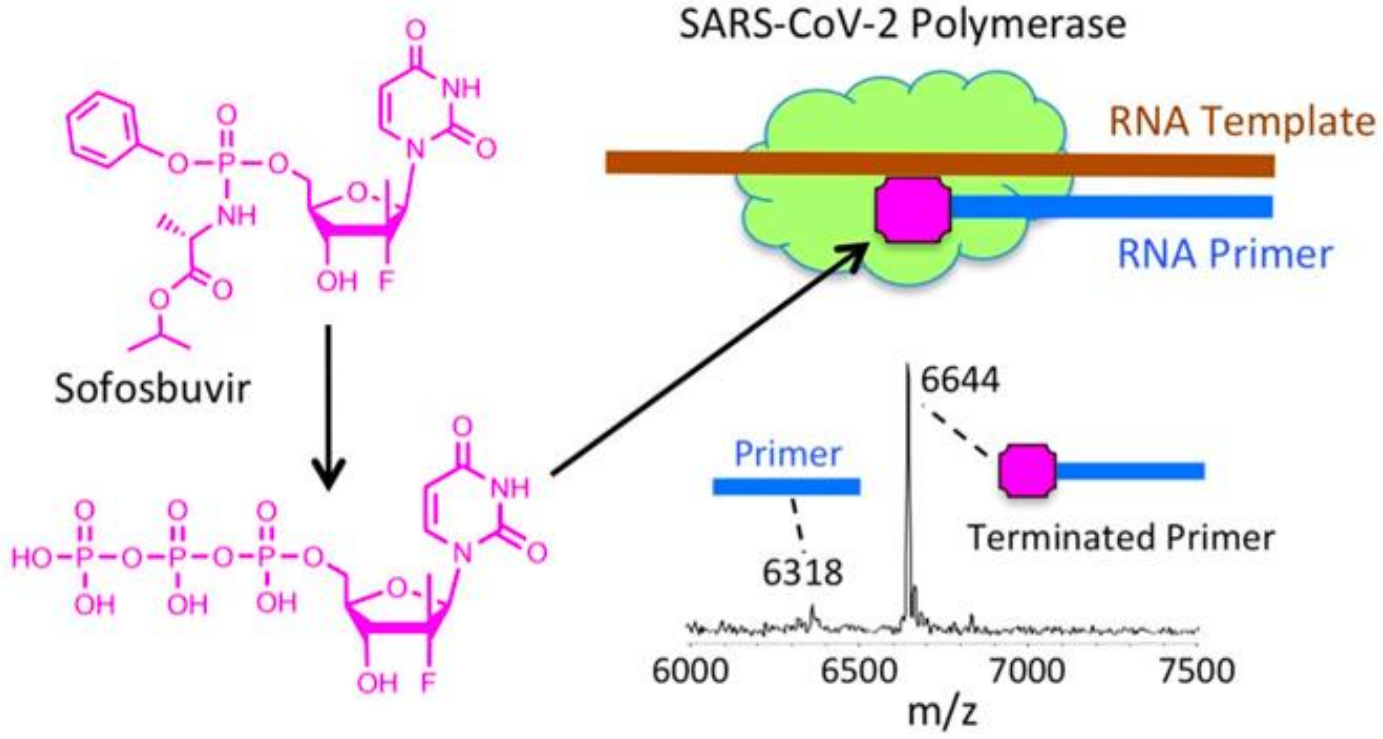


Image from Chien et al., 2020

2. Mpro (Main Protease) Inhibitors

- Main protease (Mpro) is essential for post-translational modifications of viral proteins[20]

- **How it Works[20]:**
  - **Ebselen** covalently binds to catalytic region of Mpro

- **Limitations[21]:**
  - Differences in catalytic site of SARS-CoV and SARS-CoV-2

