

Journal Pre-proof

Effective anti-SARS-CoV-2 immune response in patients with clonal mast cell disorders.

Julien Rossignol, MD, PhD, Amani Ouedrani, PhD, Cristina Bulai Livideanu, MD, Stéphane Barete, MD, PhD, Louis Terriou, MD, David Launay, MD, PhD, Richard Lemal, MD, PhD, Celine Greco, MD, PhD, Laurent Frenzel, MD, PhD, Cecile Meni, MD, Christine Bodemere-Skandalis, MD, PhD, Laura Polivka, MD, PhD, Anne-Florence Collange, Hassiba Hachichi, MSc, Sonia Bouzourine, Djazira Nait Messaoud, Mathilde Negretto, Laurence Vendrame, Marguerite Jambou, Marie Gousseff, MD, Stéphane Durupt, MD, Jean-Christophe Lega, MD, PhD, Jean-Marc Durand, MD, PhD, Caroline Gaudy, MD, PhD, Gandhi Damaj, MD, PhD, Marie-Pierre Gourin, MD, Mohamed Hamidou, MD, PhD, Laurence Bouillet, MD, PhD, Edwige Le Mouel, MD, Alexandre Maria, MD, Patricia Zunic, MD, Quentin Cabrera, MD, Denis Vincent, MD, PhD, Christian Lavigne, MD, PhD, Etienne Riviere, MD, Clement Gourguechon, MD, Anne Brignier, MD, PhD, Ludovic Lhermitte, MD, PhD, Thierry Jo Molina, MD, PhD, Julie Bruneau, MD, PhD, Julie Agopian, Patrice Dubreuil, PhD, Dana Ranta, MD, Alexandre Mania, MD, Michel Arock, MD, PhD, Isabelle Staropoli, PhD, Olivier Tournilhac, MD, PhD, Olivier Lortholary, MD, PhD, Olivier Schwartz, PhD, Lucienne Chatenoud, MD, PhD, Olivier Hermine, MD, PhD

PII: S2213-2198(22)00015-0

DOI: <https://doi.org/10.1016/j.jaip.2021.12.038>

Reference: JAIP 4035

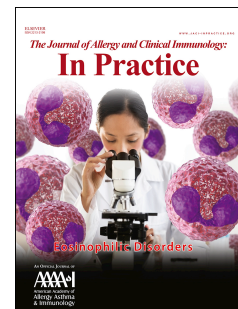
To appear in: *The Journal of Allergy and Clinical Immunology: In Practice*

Received Date: 15 September 2021

Revised Date: 5 December 2021

Accepted Date: 26 December 2021

Please cite this article as: Rossignol J, Ouedrani A, Livideanu CB, Barete S, Terriou L, Launay D, Lemal R, Greco C, Frenzel L, Meni C, Bodemere-Skandalis C, Polivka L, Collange AF, Hachichi H, Bouzourine S, Messaoud DN, Negretto M, Vendrame L, Jambou M, Gousseff M, Durupt S, Lega JC, Durand JM, Gaudy C, Damaj G, Gourin MP, Hamidou M, Bouillet L, Le Mouel E, Maria A, Zunic P, Cabrera Q, Vincent D, Lavigne C, Riviere E, Gourguechon C, Brignier A, Lhermitte L, Molina TJ, Bruneau J, Agopian J, Dubreuil P, Ranta D, Mania A, Arock M, Staropoli I, Tournilhac O, Lortholary O, Schwartz



O, Chatenoud L, Hermine O, Effective anti-SARS-CoV-2 immune response in patients with clonal mast cell disorders., *The Journal of Allergy and Clinical Immunology: In Practice* (2022), doi: <https://doi.org/10.1016/j.jaip.2021.12.038>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2022 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology

Effective anti-SARS-CoV-2 immune response in patients with clonal mast cell disorders.

Julien Rossignol^{1,2*} MD, PhD, Amani Ouedrani^{3,4*} PhD, Cristina Bulai Livideanu⁵ MD, Stéphane Barete⁶ MD, PhD, Louis Terriou⁷ MD, David Launay⁷ MD, PhD, Richard Lemal⁸ MD, PhD, Celine Greco^{1,2} MD, PhD, Laurent Frenzel^{1,2} MD, PhD, Cecile Meni¹ MD, Christine Bodemere-Skandalis^{1,2} MD, PhD, Laura Polivka^{1,2} MD, PhD, Anne-Florence Collange^{1,2}, Hassiba Hachichi MSc¹, Sonia Bouzourine¹, Djazira Nait Messaoud¹, Mathilde Negretto⁵, Laurence Vendrame³, Marguerite Jambou³, Marie Gousseff⁹ MD, Stéphane Durupt¹⁰ MD, Jean-Christophe Lega¹⁰ MD, PhD, Jean-Marc Durand¹¹ MD, PhD, Caroline Gaudy¹¹ MD, PhD, Gandhi Damaj¹² MD, PhD, Marie-Pierre Gourin¹³ MD, Mohamed Hamidou¹⁴ MD, PhD, Laurence Bouillet¹⁵ MD, PhD, Edwige Le Mouel¹⁶ MD, Alexandre Maria¹⁷ MD, Patricia Zunic¹⁸ MD, Quentin Cabrera¹⁸ MD, Denis Vincent¹⁹ MD, PhD, Christian Lavigne²⁰ MD, PhD, Etienne Riviere²¹ MD, Clement Gourguechon²² MD, Anne Brignier²³ MD, PhD, Ludovic Lhermitte²⁴ MD, PhD, Thierry Jo Molina^{2,25} MD, PhD, Julie Bruneau^{2,25} MD, PhD, Julie Agopian²⁶, Patrice Dubreuil²⁶ PhD, Dana Ranta²⁷ MD, Alexandre Mania⁸ MD, Michel Arock²⁸ MD, PhD, Isabelle Staropoli²⁹ PhD, Olivier Tournilhac⁸ MD, PhD, Olivier Lortholary^{1,2} MD, PhD, Olivier Schwartz²⁹ PhD, Lucienne Chatenoud^{3,4} MD, PhD and Olivier Hermine^{1,2†} MD, PhD.

* Co-authorship

† Corresponding author

Authors' affiliations.

¹ French Reference Center for Mastocytosis (CEREMAST), Necker-Enfants Malades University Hospital, AP-HP, Paris, France

² Paris University, Imagine Institute, INSERM U1163, Necker-Enfants Malades University Hospital, Paris, France

³ Paris University, Necker-Enfants Malades Institute, CNRS UMR 8253 and INSERM UMR1151, Necker-Enfants Malades University Hospital, Paris, France

⁴ Laboratory of Immunoregulation and Immunopathology, Necker-Enfants Malades University Hospital, AP-HP, Paris, France

⁵ French Reference Center for Mastocytosis (CEREMAST), Department of Dermatology, Hôpital Larrey, Toulouse University Hospital, Toulouse, France

⁶ French Reference Center for Mastocytosis (CEREMAST), Department of Dermatology, Pitié-Salpêtrière University Hospital, AP-HP, Paris, France

⁷ University Lille, INSERM, CHU Lille, Department of Internal Medicine and Clinical Immunology, U1286 - INFINITE - Institute for Translational Research in Inflammation, Lille, France

⁸ Adult Clinical Hematology, Clermont-Ferrand University Hospital, INSERM CIC501, EA 7453 -Université Clermont Auvergne, Clermont-Ferrand, France

⁹ Department of Internal Medicine, Bretagne Atlantique Hospital, Vannes, France

¹⁰ Department of Internal Medicine, Adult Cystic Fibrosis Care Center, Hospices Civils de Lyon, Lyon, France

¹¹ Department of Internal Medicine, Aix-Marseille University, Timone University Hospital, Marseille, France

¹² Haematology Institute, Normandy University School of Medicine, Caen, France.

