# Immune dysregulation in Covid-19

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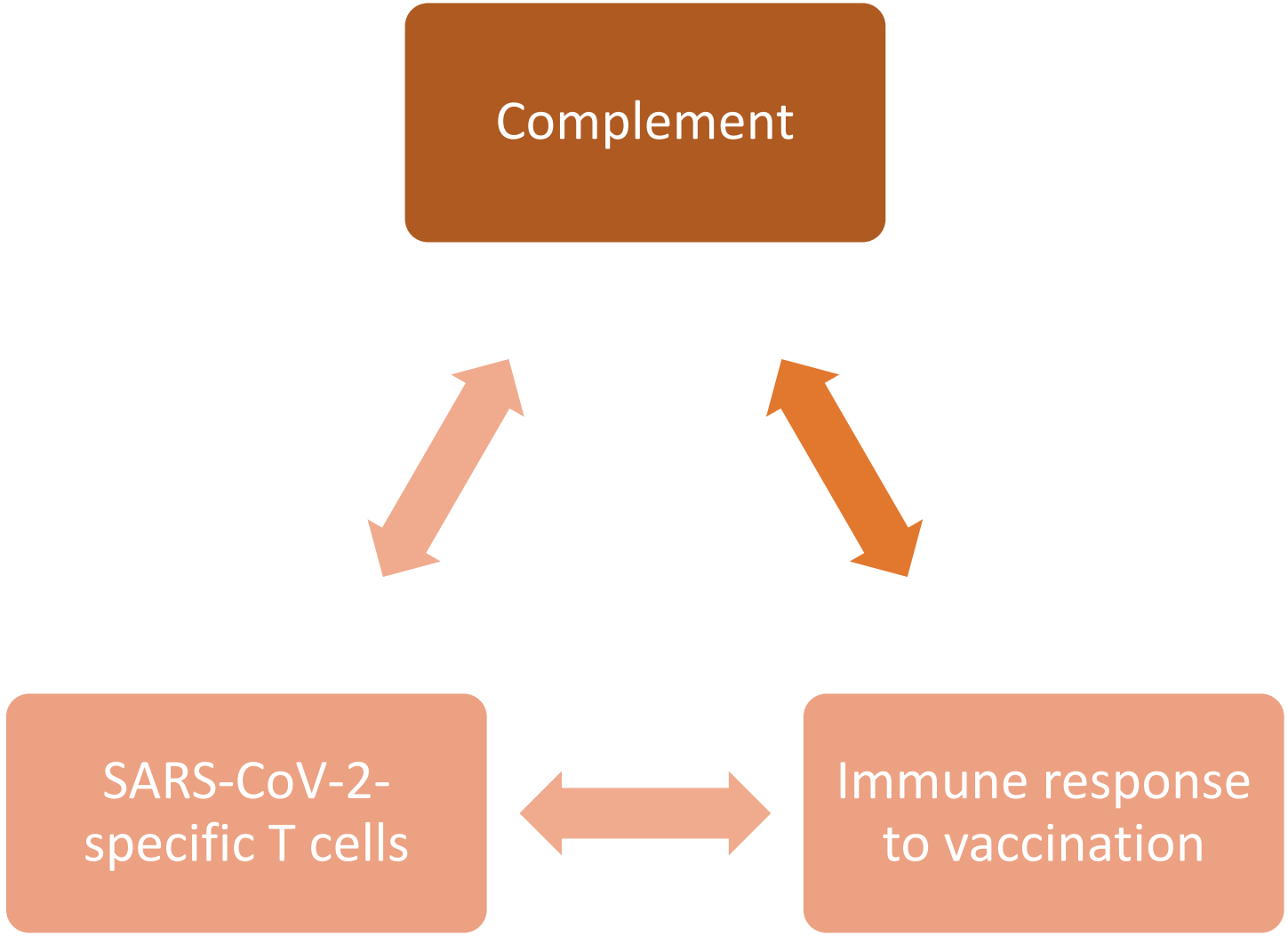
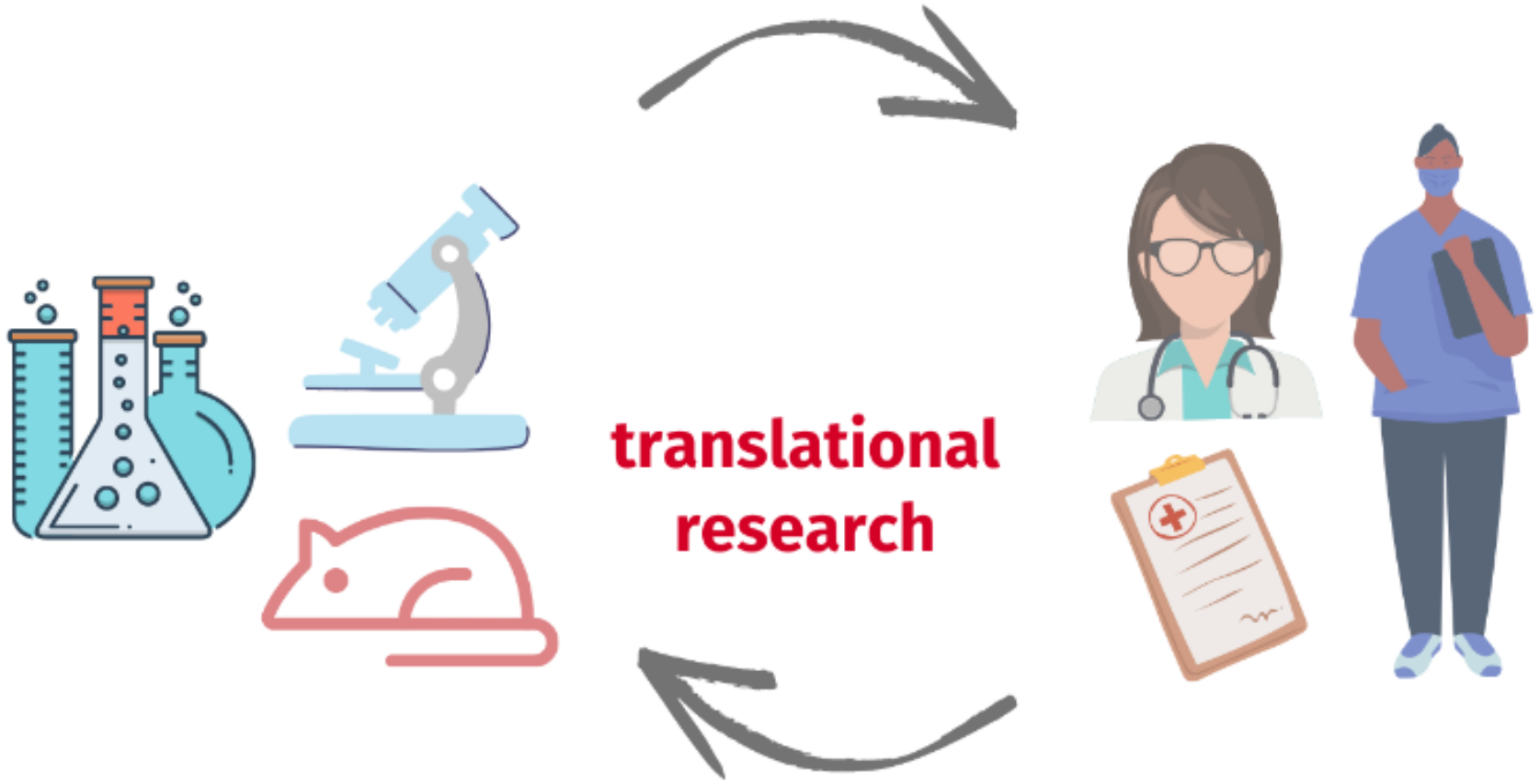
# Immune dysregulation in Covid-19