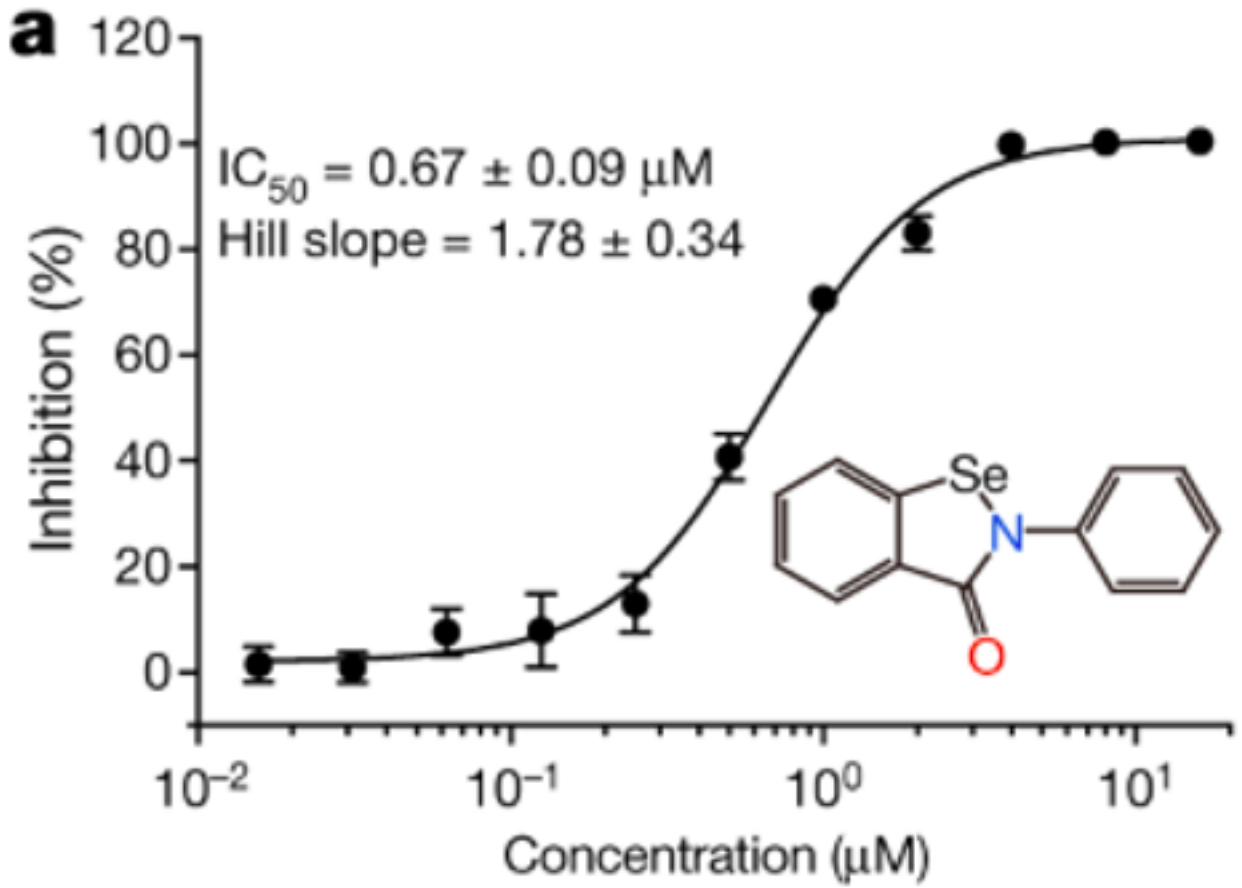


Image from Jin et al., 2020

3. Blocking S Protein-ACE2 Interaction

- S-protein and ACE-2 binding is essential for viral entry into cells[2]
- **How it Works[22]:**
  - **Reproterol** binds to His34 and Asp30 residues of ACE-2
- **Limitations[22]:**
  - More research must be done
  - Most studies focus on neutralizing antibodies with the same purpose