¹³ Laboratory of Hematology, Dupuytren University Hospital, Limoges, France

¹⁴ Department of Internal Medicine, Hôtel-Dieu University Hospital, Nantes, France

¹⁵ Clinical Immunology/Internal Medicine Department, National Reference Center for Angioedema, Grenoble University Hospital, Grenoble, France

¹⁶ Department of Internal Medicine and Clinical Immunology, Rennes University Hospital, Rennes, France

¹⁷ Department of Internal Medicine, Montpellier University Hospital, Montpellier, France

¹⁸ Department of Haematology, Sud Réunion University Hospital, Saint Pierre, La Réunion, France

¹⁹ Department of Pneumology and Internal Medicine, Caremeau University Hospital, Nîmes, France

²⁰ Department of Internal Medicine, Angers University Hospital, Angers, France

²¹ Department of Internal Medicine, Bordeaux University Hospital, Haut-Lévêque Hospital, Pessac, France

²² Department of Hematology, Amiens University Hospital, Amiens, France

²³ Therapeutic Apheresis Unit, Saint-Louis University Hospital, AP-HP, Paris, France

²⁴ Laboratory of Onco-Hematology, Necker-Enfants Malades University Hospital, APHP, Paris, France

²⁵ Pathology Department, Necker-Enfants Malades University Hospital, APHP, Paris, France

²⁶ CRCM, [Signaling, Hematopoiesis and Mechanism of Oncogenesis, CEREMAST, AFIRMM], INSERM U1068; Institut Paoli-Calmettes; Aix-Marseille Univ, UM105; CNRS, UMR7258, Marseille, F-13009, France

²⁷ Department of Haematology, Nancy University Hospital, Nancy, France

²⁸ Laboratory of Haematology, Pitié-Salpêtrière University Hospital, Paris, France

²⁹ Virus & Immunity Unit, Department of Virology, Institut Pasteur, CNRS UMR3569, Paris, France

[†] Corresponding author:

Professor Olivier Hermine

Service d'hématologie adulte

Assistance Publique-Hôpitaux de Paris, Hôpital Necker-Enfants Malades

149-161 Rue de Sèvres

F-75743 Paris Cedex 15, France

E-mail: ohermine@gmail.com

Phone: +33-144-495-282

Funding sources, Disclosure of Conflicts of Interest for this study:

All authors declare no conflicts of interest for this work

Julien Rossignol MD PhD: Consulting fees from Blueprint and Novartis

Amani Ouedrani PhD: Author has no conflict of interest to declare

Cristina Bulai Livideanu MD: Author has no conflict of interest to declare

Stéphane Barete MD PhD: Author has no conflict of interest to declare

Louis Terriou MD: Author has no conflict of interest to declare

David Launay MD PhD: Author has no conflict of interest to declare

Richard Lemal MD PhD: Author has no conflict of interest to declare

Celine Greco MD PhD: Author has no conflict of interest to declare

Laurent Frenzel MD PhD: Author has no conflict of interest to declare

Cecile Meni MD: Author has no conflict of interest to declare

Christine Bodemere-Skandalis MD PhD: Author has no conflict of interest to declare

Laura Polivka MD PhD: Author has no conflict of interest to declare

Anne-Florence Collange: Author has no conflict of interest to declare

Hassiba Hachichi MSc: Author has no conflict of interest to declare

Sonia Bouzourine: Author has no conflict of interest to declare

Djazira Nait Messaoud: Author has no conflict of interest to declare

Mathilde Negretto: Author has no conflict of interest to declare

Laurence Vendrame: Author has no conflict of interest to declare

Marguerite Jambou: Author has no conflict of interest to declare

- 101 Marie Gousseff MD: Author has no conflict of interest to declare
- 102 Stéphane Durupt MD: Author has no conflict of interest to declare
- 103 Jean-Christophe Lega MD PhD: Author has no conflict of interest to declare
- 104 Jean-Marc Durand MD PhD: Author has no conflict of interest to declare
- 105 Caroline Gaudy MD PhD: Consulting fees and/or honoraria from BMS, Blueprint, MSD,
- 106 Roche
- 107 Gandhi Damaj MD PhD: Consulting fees and/or honoraria from Takeda, Iqone, Blueprint
- 108 Marie-Pierre Gourin MD: Author has no conflict of interest to declare
- 109 Mohamed Hamidou MD: Consulting fees from Blueprint
- 110 Laurence Bouillet MD PhD: Research grants, and/or consulting fees, and/or honoraria from
- 111 Takeda, CSL Behring, Biocryst, Biomarin, Novartis, GSK.
- 112 Edwige Le Mouel MD: Author has no conflict of interest to declare
- 113 Alexandre Maria MD: Author has no conflict of interest to declare
- 114 Patricia Zunic MD: Author has no conflict of interest to declare
- 115 Quentin Cabrera MD: Author has no conflict of interest to declare
- 116 Denis Vincent MD PhD: Author has no conflict of interest to declare
- 117 Christian Lavigne MD MSc: Consulting fees and/or honoraria from Sanofi-Genzyme,
- 118 Boehringer-Ingelheim, Shire, Ultragenyx, Biomarin
- 119 Etienne Riviere MD, PhD: Author has no conflict of interest to declare
- 120 Clement Gourguechon MD: Author has no conflict of interest to declare
- 121 Anne Brignier MD PhD: Author has no conflict of interest to declare
- 122 Ludovic Lhermitte MD PhD: Author has no conflict of interest to declare
- 123 Thierry Jo Molina MD PhD: Author has no conflict of interest to declare
- 124 Julie Bruneau MD PhD: Author has no conflict of interest to declare
- 125 Julie Agopian MSc: Author has no conflict of interest to declare

Patrice Dubreuil PhD: Author has no conflict of interest to declare

Dana Ranta MD: Author has no conflict of interest to declare

Alexandre Mania MD: Author has no conflict of interest to declare

Michel Arock MD PhD: Consultancy fees from Blueprint et Novartis

Isabelle Staropoli PhD: Author has no conflict of interest to declare

Olivier Tournilhac MD PhD: Author has no conflict of interest to declare

Olivier Lortholary MD PhD: Author has no conflict of interest to declare

Olivier Schwartz PhD: Author has no conflict of interest to declare

Lucienne Chatenoud MD PhD: Author has no conflict of interest to declare

Olivier Hermine MD PhD: AB science: Consultancy, Equity Ownership, Honoraria, Research Funding; Celgene: Research Funding; Novartis: Research Funding.

Word counts: Abstract: 247/250 words – **Manuscript:** 3689/3500 words – **Figures:** 4 –
Tables: 1 – **Supplemental Figure:** 3 – **Supplemental Table:** 1 – **References:** 26.

Abstract

Background: Mast cells are key players in innate immunity and the Th2 adaptive immune response. The latter counterbalances the Th1 response, which is critical for antiviral immunity. Clonal mast cell activation disorders (cMCADs, such as mastocytosis and clonal mast cell activation syndrome) are characterized by abnormal mast cell accumulation and/or activation. No data on the antiviral immune response in patients with MCADs have been published.

Objective: To study a comprehensive range of outcomes in cMCAD patients with PCR- or serologically confirmed COVID-19 and to characterize the specific anti-SARS-CoV-2 immune response in this setting.

Methods: Clinical follow-up and outcome data were collected prospectively over a 12-month period by members of the French *Centre de Référence des Mastocytoses* rare disease network. Anti-SARS-CoV-2-specific T-cell activity was measured with an enzyme-linked immunospot assay, and humoral responses were evaluated by assaying circulating levels of specific IgG, IgA and neutralizing antibodies.

Results: Overall, 32 cMCAD patients were evaluated. None required non-invasive or mechanical ventilation. Two patients were admitted to hospital for oxygen and steroid therapy. The SARS-CoV-2-specific immune response was characterized in 21 of the 32 patients. Most had high counts of circulating SARS-CoV-2-specific, interferon (IFN)- γ -producing T-cells and high titers of neutralizing anti-spike IgGs. The patients frequently showed spontaneous T-cell IFN- γ production in the absence of stimulation; this production was correlated with basal circulating tryptase levels (a marker of the mast cell burden).

Conclusion: Patients with cMCADs might not be at risk of severe COVID-19 – perhaps due to their spontaneous production of IFN- γ .

Highlights box:

What is already known about this topic?

Mast cells are key players in the Th2 immune response but not in antiviral immune responses. Data on antiviral responses (and especially responses against coronaviruses) in patients with clonal mast cell activation disorders have not previously been reported.

What does this article add to our knowledge?

In a comprehensive, prospective study, we did not observe any cases of severe COVID-19 among groups of patients with clonal mast cell activation disorders. The patients showed

effective anti-coronavirus immune responses. Spontaneous interferon gamma release (in the absence of T-cell stimulation) was observed frequently in patients with clonal mast cell activation disorders and was correlated with the basal tryptase level.

How does this study impact current management guidelines?

The observed spontaneous production of interferon gamma (correlated with the mast cell burden) suggests that mast cells have a role in the antiviral immune response. The four patients with serial serologic measurements became seronegative over time. Hence, anti-SARS-CoV-2 vaccination after COVID-19 is strongly recommended in patients with clonal mast cell activation disorders.

Key words: SARS-CoV-2, COVID-19, Mast cells, Mastocytosis, Clonal mast cell activation syndrome, Mast cell activation disorders, T-cells, B-cells

Abbreviations

APHP: *Assistance Publique - Hôpitaux de Paris* (Paris Public Hospital Group)

BMI: body mass index

CEREMAST: *Centre de Référence des Mastocytoses*

CM: cutaneous mastocytosis

cMCAD: clonal mast cell activation disorder

COVID-19: coronavirus disease 19

HEK: human embryonic kidney

IFN: interferon

ISM: indolent systemic mastocytosis

MIS: mastocytosis in the skin

MCAS: mast cell activation syndrome

MMAS: monoclonal mast cell activation syndrome

198 NK: natural killer

199 PBMC: peripheral blood mononuclear cell

200 PHA: phytohemagglutinin

201 PMSI: Programme de médicalisation des systèmes d'information

202 SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

203 SSM: smoldering systemic mastocytosis

204 WHO: World Health Organization

205 **Introduction**

206 Clonal mast cell activation disorders (cMCADs) constitute a heterogeneous disease spectrum
207 that ranges from monoclonal mast cell activation syndrome (MMAS) to mastocytosis and is
208 characterized by the pathological activation and/or accumulation of mast cells (MC)¹. In adults,
209 the most frequent cMCAD is indolent systemic mastocytosis (ISM). Advanced mastocytosis
210 (including aggressive systemic mastocytosis, MC leukemia, and systemic mastocytosis with an
211 associated hematological neoplasm) is rarer and is linked to a poor prognosis^{2,3}.