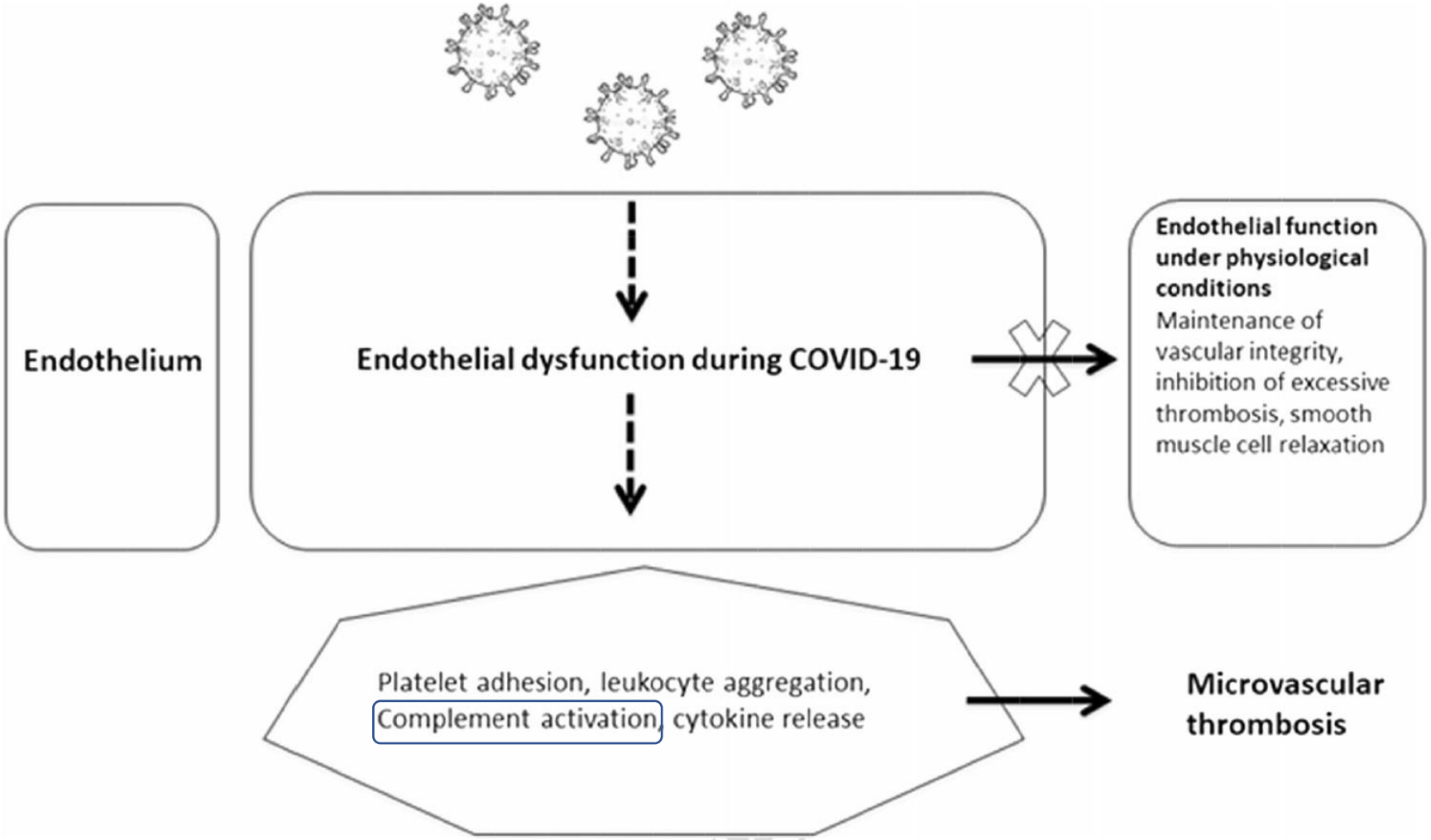



translational
research

# Immune dysregulation in Covid-19

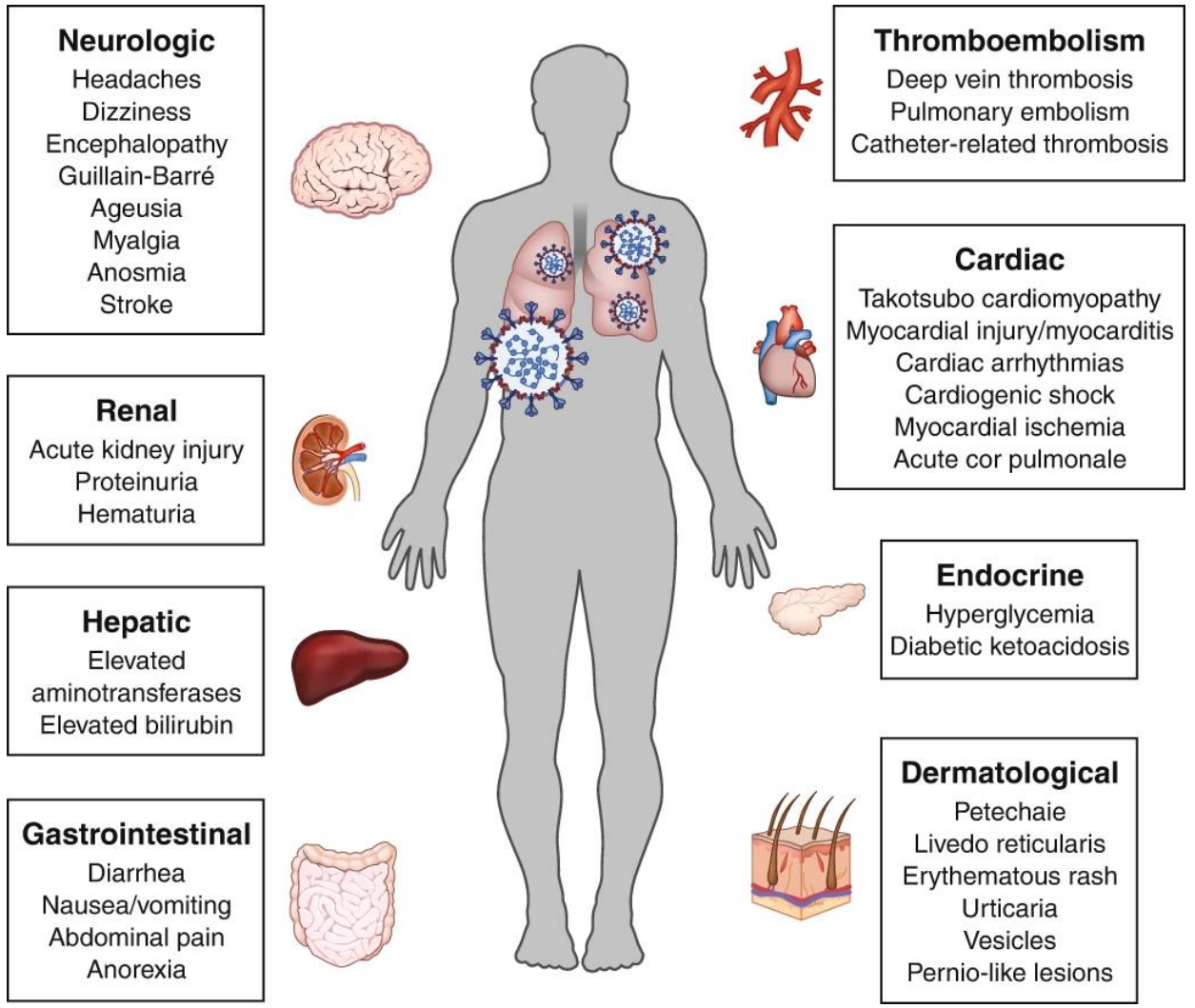
Complement

# Immuno-thrombosis and endothelial dysfunction in COVID-19



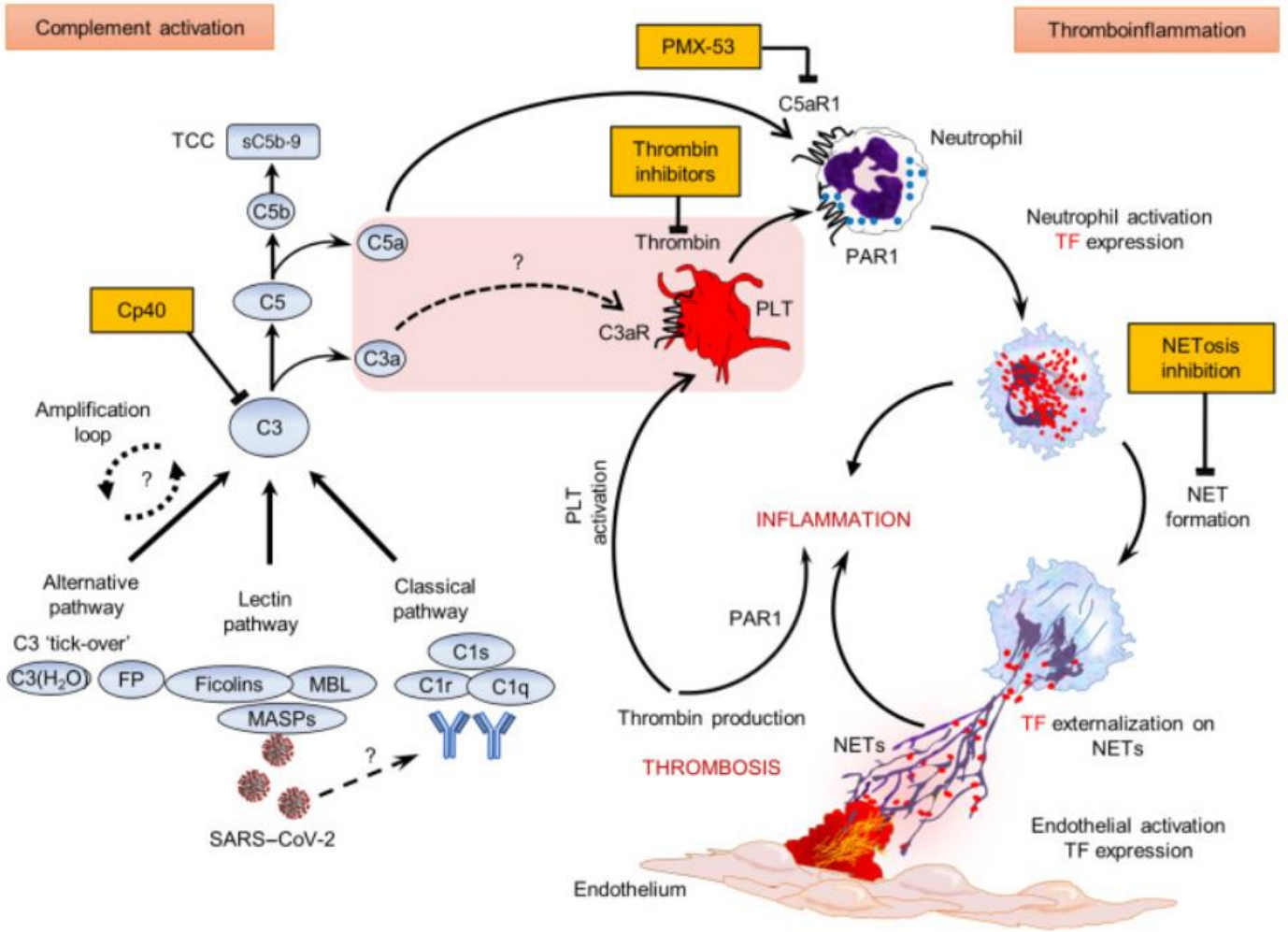

*Gavriilaki E, et al. Curr Hypert Rep 2020*

# COVID-19 immuno-thrombosis leads to multi-organ damage (…not just lung disease/ARDS)



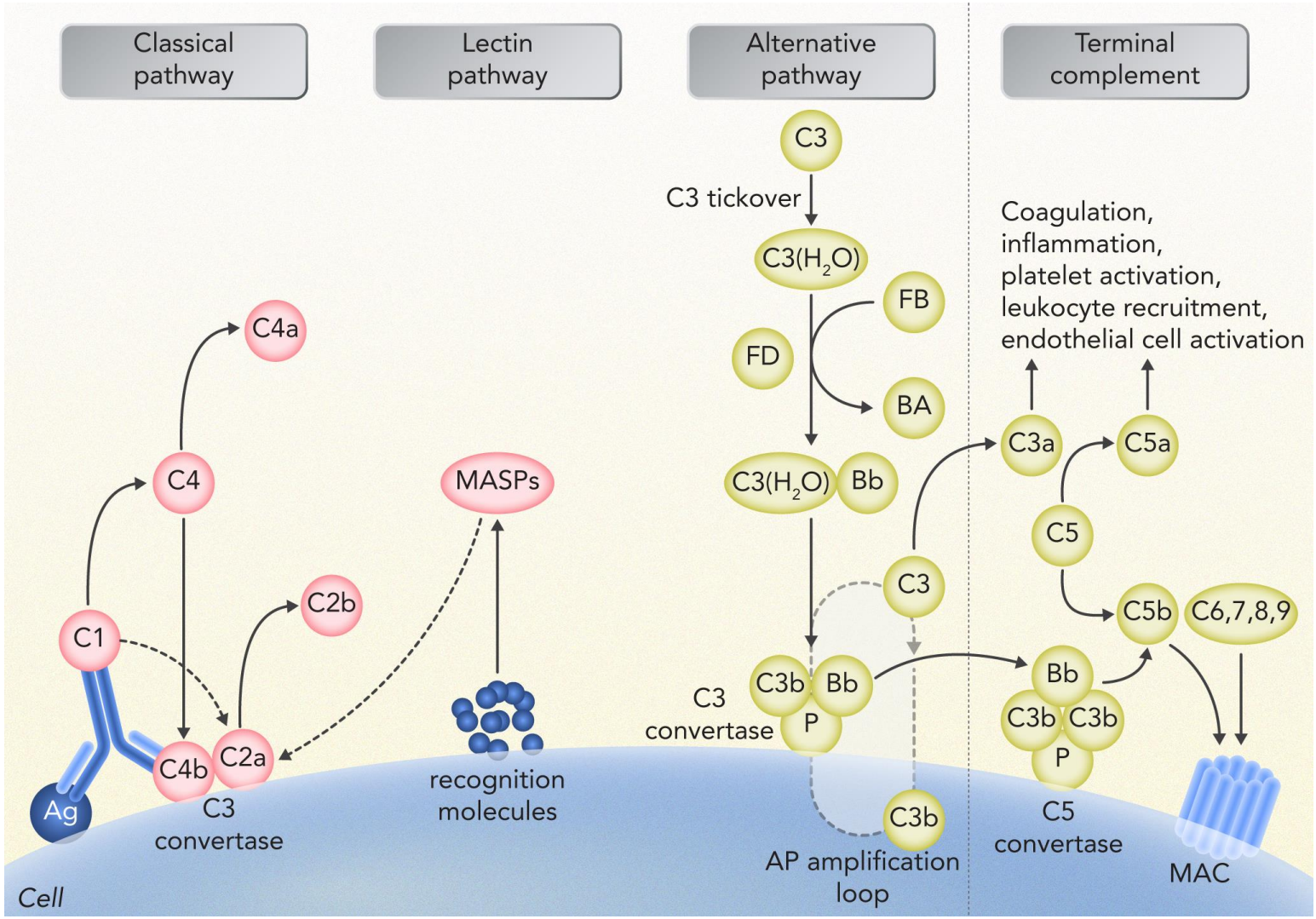

*Gupta A et al., Nature Medicine, 1017–1032(2020)*

# COVID-19 immuno-thrombosis: mechanisms



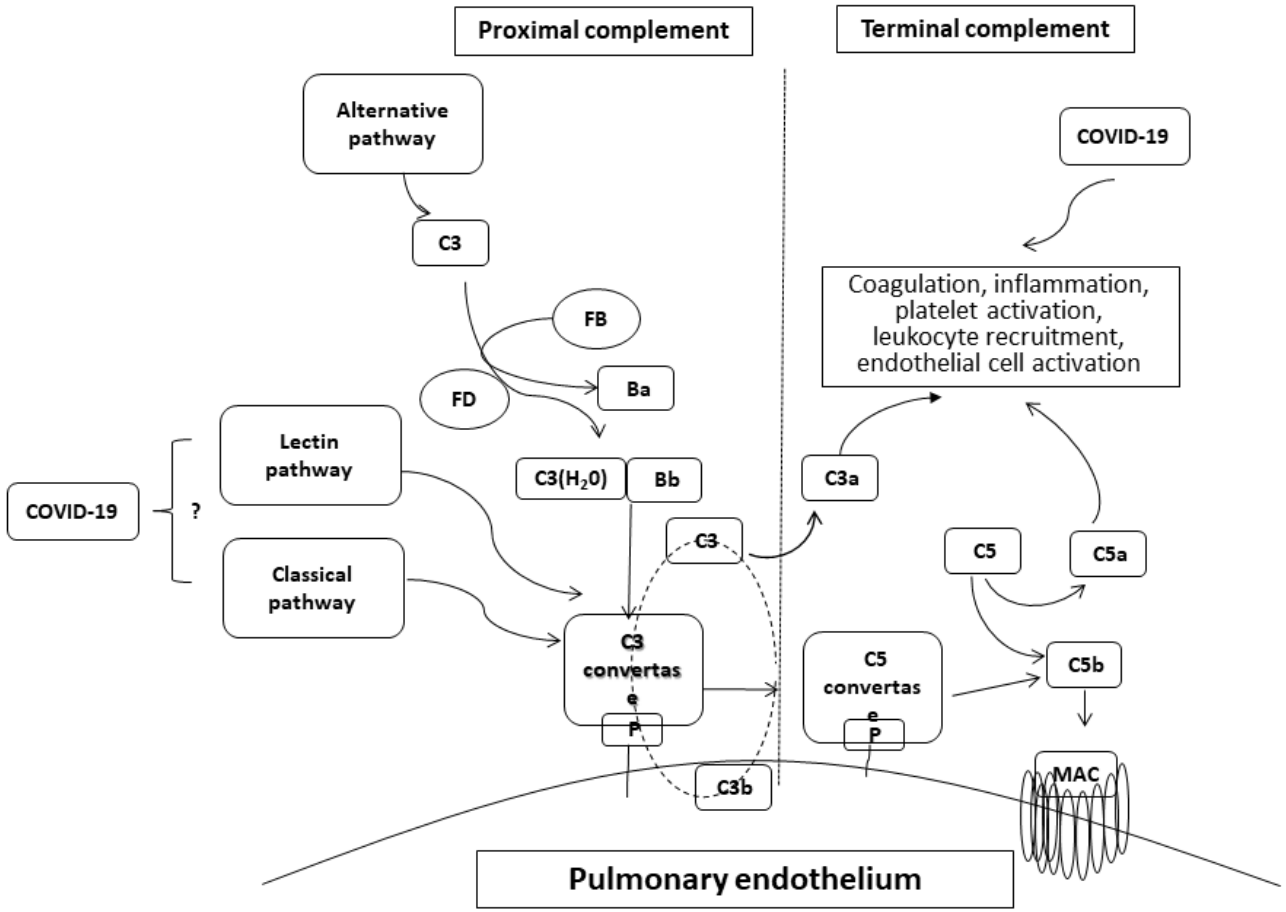

*Skendros P, et al. JCI 2020*

# Complement cascade



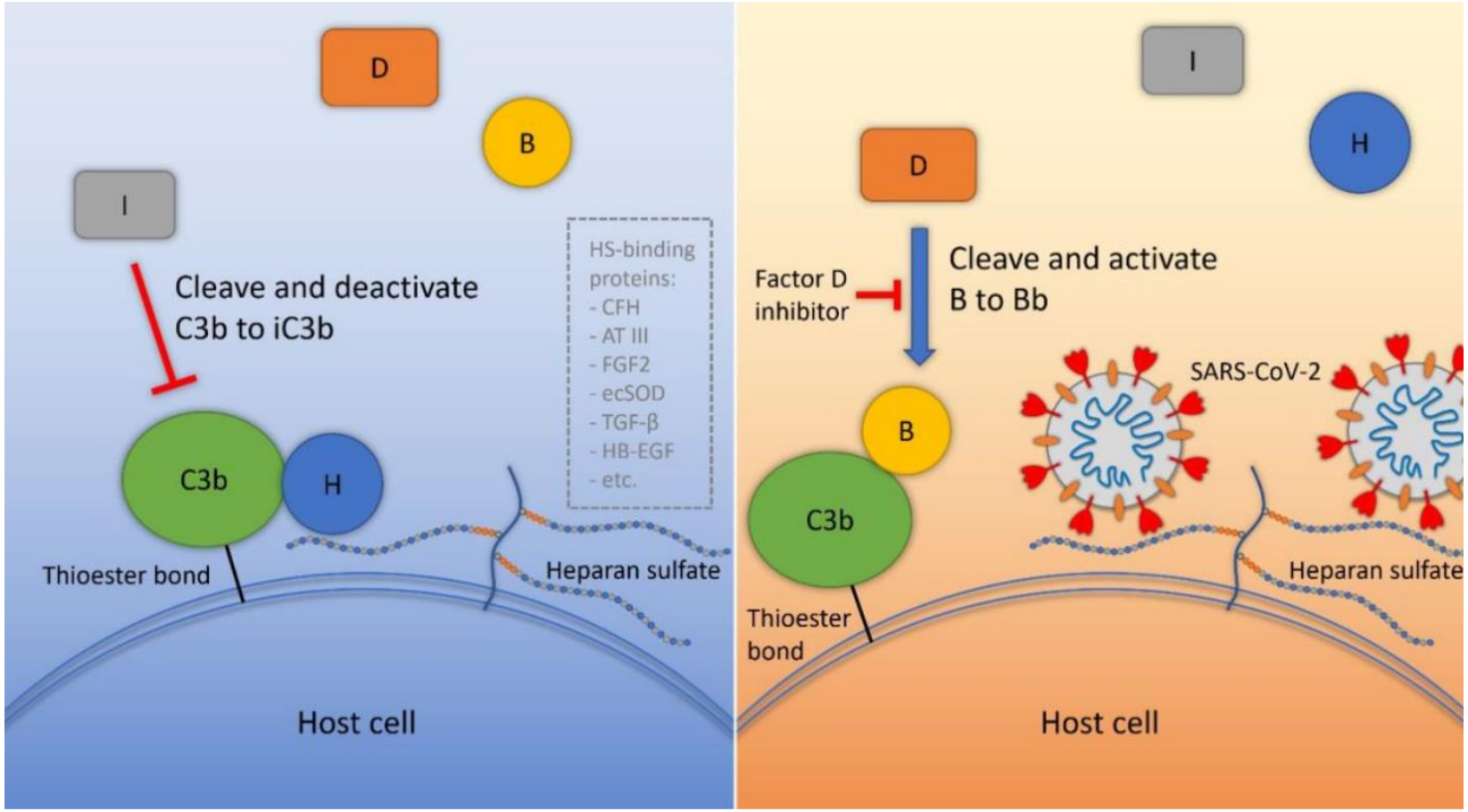

*Gavriilaki E, et al. Blood 2021*

# Complement activation in COVID-19




*Gavriilaki E, et al.. BJH 2020*
*Gavriilaki E, et al. Annals of Hematology 2020*