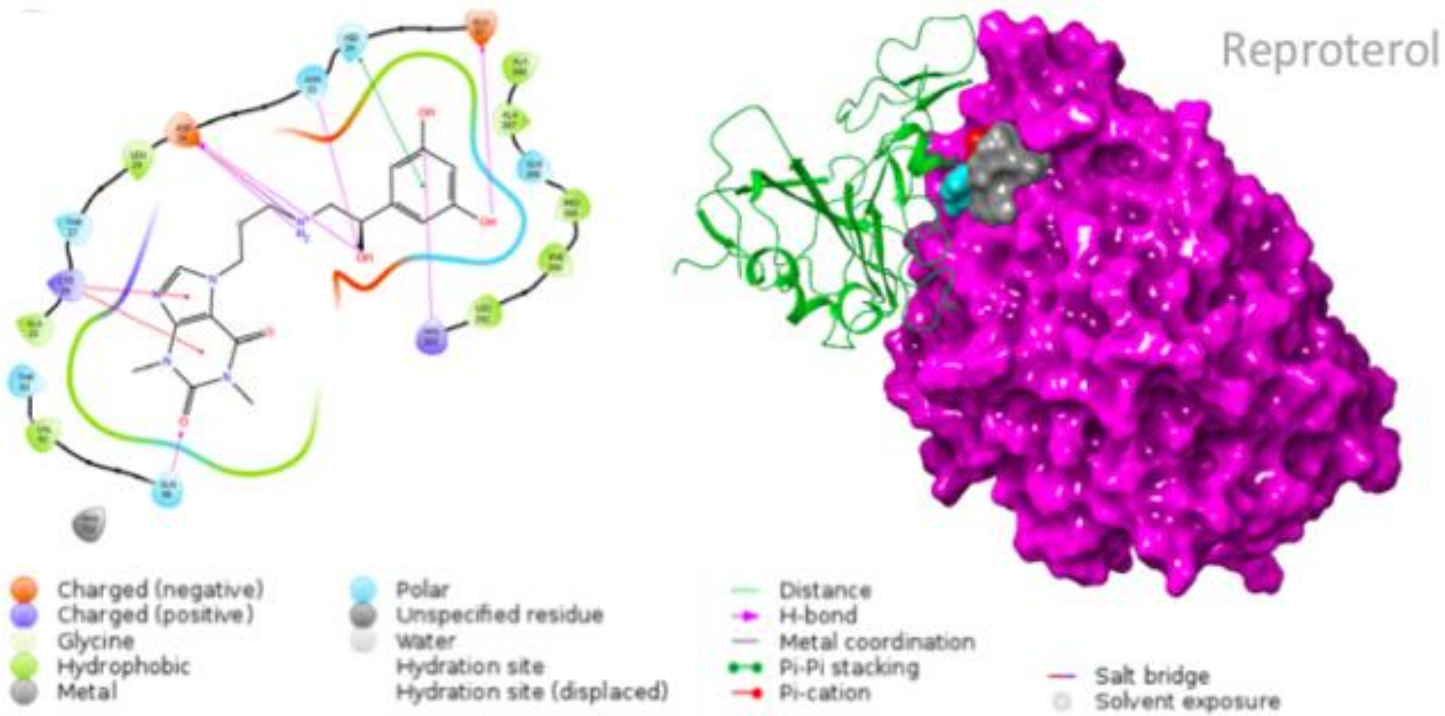


Image from Smieszek et al., 2020

4. Corticosteroids: Reduce Inflammation

- Corticosteroids like **hydrocortisone** and **dexamethasone** are being considered for COVID-19 severe cases[23]

- **How it Works[17]:**
  - Reduce inflammation caused by proinflammatory cytokines (e.g., IL-1)

- **Limitations[17]:**
  - Research findings for COVID-19 use are inconsistent
  - Chance of secondary infections

Image from Denise, 2020

Other Immunotherapies

- **Purpose**: control immune responses in diverse directions to achieve therapeutic effect [24]
- **Immunological meditated disorders** (e.g., autoimmune disease, inflammatory disorders, infectious diseases) [24]
- The use of
  - Drugs (e.g., immunosuppressors) [25]
  - Biologicals (e.g., cytokines, antibodies) [25]
  - Immunizations (e.g., therapeutic vaccines) [25]
- **Limitation**
  - Side effects [25]
  - Does not work for everyone [25]

1. Cytokine Antagonists

- Anti-inflammatory drugs limit inflammatory processes [26]
- **SARS-CoV-2**
  - **IL-1β** - Important cytokine induce further inflammatory cytokines production [26]
- **Recombinant IL-1 receptor antagonist (rIL-1Ra, Anakinra)**
  - Immunosuppressive drugs [26]
  - Blocks the binding of both IL-1α and IL-1β to the IL-1 receptor [26]
  - Inhibits IL-1 pro-inflammatory effects [26]
- **Limitation**
  - Target treatment to individuals with **hyperinflammation** [27]
  - Risk of harm by potentially targeting beneficial inflammation [27]

Image from MedPage Today, n.d.