212 Coronavirus disease 2019 (COVID-19) is a potentially fatal, pandemic infectious disease
213 caused by severe respiratory syndrome coronavirus 2 (SARS-CoV-2)⁴. A body of compelling
214 pathophysiological data suggests that interferons (IFNs) have a major role in disease control.
215 These includes type I IFN (produced by plasmacytoid dendritic cells) and type III IFN (IFN- γ)
216 produced by T-cells from the adaptive immune system in the early and late phases of the
217 disease, respectively⁵⁻⁸.

218 One can hypothesize that the MCs' ability to drive Th2 responses⁹⁻¹¹ (which counterbalance
219 Th1 responses) might impair antiviral immunity in patients with cMCADs. Furthermore,
220 histamine blocks the *in vitro* activity of plasmacytoid dendritic cells; *in vivo*, this might weaken
221 antiviral immune responses¹². MCs might therefore (i) contribute to COVID-19-induced

inflammation by releasing pro-inflammatory cytokines such as interleukin (IL-)1, IL-6, and tumor necrosis factor (TNF) and (ii) exacerbate COVID-19 lung damage *via* degranulation^{13,14}. If so, this would render patients with cMCAD more susceptible to severe COVID-19.

Over a 12-month period, members of the French *Centre de Référence des Mastocytoses* (CEREMAST) rare disease network collected data prospectively on the patients with cMCADs (MMAS and mastocytosis) and a positive PCR test or serology assay for SARS-CoV-2. Here, we describe the patients' clinical course, outcomes, and immunologic characteristics.

Methods

Patients

The members of the CEREMAST network collected data prospectively from patients with a cMCAD and COVID-19, as documented by either a positive SARS-CoV-2 PCR test on a nasal swab or symptoms suggestive of COVID-19 and a positive anti-SARS-CoV-2 serology assay. Patients with symptoms suggestive of COVID-19 but who lacked a positive PCR test or a positive serology assay for SARS-CoV-2 infection were excluded from the study. The study data were covered cases recorded between February 1st, 2020, and February 1st, 2021. cMCADs were diagnosed according to the 2016 World Health Organization (WHO) classification^{1,15}.

Firstly, we sent a questionnaire to all adult patients (aged 18 or over) with mastocytosis or MMAS and for whom recent follow-up data were available in the CEREMAST national registry (n=828) and who were participating in a study sponsored by the *Association Française pour les Initiatives de Recherche sur le Mastocyte et les Mastocytose* (AFIRMM) study. The questionnaire was designed to collect data on any signs of MC activation displayed during COVID-19, the patient's treatments, and the patient's specific signs and outcomes related to COVID-19. We then classified the patients as having had proven COVID-19 or not (Figure 1). Subsequently, we surveyed all members of the CEREMAST network (n=24) and thereby

collected data on patients who had presented with COVID-19 but had not replied to the questionnaire. To check that we had not missed any severe cases (at least in the greater Paris region, as a proxy for national coverage), we also searched the French national hospital discharge database (*Programme de médicalisation des systèmes d'information, PMSI*) for cMCAD patients admitted for COVID-19 to hospitals in the *Assistance Publique - Hôpitaux de Paris* (the Paris Public Hospital Group, which treats 8.3 million patients a year). All patients with confirmed COVID-19 were assayed for anti-SARS-CoV-2 antibodies. In order to characterize the anti-SARS-CoV-2 immune response, patients included in the AFIRMM register were asked to provide a blood sample (for serology testing) during a follow-up consultation.

For the experiment on spontaneous IFN- γ production, we incorporated a control group of patients with idiopathic mast cell activation syndrome (MCAS) and who had either experienced mild-to-moderate COVID-19 (n=2) or had no history of COVID-19 (n=9). MCAS was diagnosed according to the modified European Competence Network on Mastocytosis guidelines¹⁶. An increase in the serum total tryptase level by at least 20% above baseline plus 2 ng/mL during or within 4 hours of a symptomatic period was not investigated in the majority of patients.

To measure the spontaneous production of IFN γ in the ELISPOT assay, we studied PBMCs from patients with cMCADs and MCAS (with and without a history of COVID-19) and healthy donors with a history of COVID-19.

Ethic Statements

All the patients with cMCADs were followed up in CEREMAST mastocytosis network reference centers and were enrolled in a prospective, nationwide, multicenter study sponsored by AFIRMM. The AFIRMM study was approved by the local investigational review board

(Comité de Protection des Personnes Ile-de-France, France; reference: 93-00) and was carried out in compliance with the principles of the Declaration of Helsinki. The patients gave their written informed consent to participation. The patients' blood samples were obtained as part of routine follow-up care for their cMCADs. Data on the control cohorts were collected prospectively and analyzed as part of the COVID-HOP study (Clinicaltrials.gov: NCT04418375; other study identifier: APHP200609).

Immunological assays

SARS-CoV-2 specific T-cell responses were evaluated by measuring the cells' IFN- γ production in an enzyme-linked immunospot (ELISpot) assay. Briefly, peripheral blood mononuclear cells (PBMCs) were isolated from fresh blood collected during a follow-up consultation. After isolation on a Ficoll density gradient, the PBMCs were stimulated for 18 to 20 h with individual 15-mer 11-aa overlapping peptide pools for various SARS-CoV-2 proteins or common coronavirus proteins. Each responding cell generated a spot. The ELISpot results were expressed as the number of spot-forming cell (SFCs)/ 10^6 CD3⁺ T-cells after the subtraction of background values from wells with non-stimulated cells.

The negative controls were PBMCs cultured in RPMI-1640 medium supplemented with L-glutamine, sodium bicarbonate (Sigma-Aldrich, Molsheim, France) and 10% human AB serum (Biowest, Nuaille, France), in the absence of stimulation. The positive controls were phytohemagglutinin (PHA) (Sigma-Aldrich) and the CEFX Ultra SuperStim Pool (JPT Peptide Technologies GmbH, BioNTech AG, Berlin, Germany). The tested SARS-CoV-2 peptide pools were derived from a peptide scan through the SARS-CoV-2 spike glycoprotein (two pools: S1 for the N-terminal fragment, and S2 for the C-terminal fragment), membrane protein (M), nucleoprotein (N), envelope small membrane protein (E), and ORF3a protein. We also tested

peptide pools derived from the spike glycoprotein of the common human alpha-coronaviruses (HCoV-229E and HCoV-NL63) and beta-coronaviruses (HCoV-OC43 and HCoV-HKU1).

The humoral immune response (including the production of anti-spike SARS-CoV-2 IgG, IgA antibodies and the neutralizing ability of anti-spike IgGs) was characterized using previously described S-flow and S-pseudotype neutralization assays¹⁷. Briefly, the S-flow assay used human embryonic kidney (HEK)293T cells transduced with the SARS-CoV-2 spike protein. Cells were incubated with sera from patients (dilution: 1:300) and stained with either anti-IgG or anti-IgA antibodies. The fluorescent signal was measured by flow cytometry. For the S-pseudotype neutralization assay, pseudotyped viruses carrying SARS-CoV-2 spike protein were used. The viral pseudotypes were incubated with the sera to be tested (dilution: 1:100), added to transduced HEK293T cells expressing ACE2, and incubated for 48 h at 37°C. The assay measures the anti-S antibodies' ability to neutralize an infection, as described elsewhere¹⁷.

Non-cMCAD control groups

For each group, the number of patients, the median age and the sex ratio are reported in Supplemental Table E1. Four control groups were used in this study: (i) non-MCAD patients convalescing from mild-to-moderate (n=17) and (ii) severe (n=15) forms of COVID-19 and who had already been evaluated with SARS-CoV-2 ELISpot and serology assays in Necker Hospital's immunology laboratory, (iii) idiopathic MCAS patients with or without a history of COVID-19, and (iv) non-MCAD patients with no history of COVID-19 (n=15). We compared the cMCAD patients with age-matched MCAS patients who presented symptoms of MC activation and were being treated with antimediator agents; at the time of the SARS-CoV-2 infection and the immunoassays, all 11 of the MCAS patients were being treated with H1 antihistamines, 8 were being treated with H2 antihistamines, and 5 were being treated with

montelukast. The cMCAD and MCAS patient subgroups did not differ significantly with regard to age (mean age: 45.6 vs. 49.2, respectively; $p=0.35$).