# Direct activation of alternative pathway from SARS-CoV-2 (spike proteins)




*Yu J, et al. Blood 2020*

# Hypothesis

## Could severe COVID-19 resemble Complementopathies?

# Disorders associated with complement activation (complementopathies)

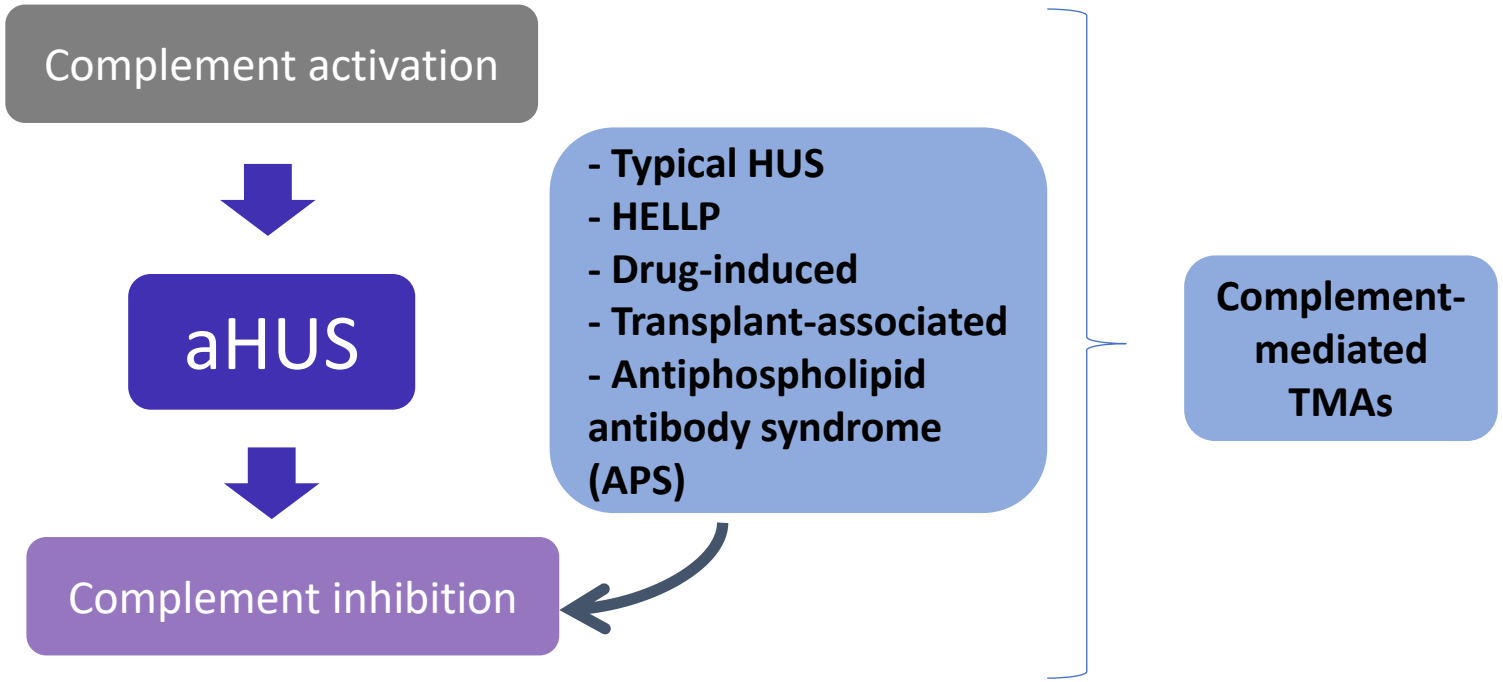
**Table 2. Disorders in which complement inhibition is beneficial**

| Disorder | Mechanism of complement activation | Complement pathway implicated | Proof of benefit from complement inhibition |
|---|---|---|---|
| Age-related macular degeneration | Genetic variants of complement-regulatory proteins | Alternative | Phase III clinical trials |
| ANCA vasculitis | C5a-mediated effects of complement-activating autoantibodies | Alternative/classical | Phase III clinical trial |
| Antiphospholipid antibody syndrome | Genetic variants of complement-regulatory proteins | Alternative/classical | Case reports/series |
| Atypical hemolytic uremic syndrome | Genetic variants of complement-regulatory proteins; autoantibodies | Alternative | Approved treatment |
| Cold agglutinin disease | Complement-activating antibodies | Classical | Phase III clinical trials |
| Glomerulopathies | Genetic variants of complement-regulatory proteins; autoantibodies | Alternative/classical | Phase II clinical trials |
| HELLP syndrome | Genetic variants of complement-regulatory proteins | Alternative | Case reports |
| Myasthenia gravis | Complement-activating antibodies | Classical | Approved treatment |
| Neuromyelitis optica | Complement-activating antibodies | Alternative/classical | Approved treatment |
| Paroxysmal nocturnal hemoglobinuria | GPI anchor deficiency | Alternative | Approved treatment |
| Periodontitis | Local microbially induced complement activation | Alternative | Phase II clinical trial |
| Transplant-associated TMA | Genetic variants of complement-regulatory proteins | Alternative | Phase III clinical trial |

Disorders are listed alphabetically. ANCA, anti-neutrophil cytoplasmic antibody; GPI, glycosylphosphatidylinositol; HELLP, hemolysis, elevated liver enzymes, and low platelets; TMA, thrombotic microangiopathy.

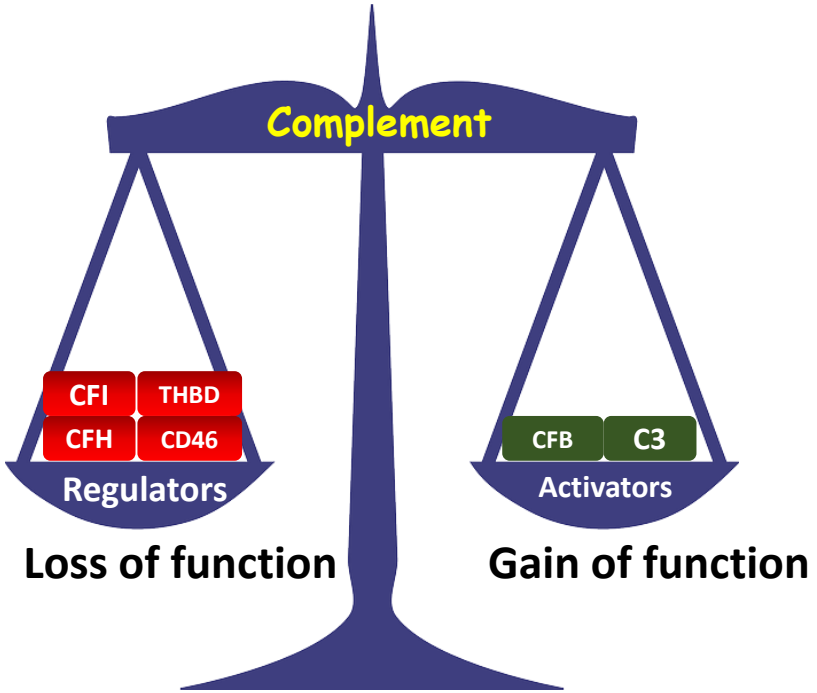
*Gavriilaki E, Brodsky RA. JCI 2020*

# Atypical hemolytic uremic syndrome: the prototype disease of complementopathies




*Gavriilaki E, et al. Blood 2015*

# Atypical hemolytic uremic syndrome: the prototype disease of complementopathies





*Gavriilaki E, et al. Blood 2015*

Genetic susceptibility in COVID-19

Clinical Immunology 226 (2021) 108726

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

Full Length Article

# Genetic justification of severe COVID-19 using a rigorous algorithm

Eleni Gavriilaki [a,*], Panagiotis G. Asteris [b], Tasoula Touloumenidou [a], Evaggelia-Evdoxia Koravou [a], Maria Koutra [a], Penelope Georgia Papayanni [a], Vassiliki Karali [c], Apostolia Papalexandri [a], Christos Varelas [a], Fani Chatzopoulou [d], Maria Chatzidimitriou [e], Dimitrios Chatzidimitriou [d], Anastasia Veleni [f], Savvas Grigoriadis [g], Evdoxia Rapti [h], Diamantis Chloros [i], Ioannis Kioumis [j], Evaggelos Kaimakamis [k], Milly Bitzani [k], Dimitrios Boumpas [c], Argyris Tsantes [h], Damianos Sotiropoulos [a], Ioanna Sakellari [a], Ioannis G. Kalantzis [l], Stefanos T. Parastatidis [b], Mohammadreza Koopialipoor [m], Liborio Cavaleri [n], Danial J. Armaghani [o], Anastasia Papadopoulou [a], Robert Alan Brodsky [p], Styliani Kokoris [h], Achilles Anagnostopoulos [a]

# Aims

We hypothesized that genetic susceptibility would be :

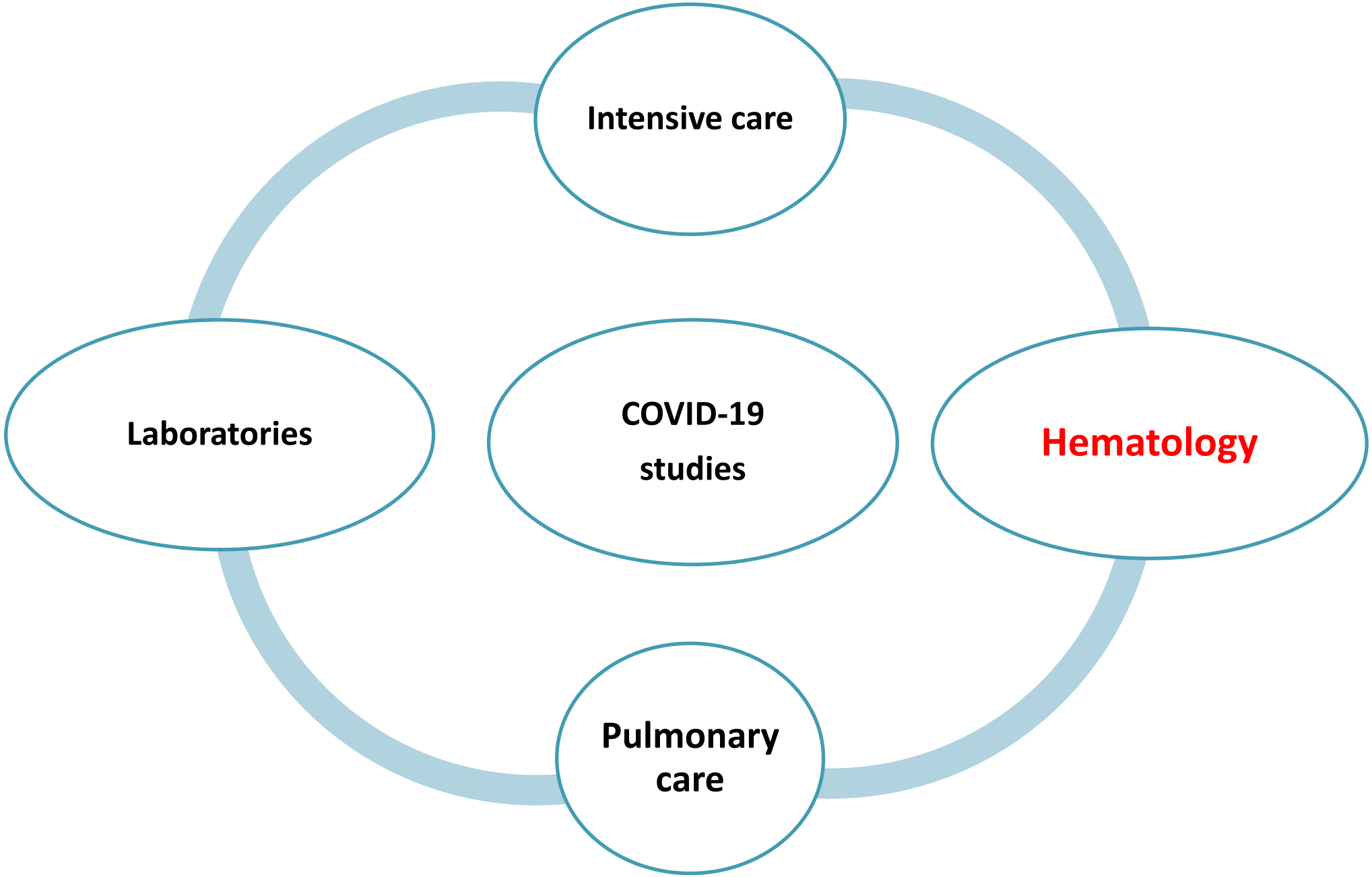
- **evident in patients with severe COVID-19 and**
- **associated with TMA-associated features**

# Methods

- **Consecutive patients hospitalized with COVID-19**

(March-April 2020, first wave in Greece)

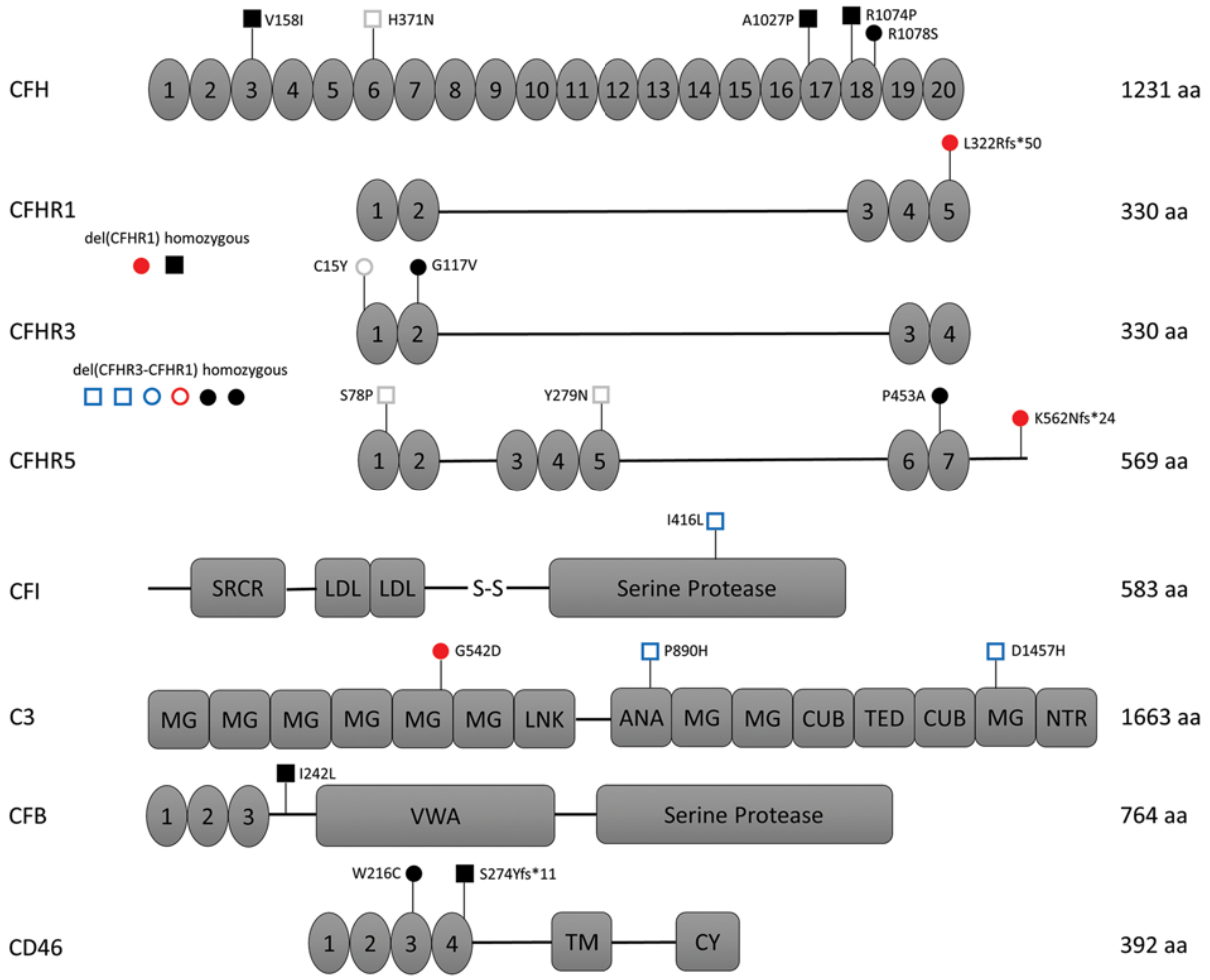
- **COVID-19 severity:** World Health Organization's (WHO) criteria
- **DNA extraction** (peripheral blood)
- **NGS targeted gene panel**


Intensive care
Laboratories
COVID-19 studies
Hematology
Pulmonary care

# NGS targeted gene panel

- Probes were designed using the Design studio (Illumina).
- Amplicons cover **exonic regions of TMA-associated genes** (*Complement factor H/CFH, CFH-related, CFI, CFB, CFD, C3, CD55, C5, MCP, Thombomodulin/THBD, ADAMTS13*).
- Libraries were sequenced on a MiniSeq System
- Analysis was performed with TruSeq Amplicon, and variant calling with Somatic Variant Caller in germline mode and variant allele frequency higher than 20%.
- Variants' clinical significance: based on ClinVar and the current version of the Complement Database, as we have previously described.

# NGS targeted gene panel




CD55
CFD
C5
THBD
ADAMTS13



*Gavriilaki E, et al. Thromb Haem 2020*

# Patient population
# 133 COVID-19 patients

- **80 with moderate disease hospitalized in COVID-19 general ward**
- **53 with severe disease hospitalized in ICU**

# Patient characteristics at hospitalization

| Characteristics | General Ward patients | Intensive Care Unit patients | p-value |
|---|---|---|---|
| Total lymphocyte count | 1.2[0.61] | 1.1[0.89] | 0.088 |
| Neutrophil-to-lymphocyte ratio | 3.1[3.9] | 7.5[5.2] | <0.001 |
| Platelets (x 103/mm3) | 229±105 | 326±155 | 0.015 |
| Alanine aminotransferase (U/L) | 26[32] | 50[108] | 0.061 |
| Asparate aminotransferase (U/L) | 21[62] | 30[54] | 0.367 |
| Direct bilirubin (mg/dl) | 0.19[0.05] | 0.51[0.42] | 0.012 |
| Lactate dehydrogenase (U/L) | 294±1a14 | 417±160 | 0.001 |
| Creatinine (mg/dl) | 0.7[0.6] | 0.9[0.7] | 0.771 |
| Activated partial thromboplastin time (sec) | 34.3±7.6 | 37.5±5.6 | 0.201 |
| D-dimer (ng/mL) | 875[542] | 2115[2312] | 0.011 |
| C-reactive protein (mg/dl) | 51±37 | 126±76 | <0.001 |
| Procalcitonin (ng/ml) | 0.08±0.01 | 0.17±0.09 | 0.236 |
| Ferritin (ng/ml) | 667±561 | 1221±908 | 0.012 |

Values are presented as mean±standard deviation for normal variables or median[interquartile range] for variables without normal distribution. Results were measured at the first day of hospitalization in General Ward or Intensive Care Unit.