2. Passive immunotherapy

- **Convalescent plasma treatment**
  - Passive polyclonal antibody administration [26]
  - Supply immunity against viral infection & improves survival rate [26]
- **SARS-CoV-2**
  - Immunomodulatory effect via inhibiting cytokine storm [26]
  - Presence of antibodies against SARS-CoV-2 in serum such as IgG, IgM, and IgA [26]
  - Improved clinical symptoms & decreased CRP level [26]
- **Limitation**
  - Immunological reactions [28]
  - Risk of reinfection [28]

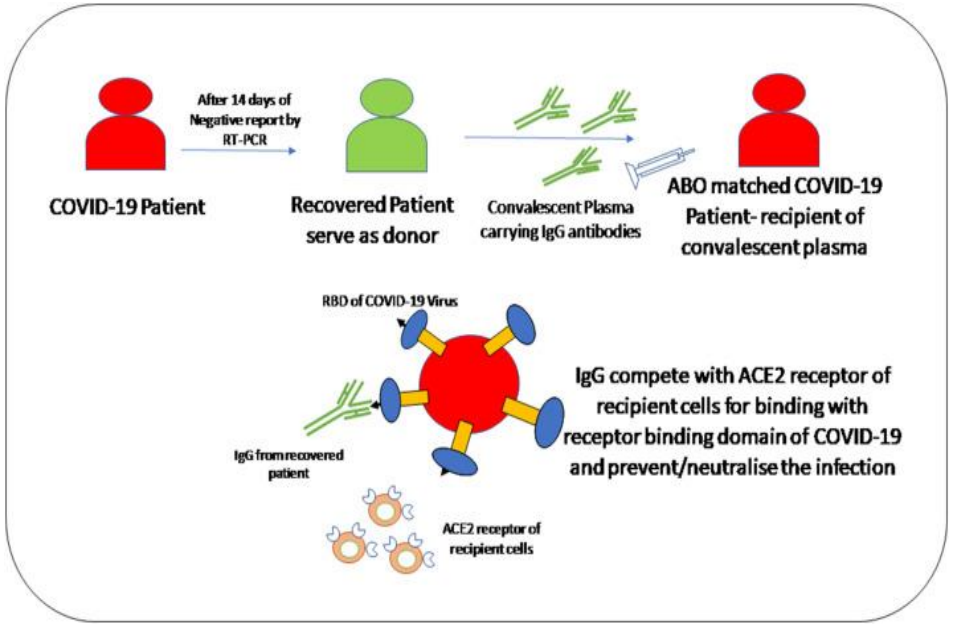
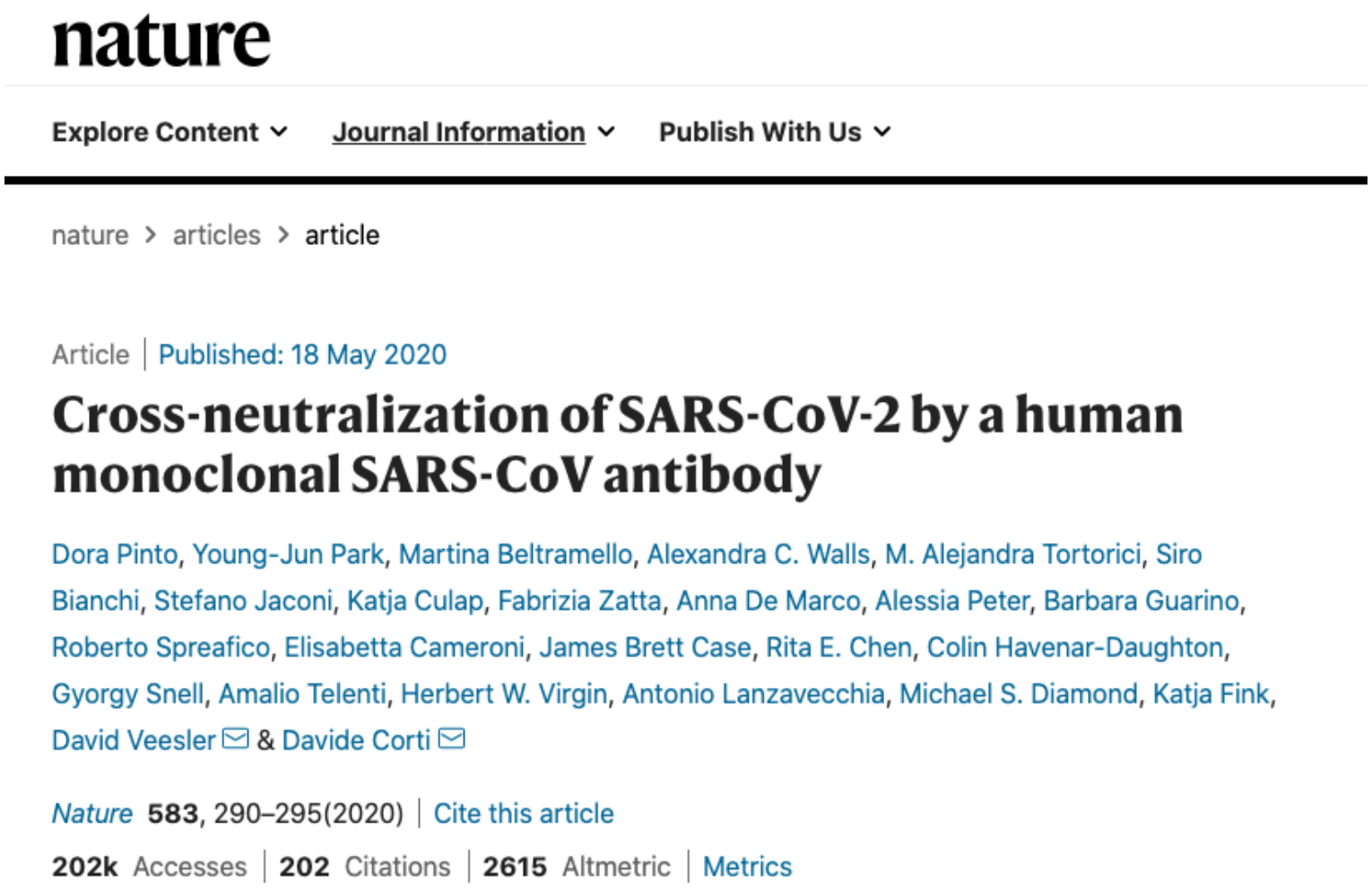