Statistics

Statistical analyses were performed using GraphPad Prism software (version 6.0; GraphPad Software, Inc., La Jolla, CA). Groups were compared using Student's *t* test, a chi-square test or Fisher's exact test, as appropriate. Data were expressed as the mean, median [interquartile range], or median (range). The threshold for statistical significance was set to $p<0.05$ (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$, ****, $p<0.0001$, in figure legends).

Results

From February 1st, 2020, to February 1st, 2021, 32 patients with cMCADs and proven COVID-19 were prospectively identified by the CEREMAST network (Figure 1). Eighteen of the 32 had replied to the questionnaire, and 14 patients had been subsequently identified by referring physicians in CEREMAST centers. No additional inpatient cases were found in the APHP database.

The characteristics and outcomes of the 32 patients with cMCADs and COVID-19 are summarized in Table 1. The median (range) age was 49.7 years (range: 25.6–76.4), with female predominance (59.4%). The cMCAD subtype was cutaneous mastocytosis or mastocytosis in the skin in 14 patients, ISM in 15 patients, smoldering systemic mastocytosis (SSM) in one patient, and MMAS in 2 patients. Of the 21 patients having undergone genetic testing, 18 (85.7%) carried the D816V *KIT* mutation. Ten patients (31.3%) had experienced a severe anaphylactic reaction, and the median (range) basal serum tryptase level before the appearance of clinical or laboratory signs of COVID-19 was 13.0 $\mu\text{g/L}$ (2.7–163.0 $\mu\text{g/L}$). Risk factors for

severe COVID-19 were present in 13 of the 32 patients (40.6%), and four had at least two risk factors¹⁸: a body mass index >30 (n=4), age >65 (n= 4), ongoing cytoreductive therapy (midostaurin or cladribine) or recent (in the previous 12 months) administration of cladribine (n=3), cardiovascular conditions (including arterial hypertension and chronic heart failure) (n=7), and diabetes (n=2). At the time of the SARS-CoV-2 infection, 23 of the 32 patients (71.9%) were taking symptomatic medications (H1 antihistamines, H2 antihistamines, and/or montelukast), one patient with SSM was receiving midostaurin after failure to respond to cladribine, and one patient with ISM had recently received cladribine.

With regard to the diagnosis of COVID-19, 23 of the 32 patients (71.9%) had a positive SARS-CoV-2 PCR test on a nasal swab, and 9 of the 32 had a negative PCR test (or did not undergo a PCR test on a nasal swab, due to non-availability of the procedure at the time of infection) and positive serology test. The great majority of the patients (29 out of 32) became seropositive during their follow-up. As expected, patients with subsequently available serum samples (n=4) became seronegative after a median follow-up of 33.0 weeks. Regarding the symptoms of COVID-19, 22 of the 32 (68.8%) patients had fever (>38°C), and 18 (56.3%) had anosmia and/or ageusia. Only two patients were admitted to hospital for corticosteroid therapy and oxygen therapy (corresponding to stage 5 on the WHO COVID-19 clinical progression scale¹⁹) but neither required non-invasive or mechanical ventilation. Interestingly, 8 of the 32 patients (25.0%) reported an increase in signs of MC activation during the episode of COVID-19, while 3 (9.4%) reported a decrease. No recurrences of the infection were reported.

The specific cellular and humoral anti-SARS-CoV-2 immune responses were studied in 21 patients with cMCADs a median [IQR] of 24 weeks [7–36] from infection. Overall, 20 of the 21 cMCAD patients developed a specific T-cell response against at least one of the SARS-CoV-2 peptide pools tested. The responses were usually moderately intense. The median [IQR] intensity was 37 [24–130] SFC/10³ CD3 for the S1 pool, 108 [23–201] SFC/10³ CD3 for the

S2 pool, 62 [21–146] SFC/ 10^3 CD3 for the M pool, 78 [48–256] SFC/ 10^3 CD3 for the N pool and 23 [10–66] SFC/ 10^3 CD3 for the AP3a pool. The only patient (#6) who did not develop a specific T-cell response was young (38), had presented mild symptoms of COVID-19 (confirmed by a positive PCR test on a nasal swab), and did not have a history of immunodeficiency or immunosuppressive therapy.

Interestingly, the frequencies and intensities of the S2, M, N and AP3a pool responses observed for cMCAD patients were similar to those observed for the control group of patients with mild-to-moderate COVID-19 (n=17) (Figure 2). However, the median [IQR] response for the spike glycoprotein N-terminal fragment pool was significantly lower for the patients with cMCADs (37 [24–130] SFC/ 10^3 CD3) than for the mild-to-moderate COVID-19 controls (114 [52–289] SFC/ 10^3 CD3; p=0.0288). The SARS-CoV-2 specific T-cell responses were significantly lower for cMCAD patients (p<0.001) than in severe COVID-19 controls (n=15), with the exception of the N pool (Figure 2). It is noteworthy that we did not detect (or detected very few) specific T-cell responses to the SARS-CoV-2 envelope small membrane protein in any of the groups. The anti-SARS-CoV-2 immune profiles of patient #20 (grade 5 on the WHO COVID-19 clinical progression scale) and patient #30 (who had recently been given cladribine) did not appear to differ from those observed in the other patients.

To evaluate the overall anticoronavirus immune response in 18 patients with cMCADs, we studied T-cell specific responses against the spike glycoprotein of human alpha- and beta-coronaviruses HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1. Two peptide pools (S1 and S2) were tested, as had been done for the SARS-CoV-2 spike glycoprotein (Supplemental figure E1). The samples from cMCAD patients and non-cMCAD controls gave very similar responses. We did not evidence any defects in the anti-endemic coronavirus response in patients with cMCADs; the frequencies and intensities were similar to those observed for non-cMCAD controls. The same was true when comparing IFN- γ production

responses to the ELISpot positive control CEFX Ultra SuperStim Pool (containing 176 known peptide epitopes derived from a broad range of infectious agents) in cMCAD vs. non-cMCAD patients (Supplemental figure E2).

In parallel with the ELISpot assay, we also used a high-sensitivity assay (the S-flow assay) to study SARS-CoV-2-specific IgG and IgA in 15 cMCAD patients. Fourteen of the 15 patients were positive for IgG, and 7 of the 15 were positive for IgA. The IgG-negative patient (#6) had a negative ELISpot assay. Furthermore, we used a viral pseudoparticle neutralization assay to determine whether the SARS-CoV-2-specific IgGs were neutralizing. We detected neutralizing antibodies in 12 of the 14 IgG-seropositive patients (86%) and found that anti-SARS-CoV-2 seropositivity (the IgG titer) was associated with a high level of neutralizing antibody (Supplemental figure E3).

Interestingly, inspection of the ELISpot plates showed that the background signal was significantly higher in cMCAD wells than in non-cMCAD control wells. In wells containing non-stimulated PBMCs in culture medium but with no peptide pools, we counted more than 10 small spots per 2×10^5 CD3⁺ cells in 10 of the 24 cMCAD patients (with or without a history of COVID-19), 3 of the 31 non-cMCAD controls ($p=0.009$ in Fisher's exact test), and 2 of the 11 controls with idiopathic MCAS (Figure 3A).

It should be noted that the SARS-CoV-2-specific spots were much larger and more intense than the background spots (Figure 3B-E). Thus, adjustment the ELISpot reader's settings made it possible to count the SARS-CoV-2-specific spots accurately and objectively. This phenomenon resulted from spontaneous IFN- γ release in the absence of stimulation. Given that we had tested the total PBMC fraction, we were not able to identify the specific subset of IFN- γ producing cells. PBMCs include T-cells, B-cells, natural killer (NK) cells, monocytes, and other myeloid cells (such as dendritic cells). Spontaneous IFN- γ release can be associated with elevated levels of basal T-cell activation. In a study of HIV-1-seronegative people, Liu et al.²⁰ reported that the

frequency of activated CD4⁺ T-cells (CD4+CD38+HLA-DR⁺) and CD8⁺ T-cells (CD8+CD38+HLA-DR⁺) was greater in individuals with a high background than in individuals with a low background. Another hypothesis involves NK cells, which might contribute to the maintenance of an elevated baseline IFN- γ level. It has been reported that NK cells from polyallergic patients spontaneously released greater amounts of IFN- γ , IL-4, IL-5 and IL-13 than NK cells from healthy individuals did²¹ – highlighting the *in vivo* activation of NK cells in atopic patients and suggesting that NK cells might be involved in an unbalanced cytokine network in allergic inflammation.