# Genetic analysis: *single variants*

- Seven patients, each carrying one pathogenic or likely pathogenic variant in *C3*, *CD46*, *DGKE*, and *CFH* (Complement Database)
- One patient: **rare germline missense variant** in *CFI* (rs112534524), suffered from critical disease but survived after long-term ICU hospitalization
- Interestingly, **five patients** showed **a likely protective missense variant** in *CFB* (rs641153). These patients did not require ICU.

# Genetic analysis:

## *combination of variants*

- Pathogenic variant of *ADAMTS13* (rs2301612, missense) in 28 patients
- Two missense risk factor variants, previously detected in complement-related diseases: rs2230199 in *C3* (13 patients); and rs800292 in *CFH* (26 patients)
- **22 patients: combination of these characterized variants**
- This combination was significantly associated with critical disease that required intensive care (p=0.037), as well as low lymphocyte counts (p=0.021) and high neutrophil-to-lymphocyte ratio (p=0.050).
- In the multivariate model, critical disease was an independent predictor of double heterozygocity in these variants.

# Algorithms for disease risk prediction: too many variants

- **Multi-disciplinary, international collaboration (Iran, Malaysia, Italy, Greece)**
- 381 variants in total
- Combinations : more than yotta = $10^{24}$
- **Boolean algebra** (rules of logic) to determine the combination of variants that is associated with severe disease

*Gavriilaki E, et al. Clin Immunol 2021*

# Algorithms for disease risk prediction: *gender differences*

| Variant | | | Variant frequency based on Sex difference (%) | | |
|---|---|---|---|---|---|
| rs | Gene | Position | Female | Male | |
| rs1042580 | THBD | 23027621 | | | 21.09 |
| rs2230203 | C3 | 6710782 | | | 18.76 |
| rs2250656 | C3 | 6718534 | | | 17.06 |
| not available | THBD | 23027807 | | | 16.57 |
| rs2547438 | C3 | 6718078 | | | 16.47 |
| rs2073932 | ADAMTS13 | 136305439 | | | 15.02 |
| rs3124768 | ADAMTS13 | 136304497 | | | 15.02 |
| rs739469 | ADAMTS13 | 136298729 | | | 15.02 |
| rs7144 | CD46 | 207967719 | | | 14.08 |
| NA | ADAMTS13 | 136295288 | | | 14.03 |
| rs35274867 | CFH | 196712596 | | | 13.33 |
| rs1047286 | C3 | 6713262 | | | 12.09 |
| rs11085197 | C3 | 6713175 | | | 12.09 |
| rs800292 | CFH | 196642233 | | | 11.79 |
| rs551397 | CFH | 196642072 | | | 11.79 |
| rs2230199 | C3 | 6718387 | | | 11.29 |
| rs1962149 | CD46 | 207956559 | | | 11.09 |
| rs374905 | CFHR3 | 196759037 | | | 10.85 |
| rs2298749 | CFI | 110681505 | | | 10.85 |
| rs5860990 | CFI | 110678819 | | | 10.85 |

# Further genetic analysis
## *too many variants*

Algorithm for disease risk prediction

*Unpublished data*

# Functional analysis
## *THBD, C3a, C5a*

- **THBD values** were **significantly increased in patients requiring ICU** hospitalization compared to non-ICU patients (median 2.3, interquartile range [1.6] versus 1.4 [0.79] ng/ml, p=0.025), and patients **harboring the rs1042580 (*THBD*) variant** (p=0.032).
- Similarly, **C3a values** were significantly increased **in patients requiring** ICU hospitalization compared to non-ICU patients (410 [14.1] versus 312 [19.1] ng/ml, p=0.035). D
- Despite increased levels of C5a in ICU patients, this difference did not reach statistical significance (72.1 [7.2] versus 43.4 [11.3] ng/ml, p=0.244).

*Unpublished data*

# Machine learning – artificial intelligence

*Unpublished data*

# Machine learning – artificial intelligence

*Unpublished data*

# Conclusions

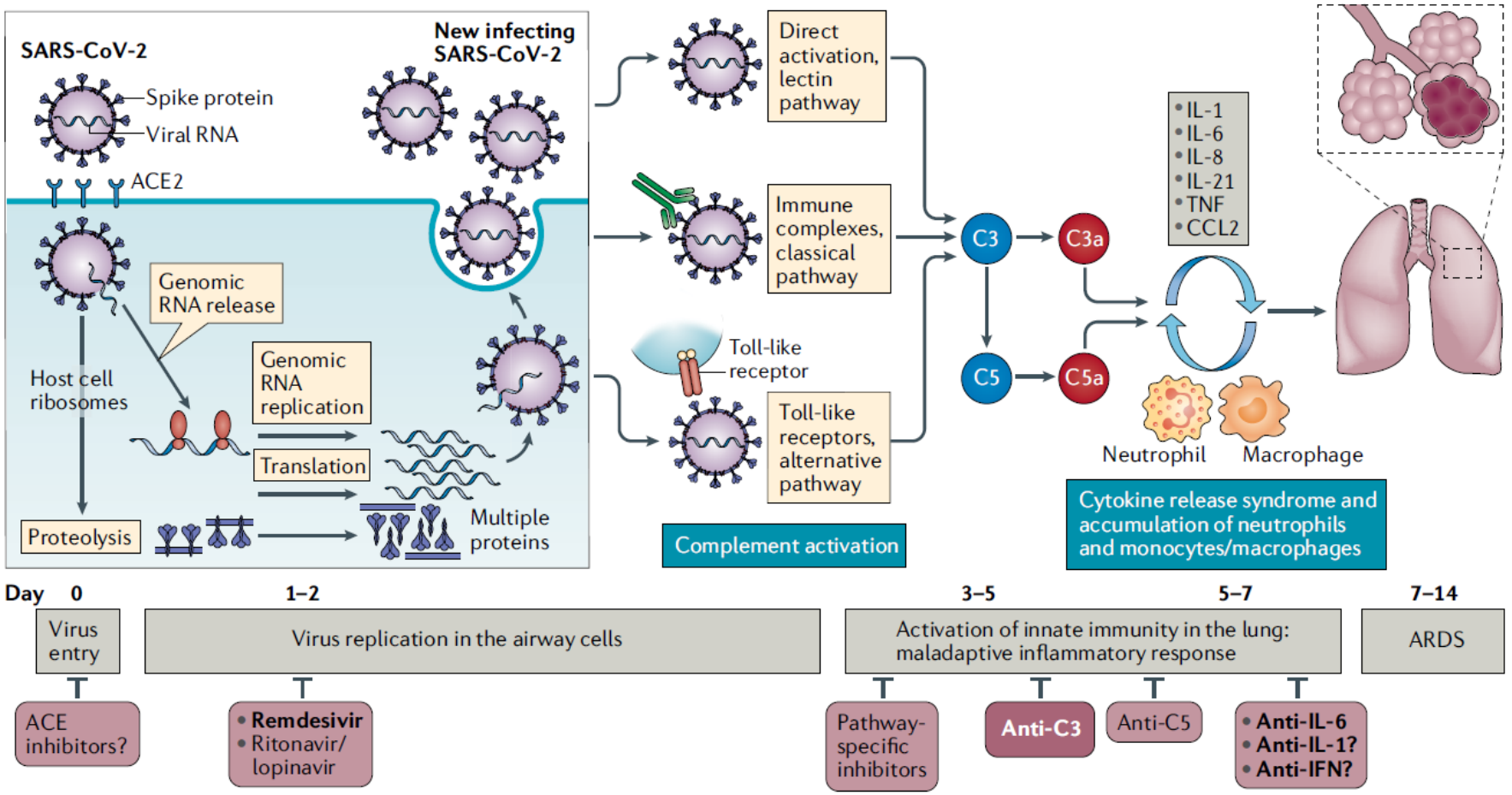
- We reveal **for the first time an ANN accurately predicting ICU hospitalization and death in COVID-19 patients**, based on genetic variants in three complement genes, age, and gender.

- Importantly, we confirm that **genetic dysregulation** is associated with **impaired complement phenotype**.

*Unpublished data*

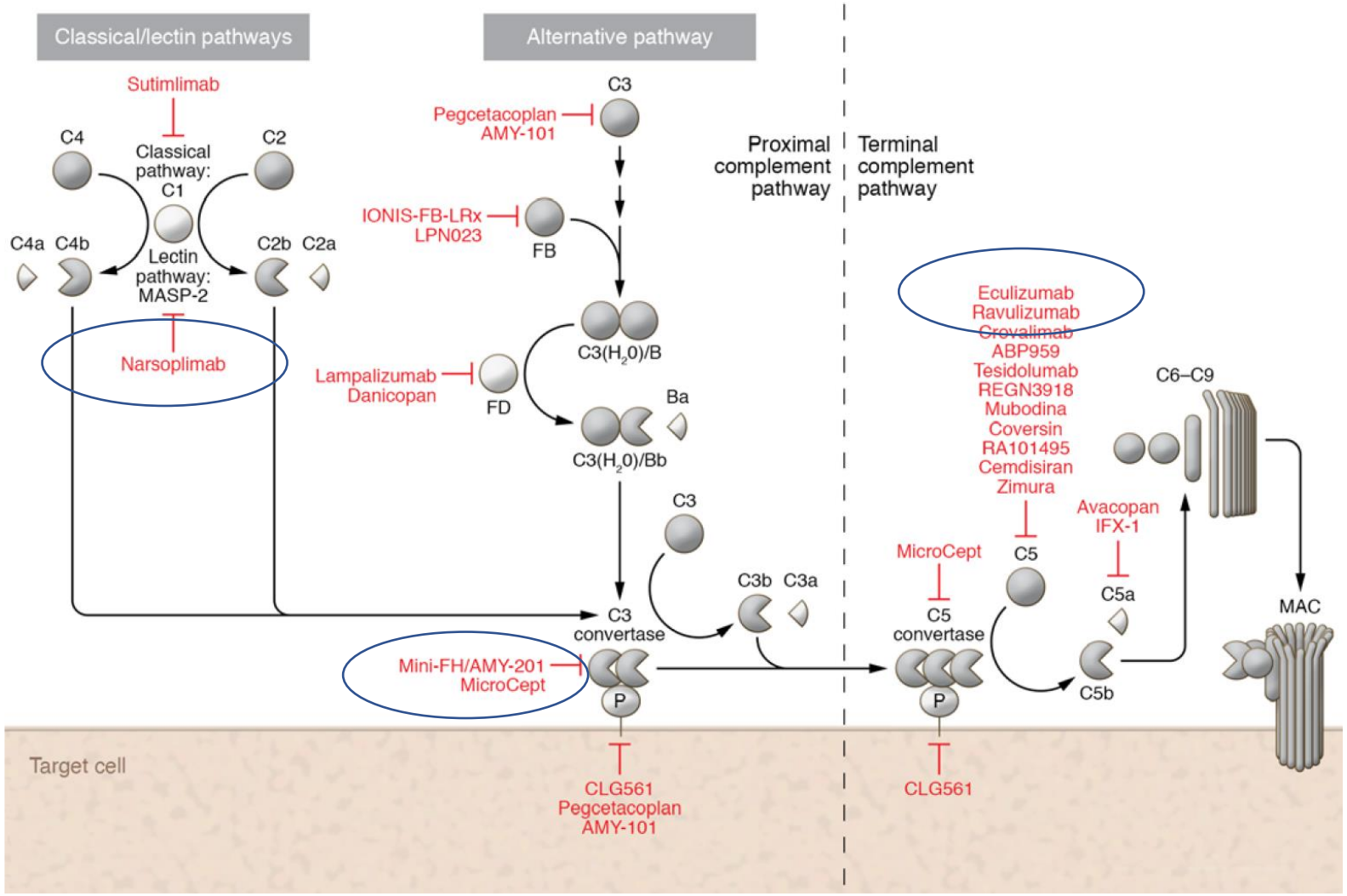

BENCH
2
BEDSIDE

# Complement : thromboinflammation in COVID-19




*Antonio M. Risitano, Dimitrios C. Mastellos, Markus Huber-Lang, Despina Yancopoulou, Cecilia Garlanda, Fabio Ciceri & John D. Lambris. Nature Reviews Immunology, 20, 343–344 (2020)*

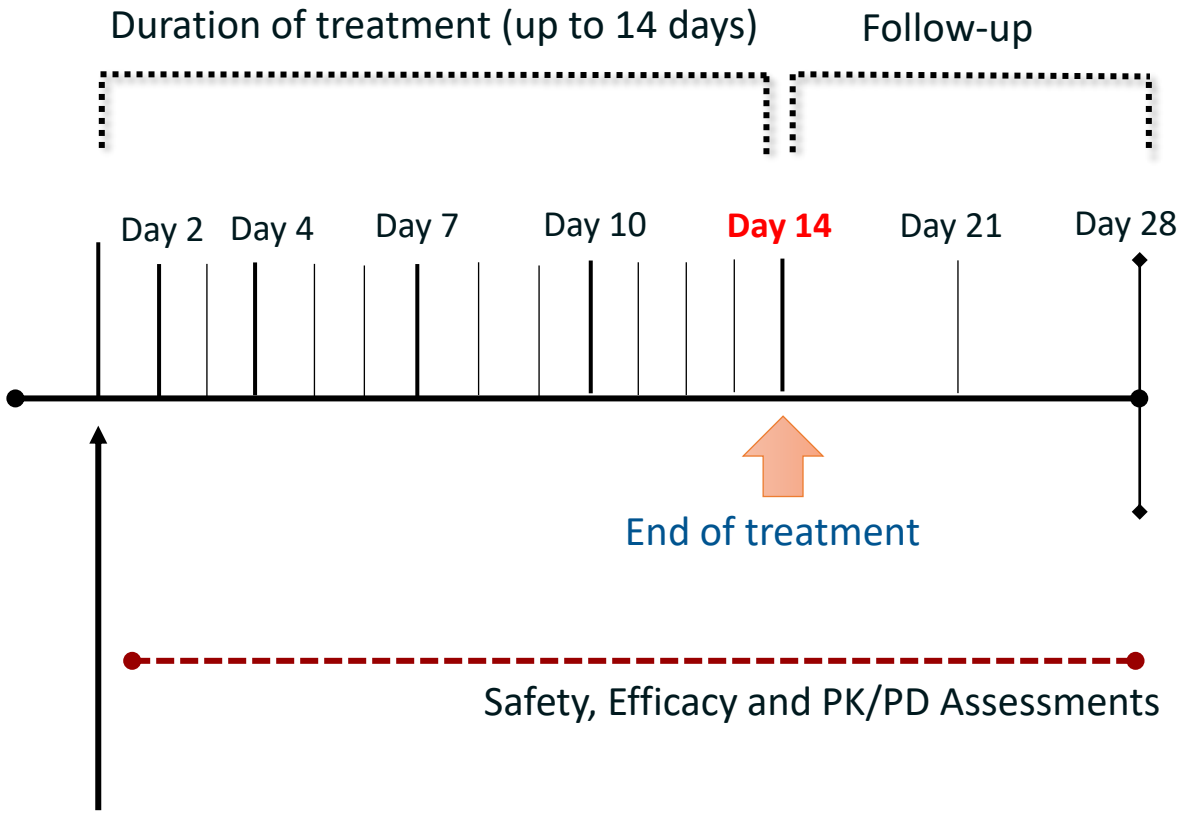
# Complement inhibitors in COVID-19




*Gavriilaki E, Brodsky RA. JCI 2020*

# AMY-101 in 3 COVID-19 ARDS patients

*San Rafaelle Hospital, Italy, March 2020*




**Loading dose of AMY-101 followed by continuous IV infusion (up to 14 days)**

**Intervention: 5 mg/Kg AMY-101**

- 1 loading dose, 6 h iv infusion
- 13 maintenance doses max, 24 h iv infusions

**+**

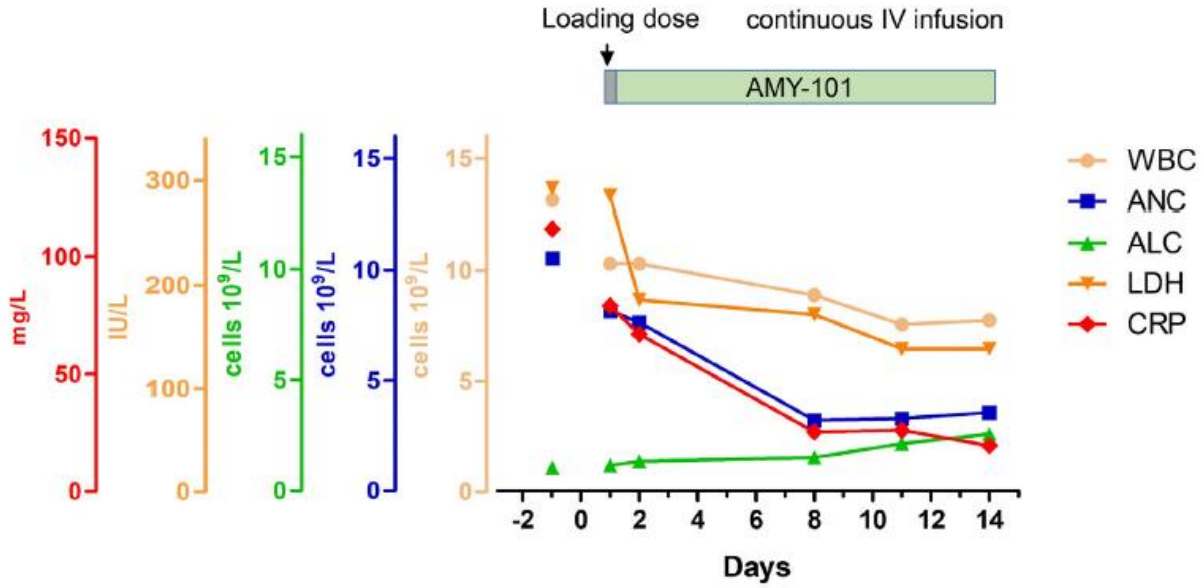
**Usual care/best available treatment**

(antibiotics given, anti-virals allowed but not given, corticosteroids allowed but not given)