Image from Kumar et al., 2020

Summary

	Benefits	Limitations
Antibody	• High-specificity for a single epitope of an antigen • Renewably generate once suitable hybridoma or CHO cells are developed • mAbs are homogenous & highly consistent • Immediately treat an existing SARS-CoV-2 infection • Neutralizing antibody: S309 • targets S glycoprotein trimer	• Mono-specificity limits their applications • Minor changes in antigen epitope structure affects the function of mAbs • Production is expensive • Protection is short-term (Weeks to Months)
Small molecule	• Can test multiple candidates with high throughput screening methods • Can be ingested orally • More affordable than other therapeutics • Target many different biochemical pathways	• Target cells and viruses may develop drug resistance mechanisms (e.g., drug inactivation, efflux pumps) • Can only target one protein/pathway
Other Immunotherapy	• Immunomodulatory effect • Supply immunity against viral infection • Target specific interleukin	• Side effects • Does not work for everyone • Immunological reaction

Conclusion



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Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody

Dora Pinto, Young-Jun Park, Martina Beltramello, Alexandra C. Walls, M. Alejandra Tortorici, Siro Bianchi, Stefano Jaconi, Katja Culap, Fabrizia Zatta, Anna De Marco, Alessia Peter, Barbara Guarino, Roberto Spreafico, Elisabetta Cameroni, James Brett Case, Rita E. Chen, Colin Havenar-Daughton, Gyorgy Snell, Amalio Telenti, Herbert W. Virgin, Antonio Lanzavecchia, Michael S. Diamond, Katja Fink, David Veesler & Davide Corti

Nature **583**, 290–295(2020) | Cite this article

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References

1. Tang, X., Wu, C., Li, X., Song, Y., Yao, X., Wu, X., . . . Lu, J. (2020). On the origin and continuing evolution of SARS-CoV-2. *National Science Review, 7*(6), 1012-1023. doi:10.1093/nsr/nwaa036

2. Fehr, A. R., & Perlman, S. (2015). Coronaviruses: An Overview of Their Replication and Pathogenesis. *Coronaviruses, 1282*, 1-23. doi:10.1007/978-1-4939-2438-7_1