Accordingly, we sought to determine whether the spontaneous release of IFN- γ by PBMCs from cMCAD patients was related to NK cell activity, as has been reported for polyallergic patients. Human NK cells can be divided into NK1 and NK2 subsets on the basis of their ability to secrete IFN- γ ²². NK1 secrete IFN- γ and inhibits IgE synthesis in allergy²¹. Levels of total IgE might therefore constitute an indirect marker of NK1 activation. Thus, we determined the total IgE titer in sera from 17 patients with cMCADs. The cohort's total IgE levels were generally low (median [IQR]: 20 IU/ml [IQR 12.8 – 36.5], and were not correlated with spontaneous IFN- γ release. Lastly, we sought to determine whether the spontaneous IFN- γ release was correlated with the patients' characteristics. Although there was no correlation between spontaneous IFN- γ release and age, current symptomatic medications, a history of anaphylaxis, or the presence of the *KIT* D816V mutation, the basal serum tryptase level was indeed correlated in patients with cutaneous mastocytosis, MIS, or ISM (Figure 4, $r^2=0.61$, $p=0.0004$).

Discussion

To the best of our knowledge, the present study is the first to have investigated the antiviral immune response in patients with cMCADs. Given that MCs are key players in the Th2 immune response, one could reasonably fear that patients with cMCADs might produce abnormally poor immune responses to infections by viruses like SARS-CoV-2. To address this hypothesis, we studied a comprehensive range of responses and outcomes in cMCAD patients having developed PCR- or serology-confirmed COVID-19 during a 12-month period in France. Overall, no cases of severe COVID-19 were observed in this comprehensive series of patients – despite the high prevalence of risk factors (obesity, advanced age, cardiovascular conditions, immunosuppressive treatments, etc.). Strikingly, only two patients (with one and three risk factors, respectively) had to be hospitalized for low-flow oxygen therapy, and the outcomes were favorable in both cases. These findings are in line with recently published data from an international study²³. However, the present study extends our knowledge of cMCADs and COVID-19 because of the exhaustive nature of our inclusion process for patients with cMCADs through the nationwide CEREMAST rare disease network. Indeed, whenever a patient with mastocytosis not referenced in the CEREMAST network was hospitalized for the treatment of COVID-19 in an intensive care unit, the local and national reference centers were systematically contacted for an expert opinion on potential drug contraindications (due to the mandatory precautions needed for anesthesia). The exhaustive recruitment of patients with cMCADs and severe or life-threatening COVID-19 was confirmed by consulting PMSI hospital discharge records for the greater Paris region; we did not retrieve any inpatients who had not been detected through the CEREMAST network. For obvious reasons, the only source of study bias was related to patients with asymptomatic, mild or moderate forms of COVID-19 who did not require hospitalization nor request advice from their referring physicians.

To characterize the antiviral immune responses in patients with cMCADs, we have investigated, using ELISpot assay, the T-cells specific responses against SARS-CoV-2, endemic coronavirus

and the CEFX Ultra SuperStim Pool containing 176 known peptide epitopes derived from a broad range infectious agent. Furthermore, we studied the specific humoral anti-SARS-CoV-2 response by assaying circulating levels of specific IgG and IgA antibodies and neutralizing antibodies.

We observed that patients with cMCADs and non-cMCAD controls with a history of mild or moderate COVID-19 had very similar T-cell profiles in response to SARS-CoV-2, endemic coronaviruses, and the CEFX Ultra SuperStim Pool. Considering these observations as a whole and in contrast to initial expectations, we believe that patients with mastocytosis were indeed able to develop an effective, protective Th1 cell response against SARS-CoV-2. Thus, MCs from patients with cMCADs do not appear to worsen the Th1 response. However, given that less intense responses against SARS-CoV-2 spike glycoprotein were observed in cMCAD patients, we cannot rule out an impact of MCs on the amplitude of the Th1 response; this would raise concerns about the post-immunization cellular response. Furthermore, recent research has shown that MCs are involved in the Th1 response in general and the Th1 response to viruses in particular²⁴⁻²⁶. MCs might have a role in the Th1/Th2 balance that is potentially important for preventing the development of severe forms of COVID-19.

Unexpectedly, our ELISpot assay results revealed that spontaneous IFN- γ release from PBMCs (i.e. release in the absence of any stimulation) was more frequent in cMCAD patients than in controls. We found that spontaneous IFN- γ release was positively correlated with the cMCAD patients' basal tryptase levels; this was the only correlated clinical and laboratory characteristic, in fact. Although tryptase is very unlikely to be directly involved in this phenotype (especially since patients with advanced mastocytosis have very high tryptase levels and are not known to be especially protected against infection), we believe that the clonal MC burden is linked to IFN- γ release in patients with non-advanced mastocytosis. To the best of our knowledge, this finding has not previously been reported in the literature and may suggest a degree of additional

protection against severe viral diseases. Our laboratory is now working to determine whether this observation is related to either a specific cytokine profile in a patient's plasma or a direct cellular interaction between MCs and T-cells. If confirmed, this specific phenotype in cMCAD patients might lead to therapeutic implications in the field of infectious diseases.

Overall, our results showed that patients with cMCADs were able to develop effective, protective cellular and humoral responses to SARS-CoV-2. However, all four of the evaluable patients with serial serology data had become seronegative after a median of 33.0 weeks. Thus, anti-SARS-CoV-2 vaccination is strongly recommended in this specific patient population, although its level of effectiveness remains to be characterized.

In conclusion, non-advanced mastocytosis and MMAS appear not to confer an elevated risk of severe COVID-19 on patients. This finding might be due to the spontaneous IFN- γ production observed in patients with cMCADs but must be confirmed by further clinical and laboratory studies. If confirmed, this specific immune profile might explain the observed protection against severe COVID-19.

Acknowledgements

We thank all the patients who filled out the questionnaire and the healthcare professional who cared for the patients with COVID-19.

References

1. Valent P, Akin C, Metcalfe DD. Review Article Mastocytosis : 2016 updated WHO classification and novel emerging treatment concepts. *Blood*. 2017;129:1420–8.
2. Lim K, Tefferi A, Lasho TL, Finke C, Patnaik M, Butterfield JH, et al. Systemic mastocytosis in 342 consecutive adults : survival studies and prognostic factors
Systemic mastocytosis in 342 consecutive adults : survival studies and prognostic factors. 2009;113:5727–36.
3. Gotlib J, Pardanani A, Akin C, Reiter A, George T, Hermine O, et al. International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) & European Competence Network on Mastocytosis (ECNM) consensus response criteria in advanced systemic mastocytosis. *Blood*. 2013;121:2393–401.
4. Helmy YA, Fawzy M, Elaswad A, Sobieh A, Kenney SP, Shehata AA. The COVID-19 Pandemic: A Comprehensive Review of Taxonomy, Genetics, Epidemiology, Diagnosis, Treatment, and Control. *J Clin Med [Internet]*. 2020;9. Available from:

<http://www.ncbi.nlm.nih.gov/pubmed/32344679>

5. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science* [Internet]. 2020;369:718–24. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/32661059>

6. Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann H-H, Zhang Y, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science* [Internet]. 2020;370. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/32972996>

7. Chen Z, John Wherry E. T cell responses in patients with COVID-19. *Nat Rev Immunol* [Internet]. 2020;20:529–36. Available from: <http://dx.doi.org/10.1038/s41577-020-0402-6>

8. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell* [Internet]. 2020;1–13. Available from: <https://doi.org/10.1016/j.cell.2020.05.015>

9. van der Ploeg EK, Hermans MAW, van der Velden VHJ, Dik WA, van Daele PLA, Stadhouders R. Increased group 2 innate lymphoid cells in peripheral blood of adults with mastocytosis. *J Allergy Clin Immunol* [Internet]. 2021;147:1490-1496.e2. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/33091410>

10. Mazzoni A, Siraganian RP, Leifer CA, Segal DM. Dendritic cell modulation by mast cells controls the Th1/Th2 balance in responding T cells. *J Immunol* [Internet]. 2006;177:3577–81. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16951316>

11. Bulfone-Paus S, Bahri R. Mast cells as regulators of T cell responses. *Front Immunol*. 2015;6:6–11.
12. Smith N, Pietrancosta N, Davidson S, Dutrieux J, Chauveau L, Cutolo P, et al. Natural amines inhibit activation of human plasmacytoid dendritic cells through CXCR4 engagement. *Nat Commun* [Internet]. 2017;8:14253. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28181493>
13. Hu Y, Jin Y, Han D, Zhang G, Cao S, Xie J, et al. Mast cell-induced lung injury in mice infected with H5N1 influenza virus. *J Virol* [Internet]. 2012;86:3347–56. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22238293>
14. Kritas SK, Ronconi G, Caraffa A, Gallenga CE, Ross R, Conti P. Mast cells contribute to coronavirus-induced inflammation: new anti-inflammatory strategy. *J Biol Regul Homeost Agents* [Internet]. 2020;34:9–14. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/32013309>
15. Valent P, Akin C, Bonadonna P, Hartmann K, Brockow K, Niedozytko M, et al. Proposed Diagnostic Algorithm for Patients With Suspected Mast Cell Activation Syndrome. *J Allergy Clin Immunol Pract* [Internet]. 2019;1–10. Available from: <https://doi.org/10.1016/j.jaip.2019.01.006>
16. Gülen T, Akin C, Bonadonna P, Siebenhaar F, Broesby-Olsen S, Brockow K, et al. Selecting the Right Criteria and Proper Classification to Diagnose Mast Cell Activation Syndromes: A Critical Review. *J allergy Clin Immunol Pract* [Internet]. 2021; Available from: <http://www.ncbi.nlm.nih.gov/pubmed/34166845>
17. Grzelak L, Temmam S, Planchais C, Demeret C, Tondeur L, Huon C, et al. A comparison of four serological assays for detecting anti-SARS-CoV-2 antibodies in

human serum samples from different populations. *Sci Transl Med* [Internet]. 2020;12. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/32817357>