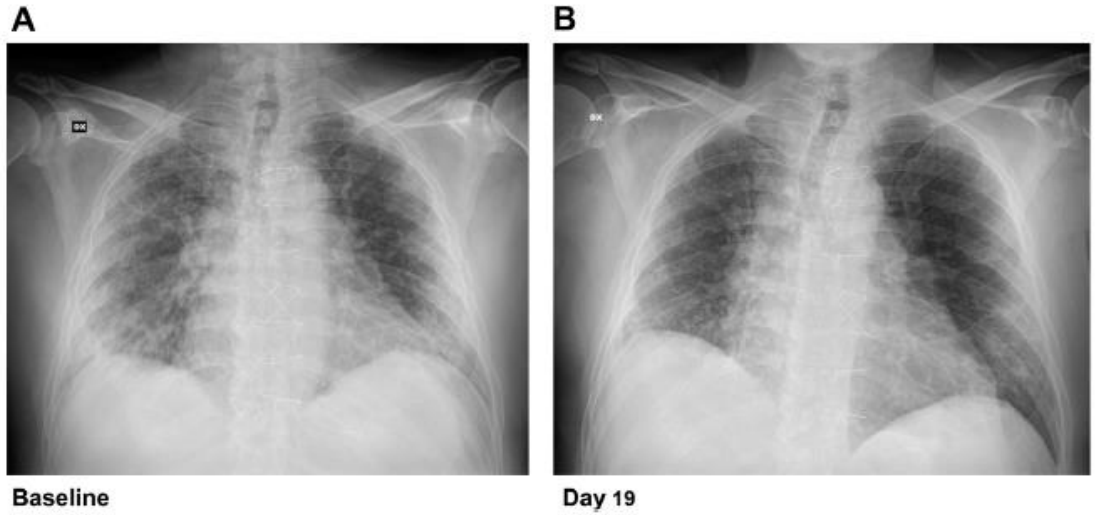
AMYNDAS PHARMACEUTICALS

# AMY-101 in 3 COVID-19 ARDS patients

*San Rafaelle Hospital, Italy, March 2020*



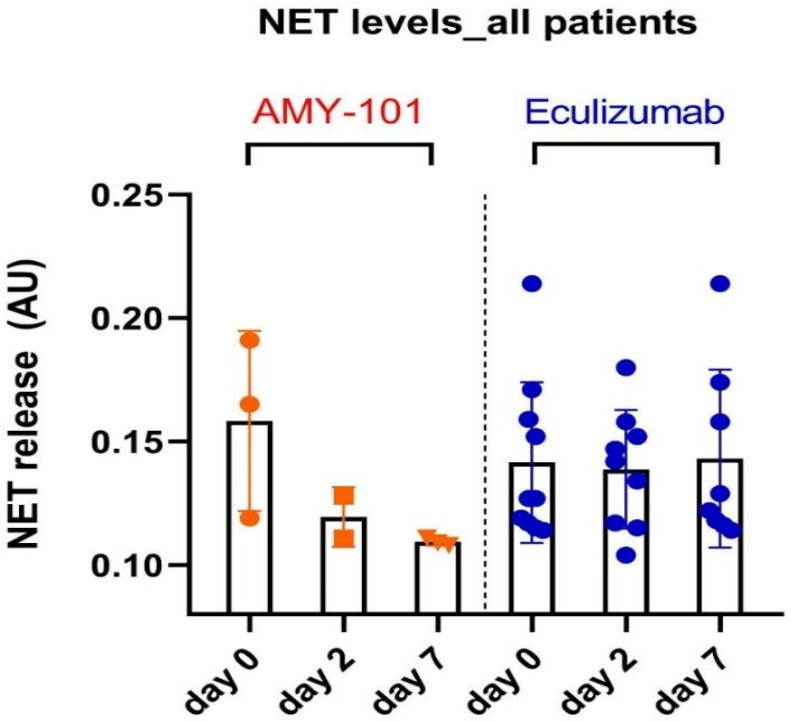

Reduction of inflammatory markers (withing 48 hours after the start of treatment) correlated with improvement in respiratory function (manifested a few days after the start of treatment).





Mastaglio S et al, *Clin Immunol*, 2020
Risitano AM et al, *Nat Rev Immunol*, 2020
Skendros P et al, *J Clin Invest*, 2020
Mastellos D et al, *Clin Immunol* 2020

# AMY-101: early efficacy in thrombo-inflammation




**Key mechanism of action**

C3 activation: a key mechanism that interlocks neutrophil with platelet responses in COVID-19 fueling NET-dependent immunothrombosis

**Mastaglio S et al, *Clin Immunol*, 2020**

**Risitano AM et al, *Nat Rev Immunol*, 2020**
**Skendros P et al, *J Clin Invest*, 2020**
**Mastellos D et al, *Clin Immunol* 2020**

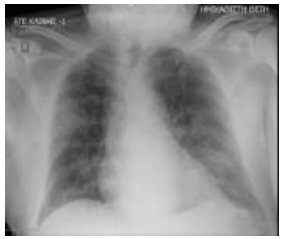
AMY-101 attenuates NET release in severe COVID-19 patients compared to a weaker effect of eculizumab (Soliris)

**Next step ITHACA:** First randomized placebo-controlled Phase 2 study of AMY-101 in severe COVID-19 patients with ARDS (EudraCT No: 2020-004408-32)

AMYNDAS PHARMACEUTICALS

# ITHACA: phase 2 randomized study in COVID-19 ARDS patients

SARS-CoV-2 (+)



P/F ≤ 300

**1:1**

**31 patients**

**AMY-101**
**DW5%**
**500 ml**

**Usual care/best available treatment**
(including antibiotics, anticoagulants, corticosteroids)

**+**

**5 mg/Kg BW AMY-101**
- 1 loading dose, 6 h iv infusion
- 13 maintenance doses max, 24 h iv infusion

**31 patients**

**DW5%**
**500 ml**

**Usual care/best available treatment**
(including antibiotics, anticoagulants, corticosteroids)

**+**

**Placebo**
- 1 loading dose, 6 h iv infusion
- 13 maintenance doses max, 24 h iv infusion

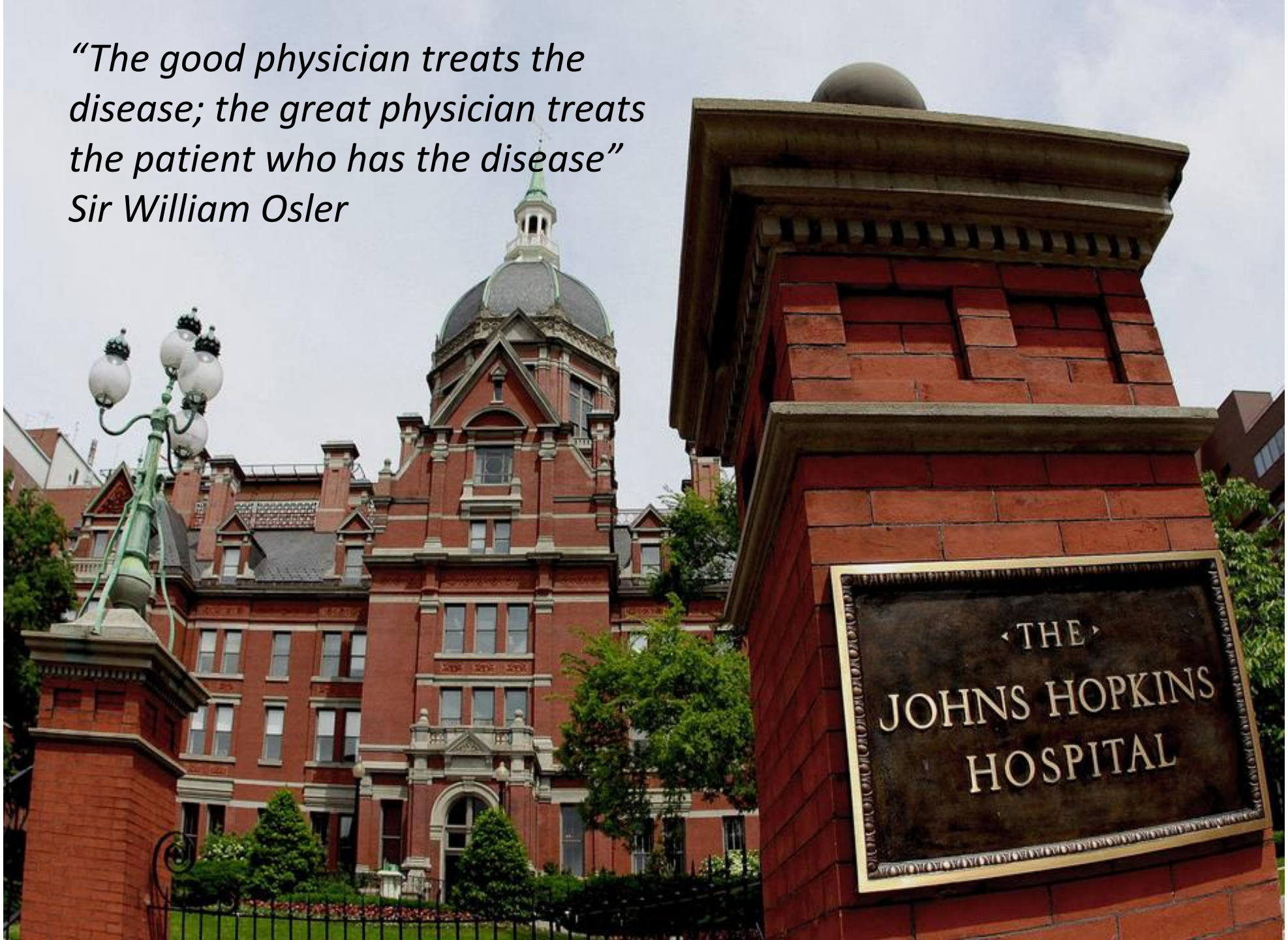
AMYNDAS PHARMACEUTICALS

# The challenges of complement inhibitors in COVID-19

Cost

Availability

Patient selection




*"The good physician treats the disease; the great physician treats the patient who has the disease"*
*Sir William Osler*

# Acknowledgements


ASH abstract achievement award

ASH press release

Best of ASH

**G. Papanikolaou Hospital**

# Immune dysregulation in Covid-19

SARS-CoV-2-specific T cells

# Immune dysregulation in Covid-19

Clin Infect Dis. 2021 Apr 27;ciab371. doi: 10.1093/cid/ciab371. Online ahead of print.

## Vaccinated and convalescent donor-derived SARS-CoV-2-specific T cells as adoptive immunotherapy for high-risk COVID-19 patients

Penelope-Georgia Papayanni [1 2], Dimitrios Chasiotis [1 2], Kiriakos Koukoulias [1 2], Aphrodite Georgakopoulou [1 2], Anastasia Iatrou [3], Eleni Gavriilaki [1], Chrysavgi Giannaki [4], Militsa Bitzani [4], Eleni Geka [5], Polychronis Tasioudis [5], Diamantis Chloros [6], Asimina Fylaktou [7], Ioannis Kioumis [8], Maria Triantafyllidou [1], Sotiria Dimou-Besikli [1], Georgios Karavalakis [1], Afroditi K Boutou [6], Eleni Siotou [1], Achilles Anagnostopoulos [1], Anastasia Papadopoulou [1], Evangelia Yannaki [1 9]

# Gene and Cell Therapy Center,  Hematology – HCT Unit, G. Papanikolaou Hospital, Thessaloniki



Polyclonal SARS-CoV-2-specific T cell products with a safe and strong cytotoxic profile against SARS-CoV-2-presenting targets, as well as SARS-CoV-2 variants, can be generated from both COVID-19 convalescent or vaccinated donors to be used as adoptive therapy of high-risk patients

› Clin Infect Dis. 2021 Apr 27;ciab371. doi: 10.1093/cid/ciab371. Online ahead of print.

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# Immune dysregulation in Covid-19

Immune response to vaccination

# Vaccination of HCT recipients

- Hematopoietic cell transplant (**HCT**) recipients with coronavirus disease 2019 (**COVID-19**): **dismal prognosis**.
- Notwithstanding the prioritization of HCT recipients to COVID-19 vaccination, **limited information** is available on whether and to what extent, they mount an **immune response to SARS-CoV-2 vaccination** as they were generally excluded from vaccination trials.

| Author, year, journal | Population, (n) | Antibodies | Response | Factors |
|---|---|---|---|---|
| Redjoul R, 2021, Lancet | Auto, Allo (88) | IgGII Quant Assay | 59% | Immunosuppression, Lymphopenia |
| Dhakal B, 2021, Blood | Auto, Allo, CAR-T (130) | EUROIMMUN | 60%, 69%, 11% | Steroids |
| Mamez AC, 2021, BMT | Allo (63) | Elecsys (anti-S) | 76% | Time from HCT, ATG |

# Aims - Methods

- We prospectively studied (April-July 2021) the **humoral and cellular immune responses** to SARS-CoV-2 vaccination in adult patients who had undergone HCT in our Unit and received two doses of a SARS-CoV-2 vaccine after written informed consent
- Responses: before each vaccination dose and 12-51 days later after the second dose

- **Neutralizing antibodies against SARS-CoV-2 (CoV-2-NAbs)**: FDA approved methodology for diagnostic use (ELISA, cPass™ SARS-CoV-2 NAbs Detection Kit; GenScript, Piscataway, NJ, USA; cut-off value for a positive result set at ≥30%)
- **SARS-CoV-2 spike-specific T cells (spike-STs)** by interferon-γ Elispot after pulsing peripheral blood mononuclear cells with spike pepmixes.

# Study population (n=65)

| Female/male (n) | 39/26 |
|---|---|
| Age (years) | 51 (21-71) |
| Time from HCT (years) | 4 (0.17-31) |
| Type of HCT (n) | |
| Autologous | 15 |
| Allogeneic | 50 |
| Type of allogeneic donor (n) | |
| Sibling | 23 |
| Unrelated | 21 |
| Haploidentical | 6 |
| Current treatment (n) | |
| No treatment | |
| Chemo- and/or immuno-therapy | 7 |
| GVHD prophylaxis | 3 |
| GVHD treatment | 14 |

HCT: hematopoietic cell transplantation; GVHD: graft-versus-host-disease. Continuous variables are presented as median (range).