3. Fan, Y., Zhao, K., Shi, Z., & Zhou, P. (2019). Bat Coronaviruses in China. *Viruses, 11*(3), 210. doi:10.3390/v11030210

4. Huang, Y., Yang, C., Xu, X., Xu, W., & Liu, S. (2020). Structural and functional properties of SARS-CoV-2 spike protein: Potential antivirus drug development for COVID-19. *Acta Pharmacologica Sinica, 41*(9), 1141-1149. doi:10.1038/s41401-020-0485-4

5. CDC. (2020, December 28). Understanding mRNA COVID-19 Vaccines. Retrieved January 22, 2021, from https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/mrna.html#:~:text=COVID%2D19%20mRNA%20vaccines%20are,protein%20piece%20on%20its%20surface

6. CDC. (2018, July). Understanding How Vaccine Work. Retrieved January 21, 2021, from https://www.cdc.gov/vaccines/hcp/conversations/downloads/vacsafe-understand-color-office.pdf

7. Pinto, D., Park, Y., Beltramello, M., Walls, A. C., Tortorici, M. A., Bianchi, S., . . . Corti, D. (2020). Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature, 583*(7815), 290-295. doi:10.1038/s41586-020-2349-y

8. Wu, Y., Li, Z., Zhao, Y., Huang, Y., Jiang, M., & Luo, H. (2021). Therapeutic targets and potential agents for the treatment of COVID-19. *Medicinal Research Reviews,* 1-23. doi:10.1002/med.21776

9. WHO. (2020, June 25). Coronavirus disease (COVID-19): Dexamethasone. Retrieved January 22, 2021, from https://www.who.int/news-room/q-a-detail/coronavirus-disease-covid-19-dexamethasone

10. Manis, J. P. (2020). Overview of therapeutic monoclonal antibodies. UpToDate. Retrieved from https://www.uptodate.com/contents/overview-of-therapeutic-monoclonal-antibodies#H1

11. Cheriyedath, S. (2018). Monoclonal Antibodies. News Medical Life Sciences. Retrieved from https://www.news-medical.net/life-sciences/Monoclonal-Antibodies.aspx

12. Nagarajan, T., Marissen, W. E., & Rupprecht, C. (2014). Monoclonal antibodies for the prevention of rabies: Theory and clinical practice. *Antibody Technology Journal*, 4(4), 1-12. doi: 10.2147/ANTI.S33533

13. Cheedarla, N., & Hanna, L. E. (2019). Functional and Protective Role of Neutralizing Antibodies (NAbs) Against Viral Infections. In *Recent Developments in Applied Microbiology and Biochemistry* (pp. 83-93). Academic Press.

14. Alexander, M. R., Sanders, R. W., Moore, J. P., & Klasse, P. J. (2015). Virion aggregation by neutralizing and nonneutralizing antibodies to the HIV-1 envelope glycoprotein. *AIDS research and human retroviruses*, *31*(11), 1160-1165.

15. Salazar, G., Zhang, N., Fu, T. M., & An, Z. (2017). Antibody therapies for the prevention and treatment of viral infections. *npj Vaccines*, *2*(1), 1-12. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5627241/

16. Ngo, H. X., & Garneau-Tsodikova, S. (2018). What are the drugs of the future?. *MedChemComm*, *9*(5), 757–758. https://doi.org/10.1039/c8md90019a

17. Prescott, H. C., & Rice, T. W. (2020). Corticosteroids in COVID-19 ARDS: evidence and hope during the pandemic. *Jama*, *324*(13), 1292-1295.

18. Chien, M., Anderson, T. K., Jockusch, S., Tao, C., Li, X., Kumar, S., ... & Ju, J. (2020). Nucleotide analogues as inhibitors of SARS-CoV-2 polymerase, a key drug target for COVID-19. *Journal of Proteome Research*, *19*(11), 4690-4697.

19. Hajdo, L., Szulc, A. B., Klajnert, B., & Bryszewska, M. (2010). Metabolic limitations of the use of nucleoside analogs in cancer therapy may be overcome by application of nanoparticles as drug carriers: A review. *Drug Development Research*, *71*(7), 383-394.

20. Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., ... & Yang, H. (2020). Structure of M pro from SARS-CoV-2 and discovery of its inhibitors. *Nature*, *582*(7811), 289-293.