18. Williamson EJ, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, et al. Factors associated with COVID-19-related death using OpenSAFELY. *Nature* [Internet]. 2020;584:430–6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/32640463>

19. WHO Working Group on the Clinical Characterisation and Management of COVID-19 infection. A minimal common outcome measure set for COVID-19 clinical research. *Lancet Infect Dis* [Internet]. 2020;20:e192–7. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/32539990>

20. Liu AY, De Rosa SC, Guthrie BL, Choi RY, Kerubo-Bosire R, Richardson BA, et al. High background in ELISpot assays is associated with elevated levels of immune activation in HIV-1-seronegative individuals in Nairobi. *Immunity, Inflamm Dis* [Internet]. 2018;6:392–401. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/29974672>

21. Aktas E, Akdis M, Bilgic S, Disch R, Falk CS, Blaser K, et al. Different natural killer (NK) receptor expression and immunoglobulin E (IgE) regulation by NK1 and NK2 cells. *Clin Exp Immunol* [Internet]. 2005;140:301–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15807855>

22. Deniz G, Akdis M, Aktas E, Blaser K, Akdis CA. Human NK1 and NK2 subsets determined by purification of IFN-gamma-secreting and IFN-gamma-nonsecreting NK cells. *Eur J Immunol* [Internet]. 2002;32:879–84. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11870632>

23. Giannetti MP, Weller E, Alvarez-Twose I, Torrado I, Bonadonna P, Zanotti R, et al.

COVID-19 infection in patients with mast cell disorders including mastocytosis does not impact mast cell activation symptoms. *J allergy Clin Immunol Pract* [Internet]. 2021; Available from: <http://www.ncbi.nlm.nih.gov/pubmed/33631409>

24. McAlpine SM, Issekutz TB, Marshall JS. Virus stimulation of human mast cells results in the recruitment of CD56 + T cells by a mechanism dependent on CCR5 ligands. *FASEB J* [Internet]. 2012;26:1280–9. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1096/fj.11-188979>
25. Ebert S, Becker M, Lemmermann NAW, Büttner JK, Michel A, Taube C, et al. Mast Cells Expedite Control of Pulmonary Murine Cytomegalovirus Infection by Enhancing the Recruitment of Protective CD8 T Cells to the Lungs. Akbari O, editor. *PLoS Pathog* [Internet]. 2014;10:e1004100. Available from: <https://dx.plos.org/10.1371/journal.ppat.1004100>
26. Becker M, Lemmermann NA, Ebert S, Baars P, Renzaho A, Podlech J, et al. Mast cells as rapid innate sensors of cytomegalovirus by TLR3/TRIF signaling-dependent and -independent mechanisms. *Cell Mol Immunol* [Internet]. 2015;12:192–201. Available from: <http://www.nature.com/articles/cmi201473>

#	Sex	Age	BMI	cMCADs	KIT mutation	History of severe anaphylaxis	Risk factors	Tryptase µg/L	Treatment: anti-H1	Treatment: anti-H2	Treatment: montelukast	Cytoreductive therapy	COVID-19 scale	cMCAD symptoms during COVID-19	Fever during COVID-19	Anosmia/ageusia during COVID-19	SARS-CoV-2 PCR test	SARS-CoV-2 serology
1	F	25.6	17.4	ISM	WT	no	no	3.9	no	yes	no	no	2	no change	yes	yes	yes	positive
2	F	26.7	24.3	CM	D816V	no	no	4.4	yes	no	yes	no	2	more severe	yes	yes	yes	negative
3	F	29.9	23.9	MIS	NA	no	no	4.7	no	no	no	no	2	no change	no	no	no	positive
4	F	33.4	28.1	CM	D816V	yes	no	6.6	yes	no	no	no	2	no change	yes	yes	no	positive
5	F	35.5	33.1	MIS	NA	no	yes	7.4	no	no	no	no	2	no change	no	yes	yes	positive
6	F	38.3	18.4	ISM	D816V	yes	no	7.0	no	no	no	no	2	more severe	no	no	yes	negative
7	F	40.3	21.0	CM	WT	no	no	8.1	yes	no	no	no	2	no change	no	yes	no	positive
8	F	41.4	22.5	ISM	NA	no	no	7.7	yes	no	no	no	2	less severe	yes	yes	yes	positive
9	F	42.3	20.0	MMAS	D816V	yes	no	18.5	yes	no	yes	no	2	more severe	no	yes	yes	positive
10	M	42.4	33.5	ISM	D816V	no	yes	2.7	yes	no	no	no	2	less severe	yes	yes	yes	positive
11	F	43.3	25.0	CM	WT	yes	no	13.0	yes	no	yes	no	2	no change	yes	no	yes	positive
12	F	43.3	24.3	ISM	D816V	yes	yes	60.0	yes	yes	no	yes	2	more severe	yes	yes	yes	positive
13	F	43.8	25.1	MIS	NA	no	no	13.0	no	no	no	no	2	no change	yes	yes	yes	positive
14	M	45.3	30.8	MMAS	D816V	yes	yes	45.0	yes	no	no	no	2	no change	yes	no	no	positive
15	F	48.1	17.6	ISM	D816V	yes	no	99.8	yes	no	yes	no	2	no change	no	yes	yes	positive
16	M	49.1	27.4	ISM	D816V	no	no	14.8	yes	yes	no	no	2	more severe	yes	yes	no	positive
17	M	50.3	23.2	MIS	NA	no	no	18.6	yes	no	no	no	2	no change	no	no	yes	positive
18	M	51.7	26.4	ISM	D816V	no	no	42.8	yes	no	no	no	2	no change	yes	yes	yes	positive
19	F	52.2	19.5	ISM	NA	no	no	7.9	yes	no	yes	no	2	no change	yes	no	no	positive
20	F	52.4	30.1	CM	D816V	no	yes	37.2	yes	no	no	no	5*	more severe	yes	no	yes	positive
21	F	52.9	17.5	ISM	D816V	no	yes	38.6	yes	no	no	no	2	no change	yes	yes	yes	negative
22	F	53.1	27.0	MIS	NA	no	no	31.4	no	no	no	no	2	no change	yes	yes	yes	positive
23	M	56.0	26.8	ISM	NA	no	yes	19.0	no	no	no	no	2	no change	yes	yes	no	positive
24	F	59.6	21.6	MIS	NA	no	no	56.0	no	no	no	no	2	more severe	no	no	yes	positive
25	M	60.7	26.6	CM	D816V	no	no	12.0	no	no	no	no	1	no change	no	no	yes	positive
26	M	62.2	26.5	ISM	D816V	no	yes	6.1	yes	no	no	no	2	no change	yes	no	no	positive
27	M	62.2	24.2	CM	D816V	no	no	18.6	yes	no	no	no	2	no change	yes	yes	yes	positive
28	M	63.2	28.1	MIS	NA	yes	yes	11.2	yes	no	yes	no	2	no change	no	yes	yes	positive
29	M	65.6	26.7	ISM	D816V	yes	yes	27.8	no	no	no	no	5*	no change	yes	no	yes	positive
30	F	73.9	21.2	SSM	D816V	no	yes	163.0	no	yes	no	yes	2	no change	yes	no	no	positive
31	M	76.2	25.4	ISM	D816V	yes	yes	8.5	yes	no	no	no	3	less severe	yes	no	yes	positive
32	M	76.5	27.8	ISM	NA	no	yes	7.6	no	yes	no	no	2	more severe	yes	no	yes	positive

Table 1: Characteristics of patients with cMCADs and COVID-19 and their outcomes. Patients are listed in order of increasing age (years). #: patient number. F: female. M: male. BMI: body mass index. MIS: mastocytosis in the skin. CM: cutaneous mastocytosis. MMAS: monoclonal mast cell activation syndrome. SSM: smoldering systemic mastocytosis. risk factors: risk factors for severe COVID-19¹⁸. NA: not available. Cytoreductive therapy: midostaurin or ongoing/recent (previous 12 months) administration of cladribine. COVID-19 scale: WHO COVID-19 clinical progression scale¹⁹. *Patients treated with corticosteroids.