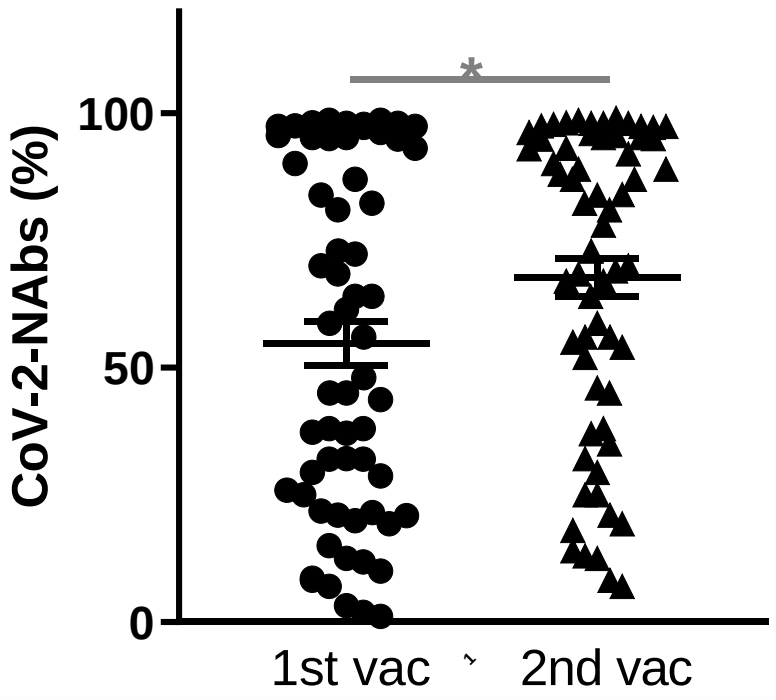
- 63 Pfizer-BioNTech, 2 Astra Zeneca
- No adverse events
- T cellular immunity: 38 / 65
- 17 health-care workers as controls