21. Bzówka, M., Mitusińska, K., Raczyńska, A., Samol, A., Tuszyński, J. A., & Góra, A. (2020). Structural and evolutionary analysis indicate that the SARS-CoV-2 Mpro is a challenging target for small-molecule inhibitor design. *International journal of molecular sciences*, *21*(9), 3099.

22. Smieszek, S., Przychodzen, B., Polymeropoulos, V., Polymeropoulos, C., & Polymeropoulos, M. (2020). Direct ACE2-Spike RBD Binding Disruption with Small Molecules: A Strategy for COVID-19 Treatment.

23. Ragab, D., Salah Eldin, H., Taeimah, M., Khattab, R., & Salem, R. (2020). The COVID-19 cytokine storm; what we know so far. *Frontiers in immunology*, *11*, 1446.

24. Wraith, D. C. (2017). The future of immunotherapy: A 20-year perspective. *Frontiers in Immunology*, *8*(NOV), 1668. https://doi.org/10.3389/fimmu.2017.01668

25. Immunological mediated: Immunotherapy - StatPearls - NCBI Bookshelf. (n.d.). Retrieved January 29, 2021, from https://www.ncbi.nlm.nih.gov/books/NBK519046/

26. Pashaei, M., & Rezaei, N. (2020). Immunotherapy for SARS-CoV-2: potential opportunities. In *Expert Opinion on Biological Therapy* (Vol. 20, Issue 10, pp. 1111–1116). Taylor and Francis Ltd. https://doi.org/10.1080/14712598.2020.1807933

27. King, A., Vail, A., O'Leary, C., Hannan, C., Brough, D., Patel, H., Galea, J., Ogungbenro, K., Wright, M., Pathmanaban, O., Hulme, S., & Allan, S. (2020). Anakinra in COVID-19: important considerations for clinical trials. The Lancet Rheumatology, 2(7), e379-e381. http://doi.org/10.1016/S665-9913(20)30160-0

28. Nagoba, B., Gavkare, A., Jamadar, N., Mumbre, S., & Selkar, S. (2020). Positive aspects, negative aspects and limitations of plasma therapy with special reference to COVID-19. *Journal of Infection and Public Health*, *13*(12), 1818-1822. http://doi.org/10.1016/j.jiph.2020.08.011

Figure References

Aronson, J. K. (2020, March 25). Coronaviruses – a general introduction [Digital image]. Retrieved January 22, 2021, from https://www.cebm.net/covid-19/coronaviruses-a-general-introduction/

BioRender [Computer software]. (2021). Retrieved from https://biorender.com/

Chem Space [Computer software]. (2021). Retrieved from https://chem-space.com/

Gordon, D. E., Jang, G. M., Bouhaddou, M., Xu, J., Obernier, K., White, K. M., . . . Krogan, N. J. (2020, April 30). A SARS-CoV-2 protein interaction map reveals targets for drug repurposing [Digital image]. Retrieved January 22, 2021, from https://www.nature.com/articles/s41586-020-2286-9#auth-Zun_Zar_Chi-Naing

King, J., Kosinski-Collins, M., & Sundberg, E. (2020, March/April). Structure and Organization of Coronaviruses [Digital image]. Retrieved January 22, 2021, from https://web.mit.edu/fnl/volume/324/king_etal.html

Ringe, R., & Bhattacharya, J. (2013). Preventive and therapeutic applications of neutralizing antibodies to Human Immunodeficiency Virus Type 1 (HIV-1). *Therapeutic advances in vaccines*, *1*(2), 67-80. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3967664/

Zost, S. J., Gilchuk, P., Chen, R. E., Case, J. B., Reidy, J. X., … & Crowe Jr, J. E. (2020). Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein. *Nature Medicine*, 26, 1422-1427. https://doi.org/10.1038/s41591-020-0998-x

Denise, M (2020, September 10). Corticosteroids to treat severe cases of COVID-19: 'It does confirm what we've been doing'. *Shaw Media,*

Anakinra Shows Benefits in Cytokine Storm | MedPage Today. (n.d.). Retrieved January 30, 2021, from https://www.medpagetoday.com/rheumatology/generalrheumatology/82198

Kumar, S., Sharma, V., & Priya, K. (2020). Battle against COVID-19: Efficacy of Convalescent Plasma as an emergency therapy. *The American Journal of Emergency Medicine*, *0*(0). https://doi.org/10.1016/j.ajem.2020.05.101