Figure legends

Figure 1: Flowchart for the selection of patients with cMCADs and proven COVID-19.

cMCAD: clonal mast cell activation disorder.

Figure 2: Quantification of specific anti-SARS-CoV-2 T-cell responses, using an ELISpot assay.

The tested SARS-CoV-2 peptide pools were derived from a peptide scan through the SARS-CoV-2 spike glycoprotein (S1: N-terminal fragment, S2: C-terminal fragment), membrane protein (M), nucleoprotein (N), and ORF3a protein (AP3a). The negative controls were PBMCs in culture medium alone, and the positive controls were PHA and the CEFX Ultra SuperStim Pool. Results were expressed as the number of spot-forming cells (SFCs)/ 10^6 CD3⁺ T-cells after subtraction of the background values from wells with non-stimulated cells. All differences between the non-COVID-19 and COVID-19 groups were statistically significant ($p < 0.001$). cMCADs: convalescent patients with clonal mast cell activation disorders. Non-cMCAD m-m COVID-19: non-cMCAD control patients convalescing from a mild-to-moderate form of COVID-19 ($n=17$). Non-cMCADs severe COVID-19: non-cMCAD control patients convalescing from a severe form of COVID-19 ($n=15$). Non-cMCAD non- COVID-19: non-cMCAD, non-COVID-19 controls ($n=15$). ns: non-significant; *, $p < 0.05$; ****, $p < 0.0001$.

Figure 3: Spontaneous IFN- γ production by PBMCs from patients, as measured in an ELISpot assay.

Graphic representation of the ELISpot assays A. cMCADs: patients with clonal mast cell activation disorders. Non-cMCAD control groups: MCAS: patients with idiopathic mast cell activation syndrome; CTR: non-cMCAD/non-MCAS control patients convalescing from

COVID-19 (n=32). Empty circles: no history of COVID-19. Filled circles: history of COVID-19. **Pictures of ELISpot assays.** **B:** A well with non-stimulated PBMCs from a COVID-19 non-cMCAD control. **C:** A well with non-stimulated PBMCs from a COVID-19 cMCAD patient. **D:** A well with PBMCs from a COVID-19 non-cMCAD control after stimulation for 18-20 h with individual 15-mer 11-aa overlapping peptide pools derived from the SARS-CoV-2 N-terminal fragment spike protein. **E:** A well with PBMCs from a COVID-19 cMCAD patient after stimulation with individual 15-mer 11-aa overlapping peptide pools derived from the SARS-CoV-2 N-terminal fragment spike protein.

Figure 4: Correlation between basal tryptase level ($\mu\text{g/L}$) and spontaneous IFN- γ production (spot-forming cells (SFCs)/ 2×10^5 CD3), as observed in ELISpot assays. n=24 patients with CM, MIS and ISM. Linear regression: $r^2=0.44$ ($p<0.0004$).

	cMCADs: whole cohort	cMCADs: SARS-CoV-2 ELISpot assay	cMCADs: common coronavirus ELISpot assay	cMCADs: humoral assay	Non-cMCADs: ELISpot assay	MCAS: ELISpot assay
Number	32	22	18	15	32	11
Median [IQR] age (years)	50 [42;60]	51 [41;55]	51 [41;56]	52 [41;54]	52 [41;62]	40 [35;58]
Female/male sex ratio	19/13	13/9	9/9	8/7	16/16	10/1

Supplemental Table E1: Numbers and characteristics of cMCAD patients and controls studied, by assay.

cMCAD: clonal mast cell activation disorder. MCAS: idiopathic mast cell activation syndrome

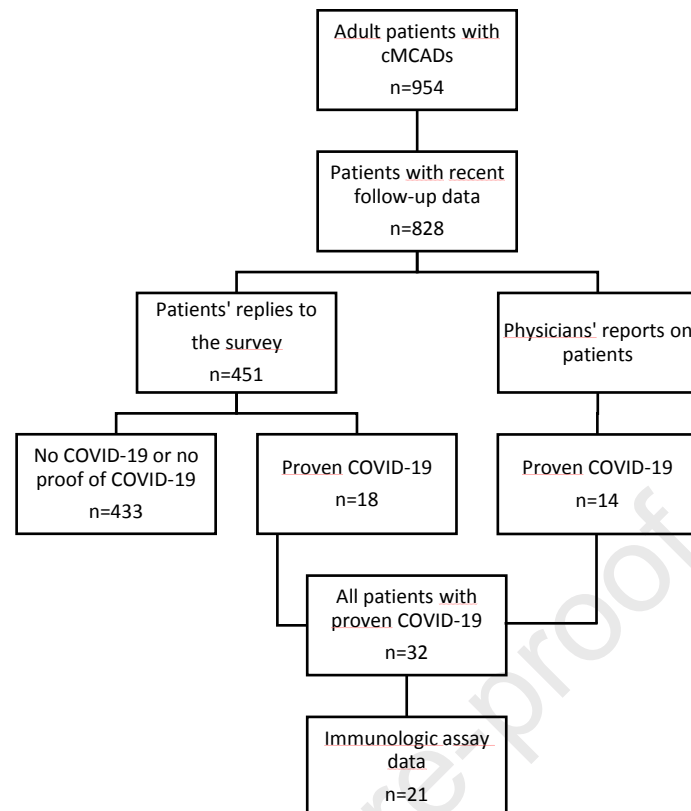


Figure 1: Flowchart for the selection of patients with cMCADs and proven COVID-19.

cMCAD: clonal mast cell activation disorder.

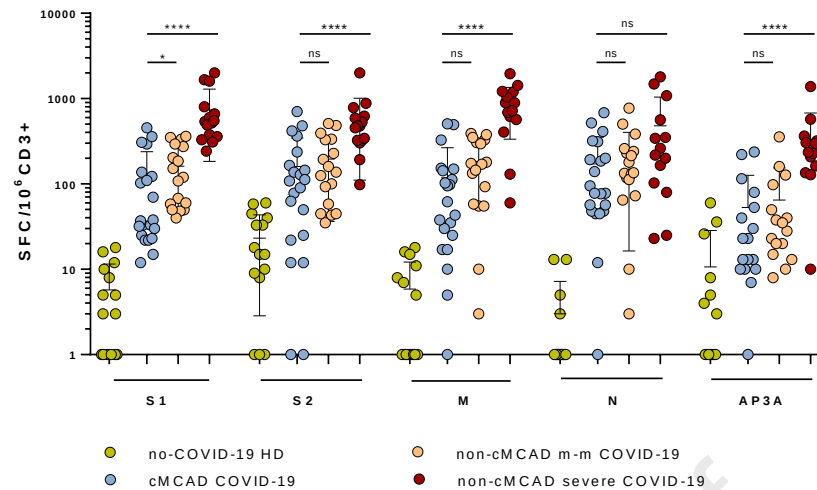


Figure 2: Quantification of specific anti-SARS-CoV-2 T-cell responses, using an ELISpot assay.

The tested SARS-CoV-2 peptide pools were derived from a peptide scan through the SARS-CoV-2 spike glycoprotein (S1: N-terminal fragment, S2: C-terminal fragment), membrane protein (M), nucleoprotein (N), and ORF3a protein (AP3a). The negative controls were PBMCs in culture medium alone, and the positive controls were PHA and the CEFX Ultra SuperStim Pool. Results were expressed as the number of spot-forming cells (SFCs)/10⁶ CD3⁺ T-cells after subtraction of the background values from wells with non-stimulated cells. All differences between the non-COVID-19 and COVID-19 groups were statistically significant ($p < 0.001$). cMCADs: convalescent patients with clonal mast cell activation disorders. Non-cMCAD m-m COVID-19: non-cMCAD control patients convalescing from a mild-to-moderate form of COVID-19 ($n = 17$). Non-cMCADs severe COVID-19: non-cMCAD control patients convalescing from a severe form of COVID-19 ($n = 15$). Non-cMCAD non-COVID-19: non-cMCAD, non-COVID-19 controls ($n = 15$). ns: non-significant; *, $p < 0.05$; ****, $p < 0.0001$.