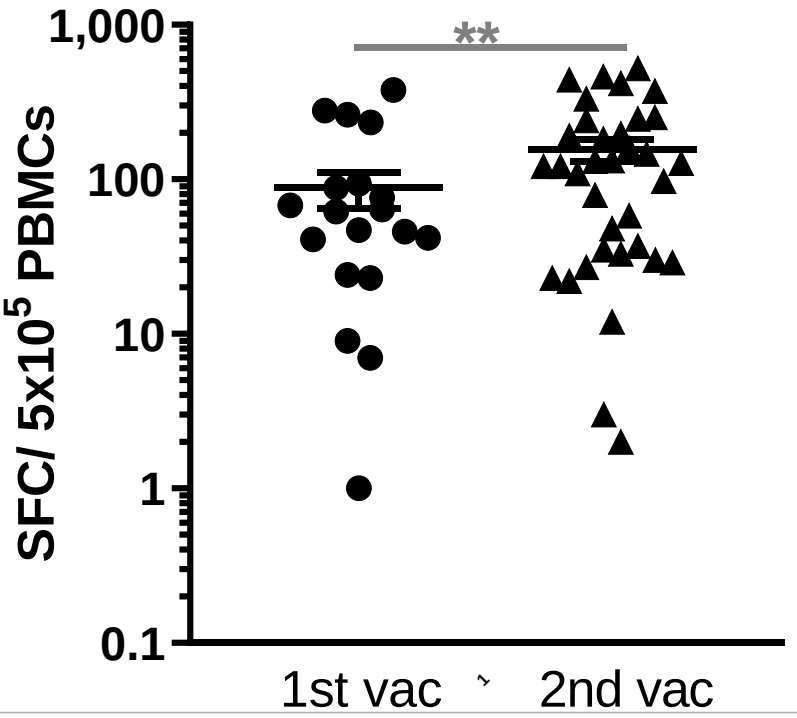
*Unpublished data*

# Significant increase in responses after 2nd vaccination

## Humoral response (87%)



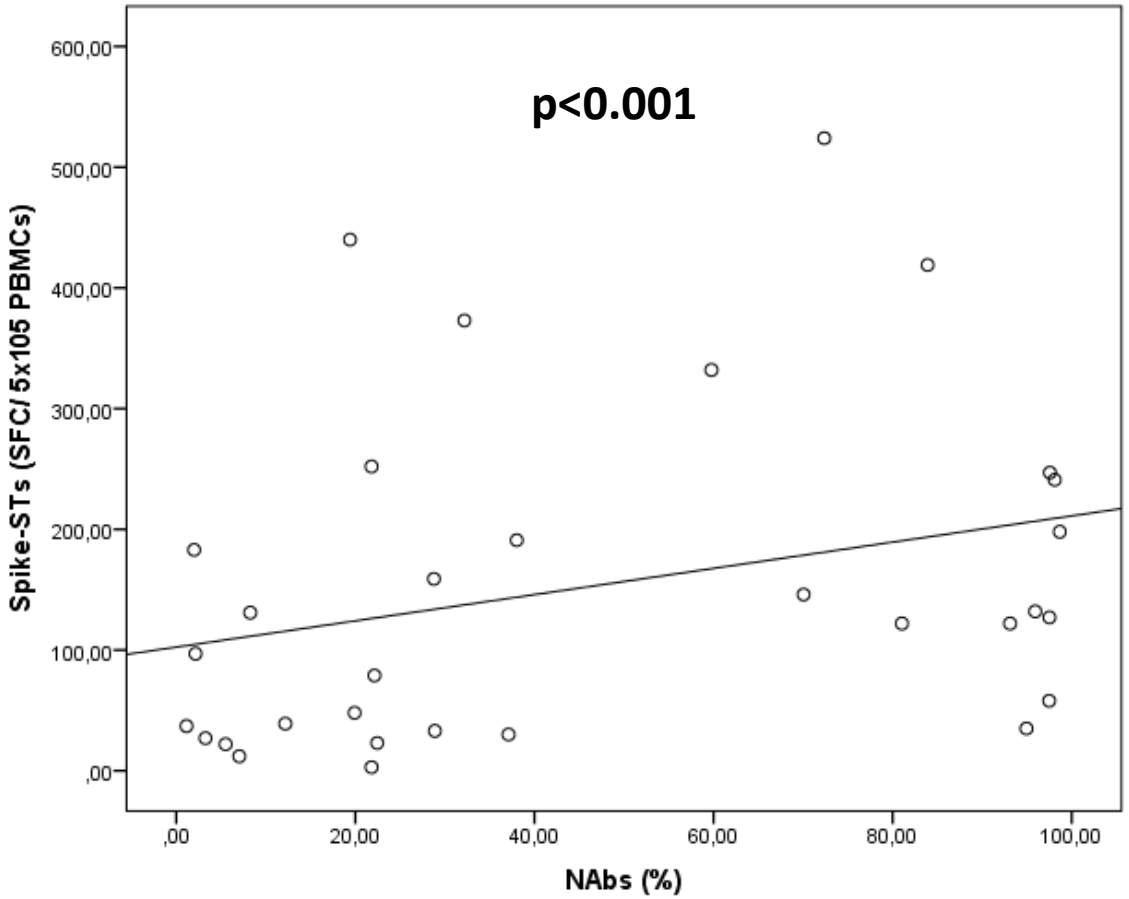

## T cellular response (80%)

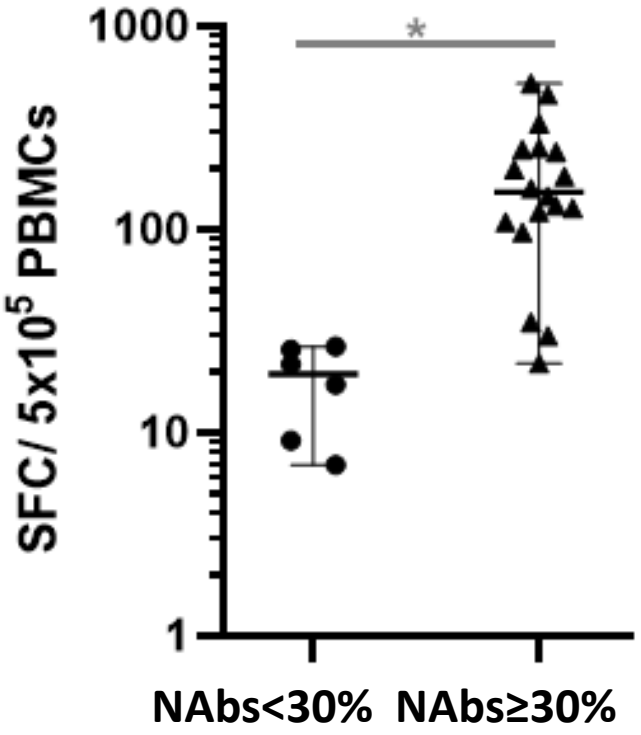





*Unpublished data*

# Significant association between humoral and cellular responses



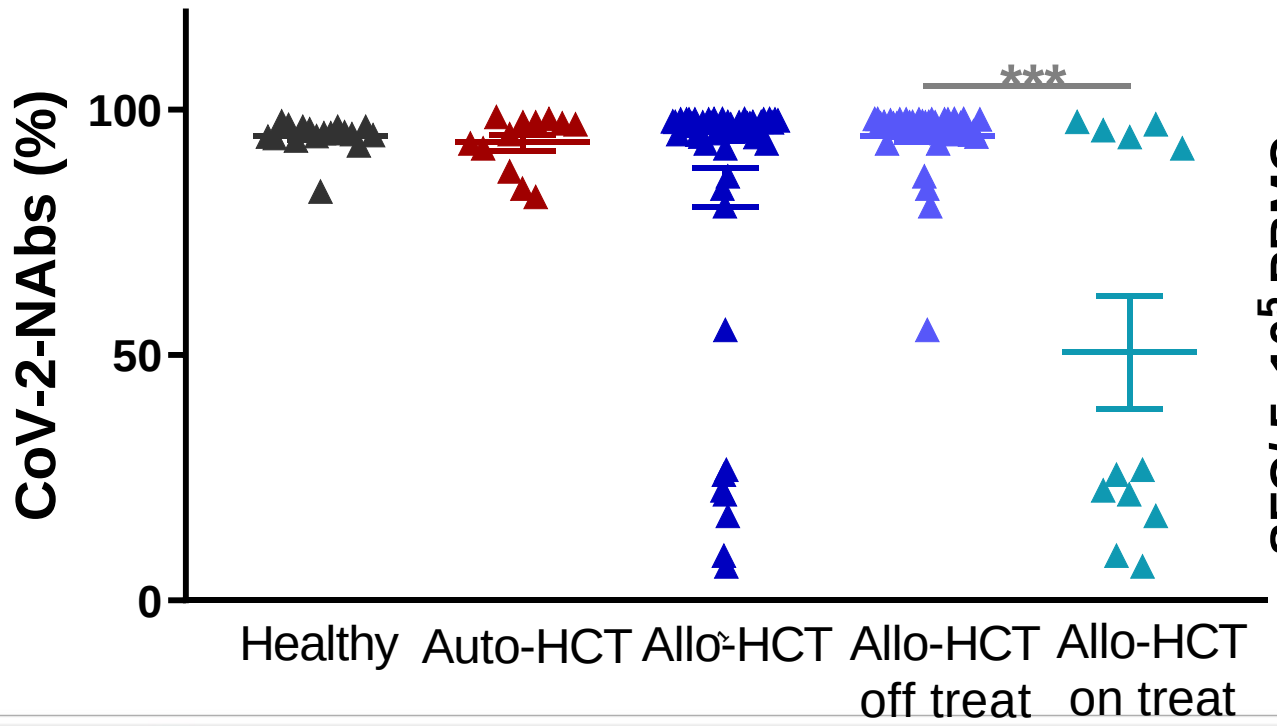





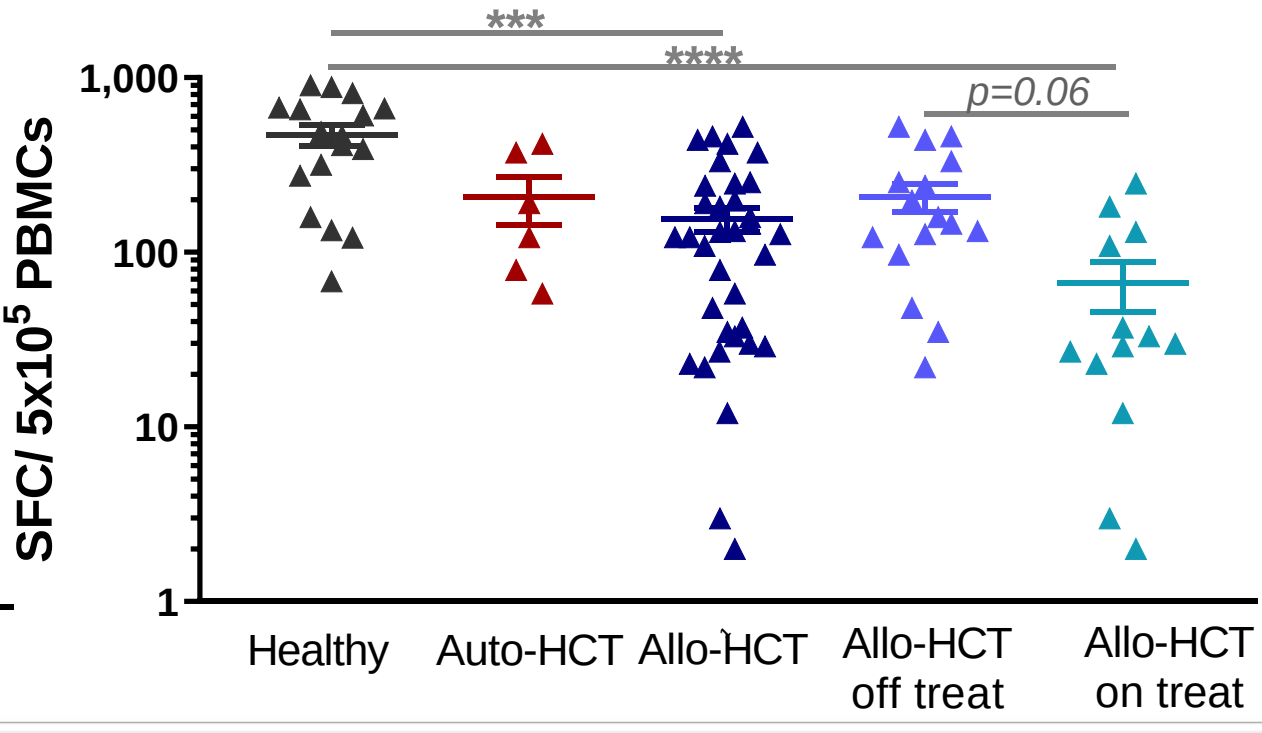

*Unpublished data*

# Significant decrease in alloHCT under immunosuppression

## Humoral response




## T cellular response

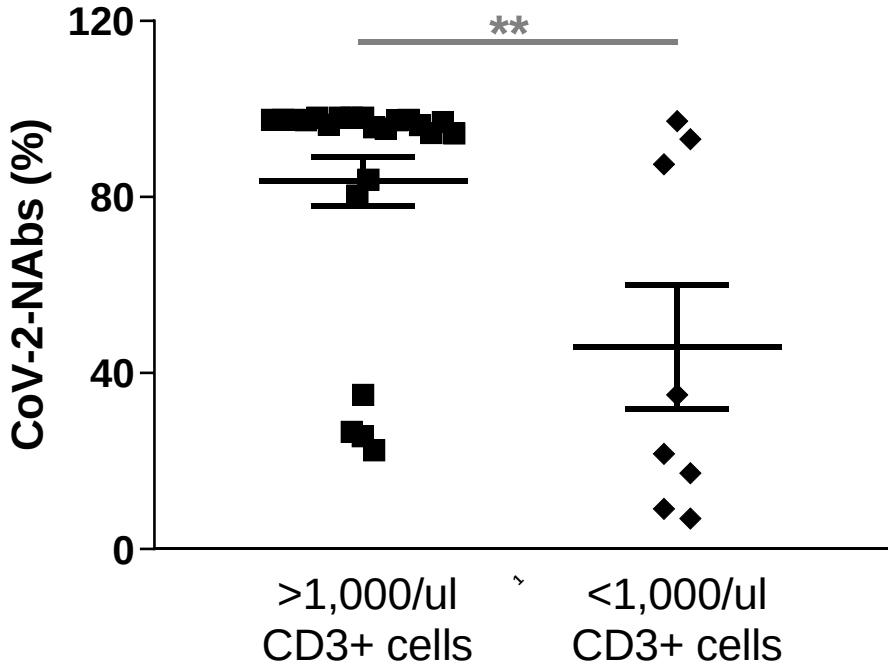




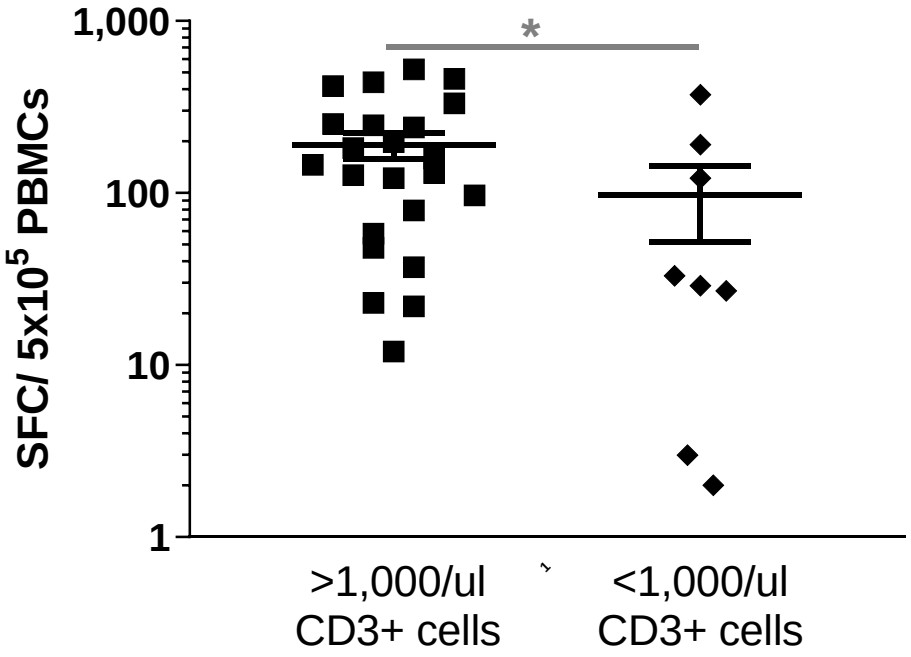

*Unpublished data*

# Significant decrease in HCT recipients with CD3+<1,000/µl

## Humoral response




## T cellular response






*Unpublished data*

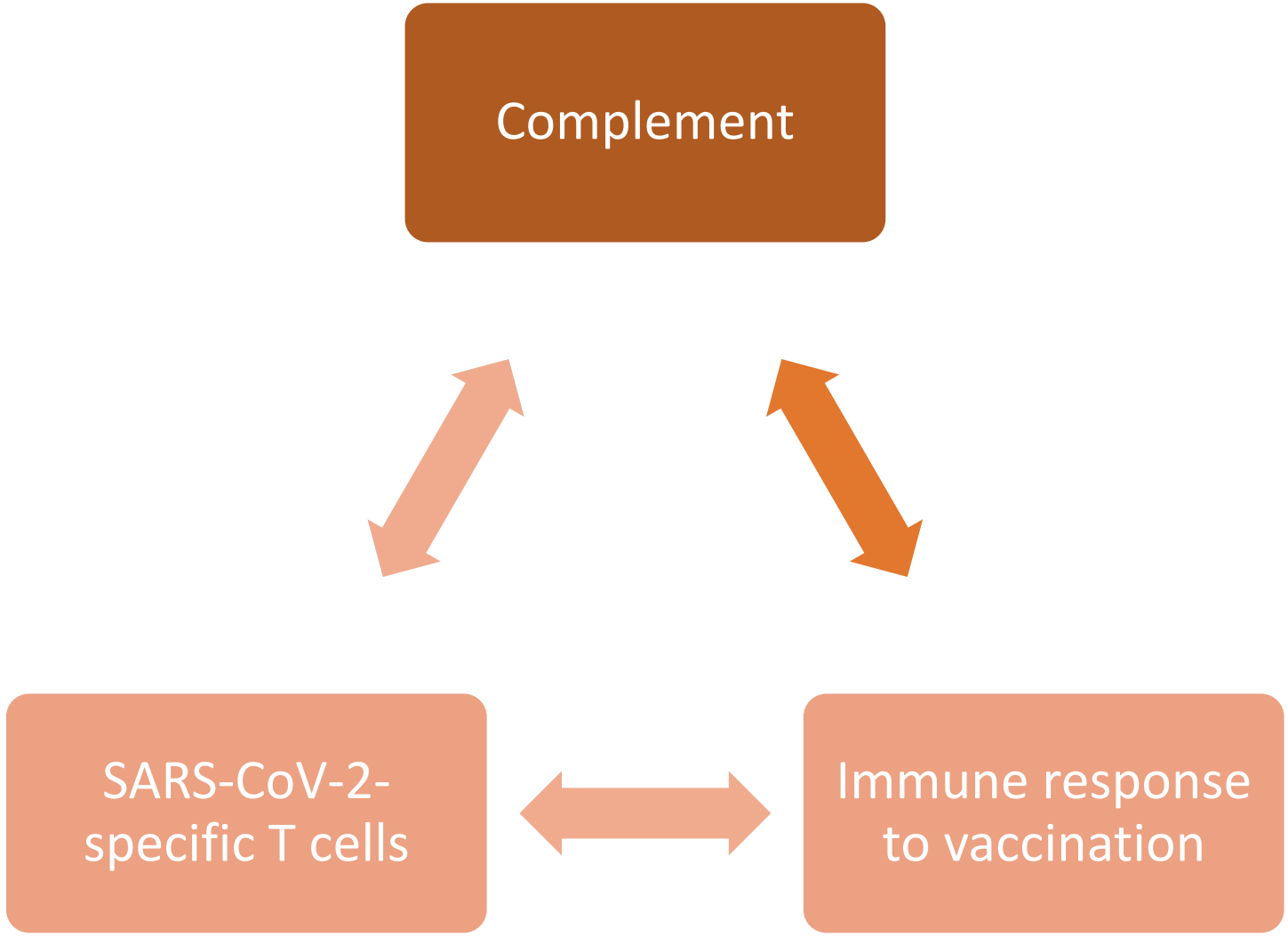
# Conclusions

- Herein, we report **for first time humoral and T cell responses** post SARSCoV-2 vaccination in HCT recipients.
- Transplant recipients not under active and intense immunosuppression at the time of vaccination may benefit significantly from COVID-19 vaccination even though these responses are blunted compared to healthy individuals.
- However, **for the severely immunocompromised patients it seems highly unlikely that they could be protected by vaccination** and for this vulnerable population, different vaccination schemes or therapeutic platforms should be developed along with collateral measures including minimal exposure and immunization of caregivers and health care providers.



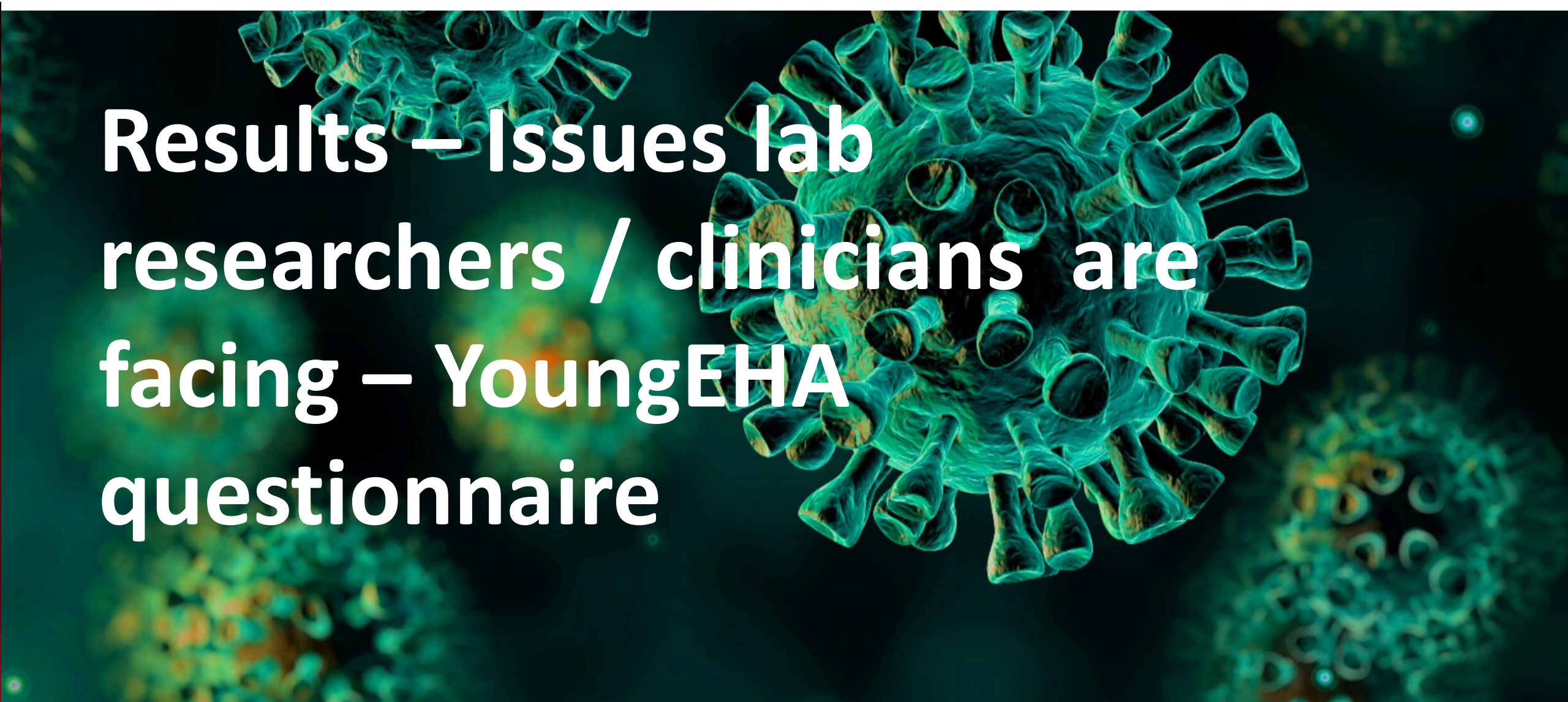
*Unpublished data*

# Immune dysregulation in Covid-19



# Acknowledgements

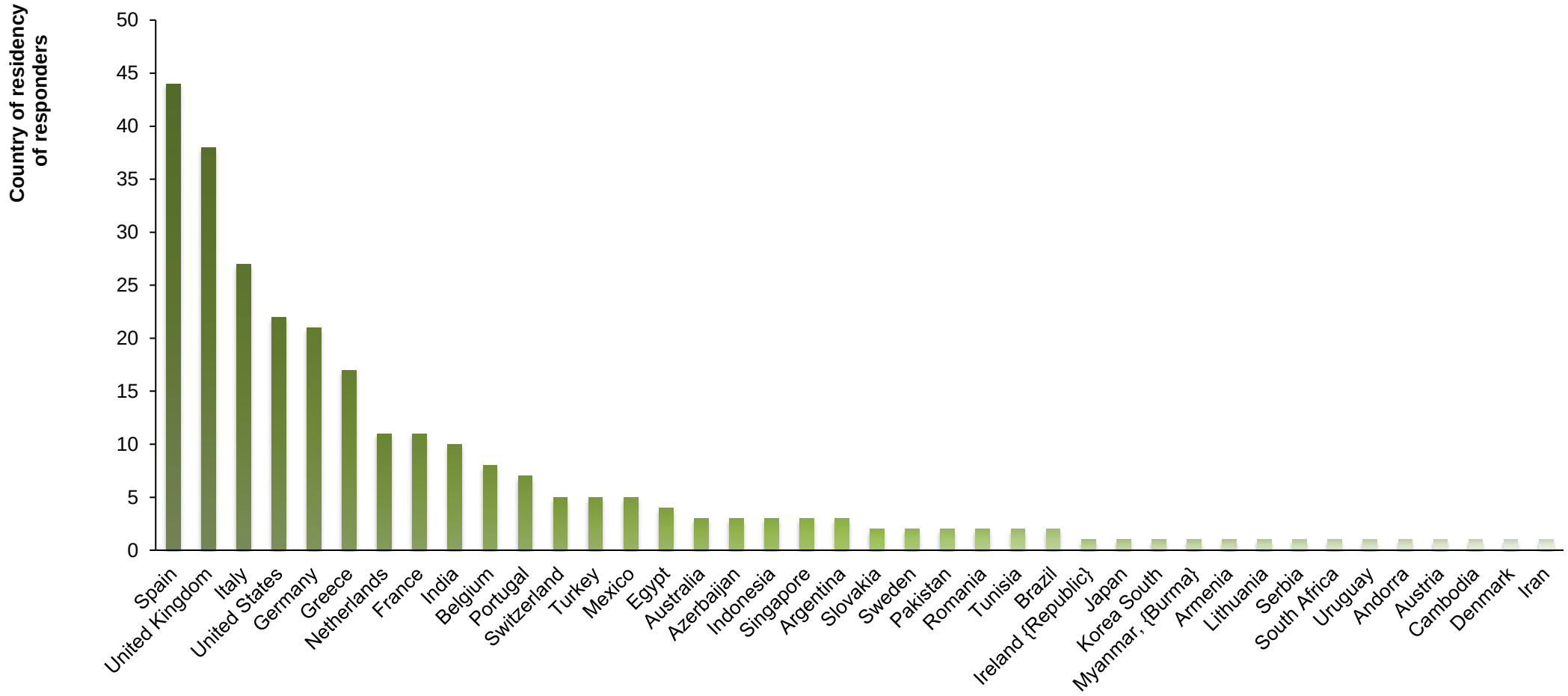




# Results – Issues lab researchers / clinicians are facing – YoungEHA questionnaire

# Effect of the COVID-19 pandemic on laboratory and clinical research:

# a testimony and a call to action from researchers

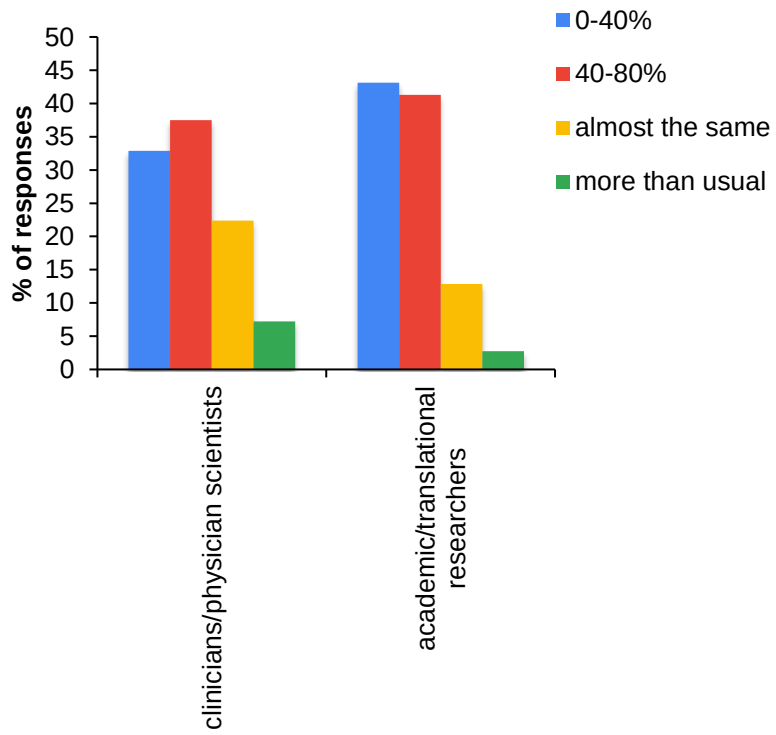



*Kabanova A, et al. Hemasphere 2020*

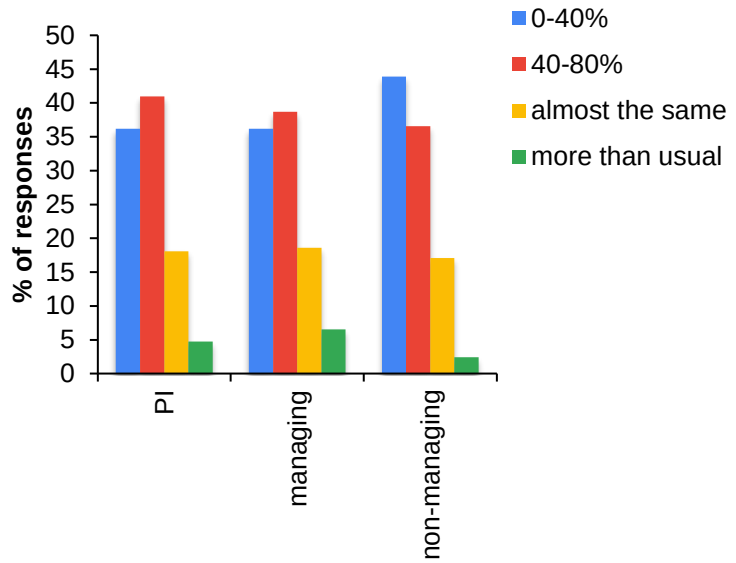
# Effect of the COVID-19 pandemic on laboratory and clinical research:

# a testimony and a call to action from researchers

## Productivity in clinical vs laboratory researchers




## Productivity according to role




*Kabanova A, et al. Hemasphere 2020*

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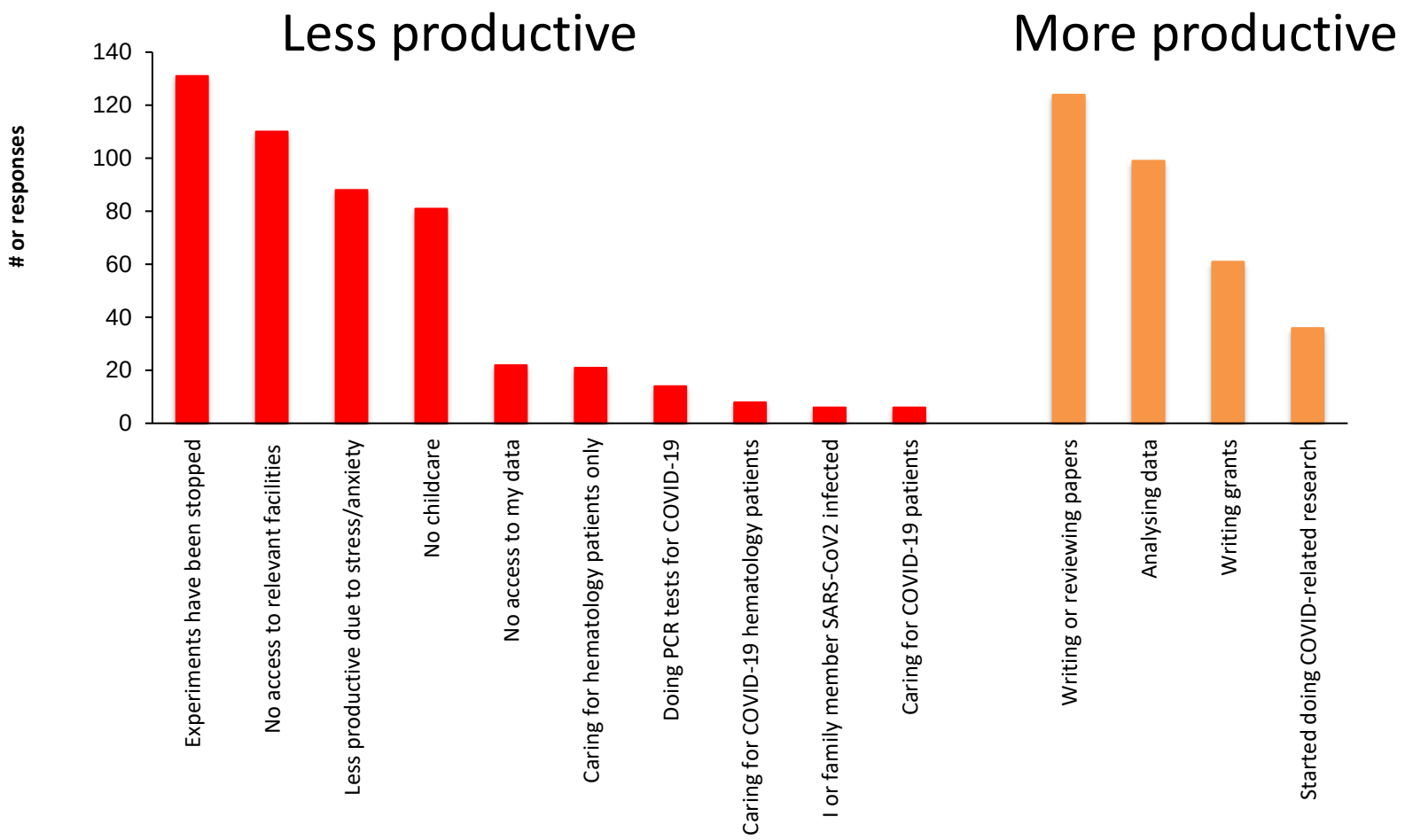
# a testimony and a call to action from researchers




*Kabanova A, et al. Hemasphere 2020*

# Strategies

„Working in the lab in security conditions with reduced number of personnel.“

„building experimental plans for future work“

„All our PhD students are now working on a review. Thus they are using this time to greatly improve their background and still having an output from this period.“

„online classes, e. g. R-coding“

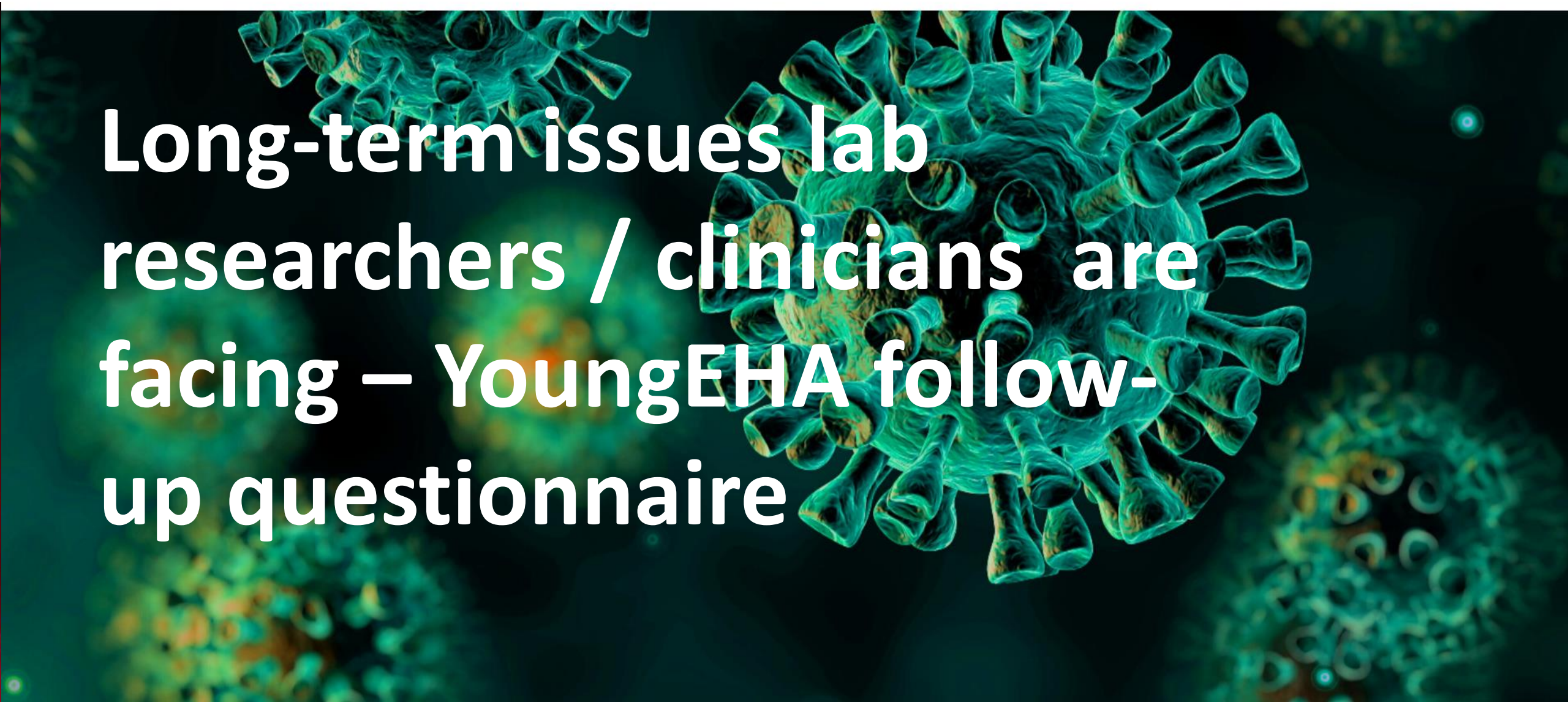
„lab meeting with external experts to discuss topics related to the scientific carreers“

„… creating an European Health Research Council.“

„…team yoga lessons online,…“

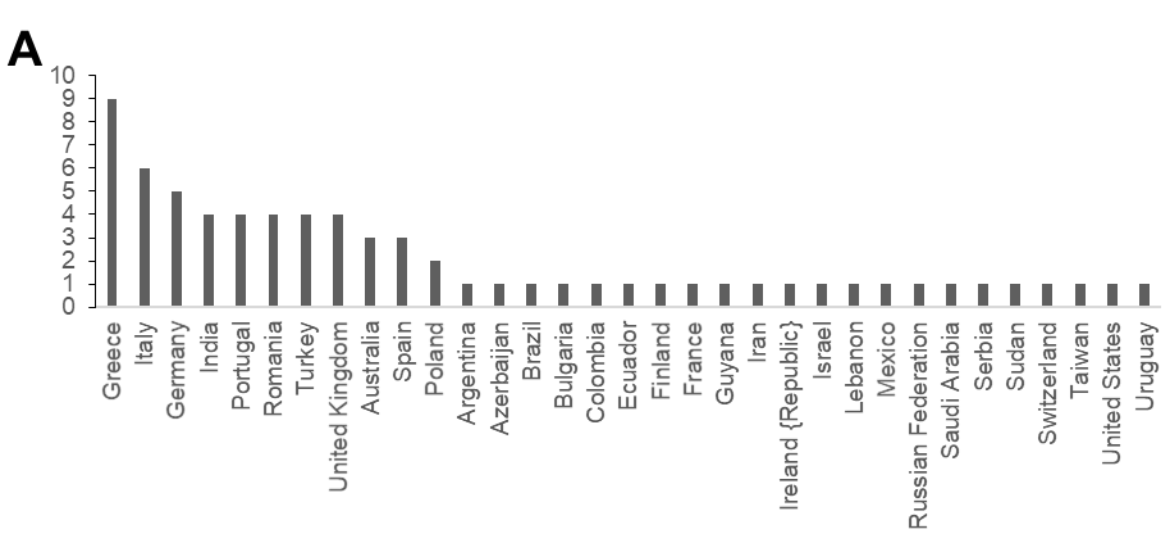
„Science & Beers club to chat about papers while drink a beer together

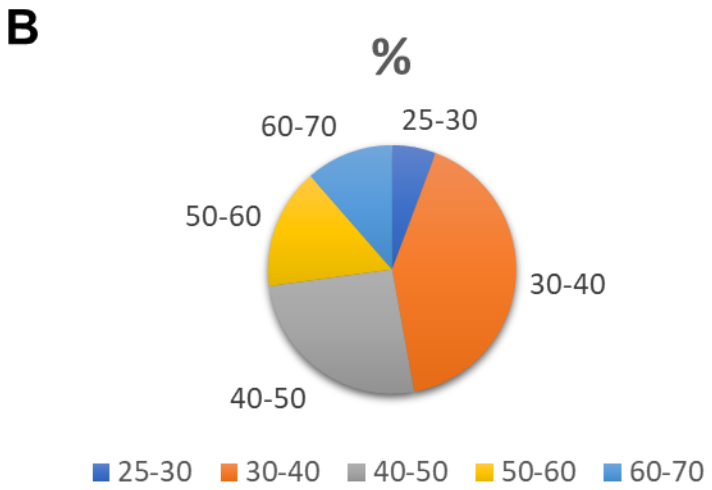
„…catch up for something fun like online codenames.“



# Long-term issues lab researchers / clinicians are facing – YoungEHA follow-up questionnaire

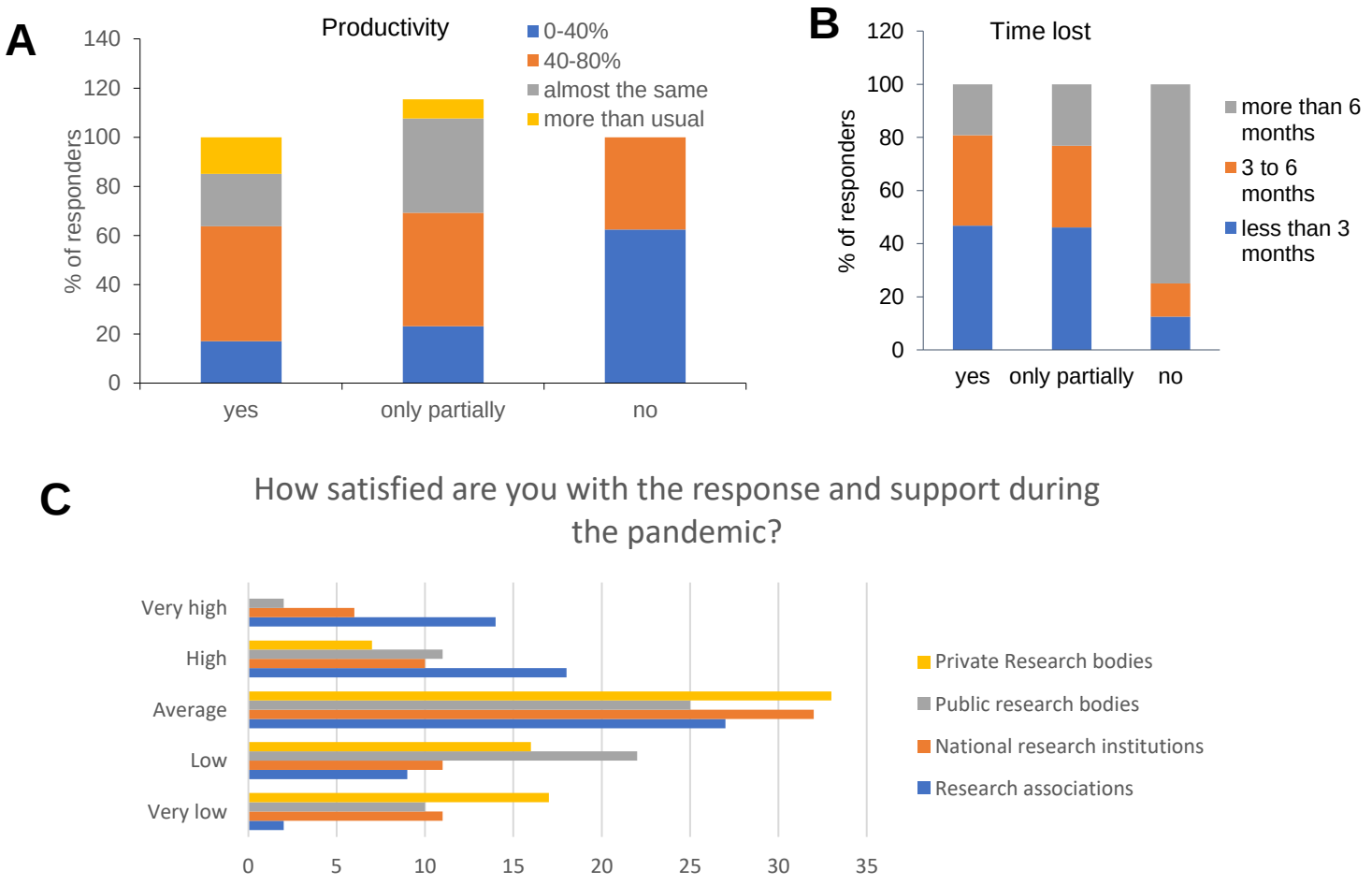
# “Long COVID-19” of researchers: what to do next




*Gavriilaki E, et al. Submitted to Hemasphere 2021*

# “Long COVID-19” of researchers: what to do next




*Gavriilaki E, et al. Submitted to Hemasphere 2021*

EUROPEAN HEMATOLOGY ASSOCIATION

# YoungEHA Research Meeting

Would you like to expand your knowledge on basic and translational research in hematology?