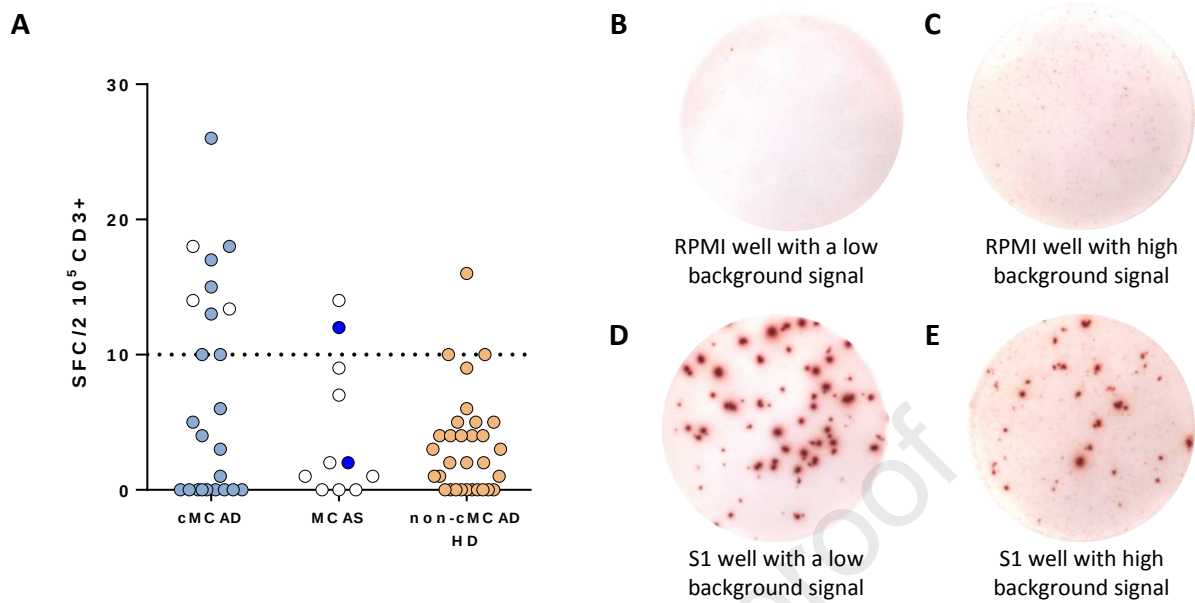


Figure 3: Spontaneous IFN- γ production by PBMCs from patients, as measured in an ELISpot assay.

Graphic representation of the ELISpot assays **A.** cMCADs: patients with clonal mast cell activation disorders. Non-cMCAD control groups: MCAS: patients with idiopathic mast cell activation syndrome; CTR: non-cMCAD/non-MCAS control patients convalescing from COVID-19 (n=32). Empty circles: no history of COVID-19. Filled circles: history of COVID-19. **Pictures of ELISpot assays.** **B:** A well with non-stimulated PBMCs from a COVID-19 non-cMCAD control. **C:** A well with non-stimulated PBMCs from a COVID-19 cMCAD patient. **D:** A well with PBMCs from a COVID-19 non-cMCAD control after stimulation for 18-20 h with individual 15-mer 11-aa overlapping peptide pools derived from the SARS-CoV-2 N-terminal fragment spike protein. **E:** A well with PBMCs from a COVID-19 cMCAD patient after stimulation with individual 15-mer 11-aa overlapping peptide pools derived from the SARS-CoV-2 N-terminal fragment spike protein.

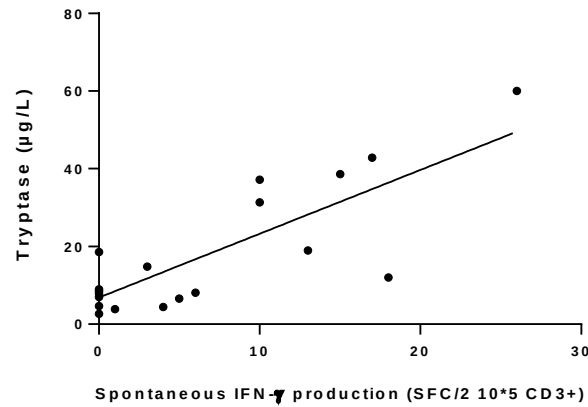
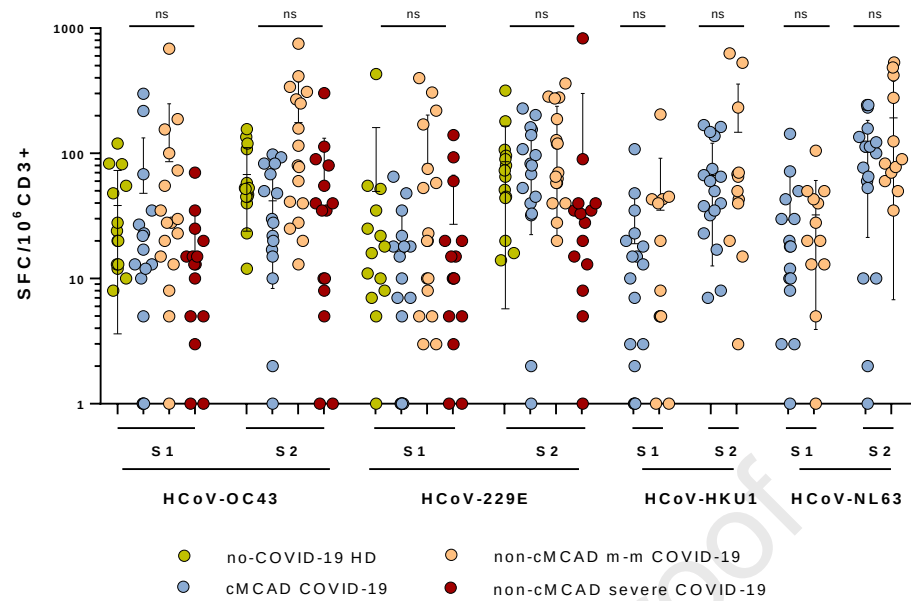
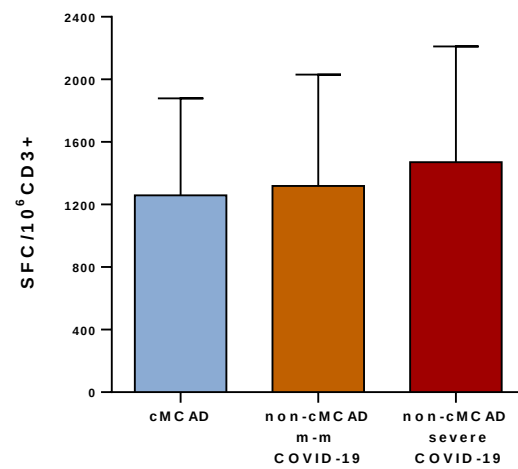


Figure 4: Correlation between basal tryptase level (µg/L) and spontaneous IFN-γ production (spot-forming cells (SFCs)/2x10⁵ CD3), as observed in ELISpot assays. n=24 patients with CM, MIS and ISM. Linear regression: $r^2=0.44$ ($p=0.0004$).



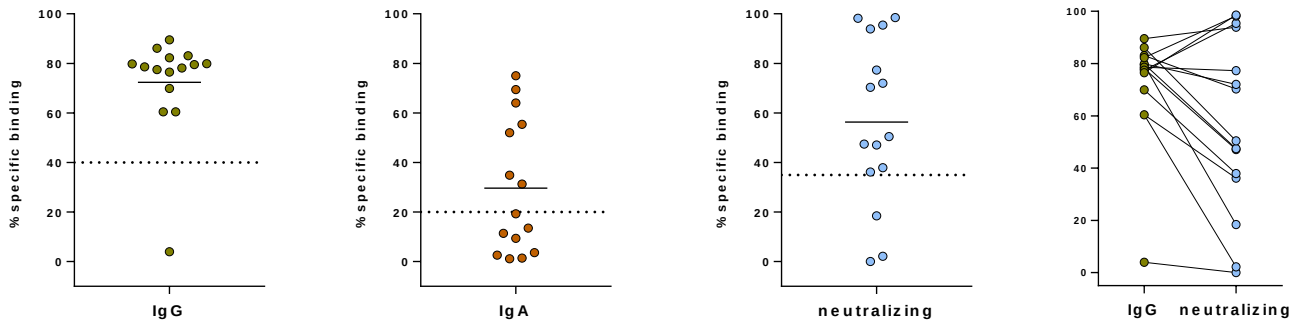
Supplemental Figure E1: T-cell responses to common coronaviruses in cMCAD patients (n=17), and non-cMCAD patients with a history of mild-to-moderate COVID-19 (n=17) or severe COVID-19 (n=15), or healthy donors (n=15).

Identification of HCoV-OC43-, HCoV-229E-, HCoV-HKU1- and HCoV-NL63-specific T-cell responses, using ELISpot assays. Results were expressed as spot forming cell (SFCs)/10⁶ CD3⁺ T-cells after subtraction of background values from wells containing non-stimulated cells. cMCADs: patients with clonal mast cell activation disorders, convalescing from mild-to-moderate (m-m) or severe COVID-19. NS: non-significant.



Supplemental Figure E2: T-cells responses to the CEFX Ultra SuperStim Pool.

cMCADs: patients with clonal mast cell activation disorders, convalescing from mild-to-moderate (m-m) or severe COVID-19.



Supplemental Figure E3

Two left-most panels: serologic status for anti-SARS-CoV-2 IgA and IgG antibodies, determined in an S-flow assay. The dashed line indicates the positivity threshold. Third panel: Percentage IgG neutralizing ability, determined in a viral pseudoparticle assay. Fourth panel: relationship between the IgG titer and the neutralizing activity.