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## Effective Anti-SARS-CoV-2 Immune Response in Patients With Clonal Mast Cell Disorders

Julien Rossignol, Amani Ouedrani, Cristina Bulai Livideanu, Stéphane Barete, Louis Terriou, David Launay, Richard Lemal, Celine Greco, Laurent Frenzel, Cecile Meni, et al.

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

2

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#### 141 **Abstract**

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

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

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

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

163 **Conclusion:** Patients with cMCADs might not be at risk of severe COVID-19 – perhaps due  
164 to their spontaneous production of IFN- $\gamma$ .

165

## 166 **Highlights box:**

### 167 **What is already known about this topic?**

168 Mast cells are key players in the Th2 immune response but not in antiviral immune responses.

169 Data on antiviral responses (and especially responses against coronaviruses) in patients with  
170 clonal mast cell activation disorders have not previously been reported.

### 171 **What does this article add to our knowledge?**

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



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

177 **How does this study impact current management guidelines?**

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

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

185

186 **Abbreviations**

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

188 *BMI: body mass index*

189 *CEREMAST: Centre de Référence des Mastocytoses*

190 *CM: cutaneous mastocytosis*

191 *cMCAD: clonal mast cell activation disorder*

192 *COVID-19: coronavirus disease 19*

193 *HEK: human embryonic kidney*

194 *IFN: interferon*

195 *ISM: indolent systemic mastocytosis*

196 *MIS: mastocytosis in the skin*

197 *MCAS: mast cell activation syndrome*

198 *MMAS: monoclonal mast cell activation syndrome*

199 NK: natural killer

200 PBMC: peripheral blood mononuclear cell

201 PHA: phytohemagglutinin

202 PMSI: Programme de médicalisation des systèmes d'information

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

204 SSM: smoldering systemic mastocytosis

205 WHO: World Health Organization

## 206 **Introduction**

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

214 Coronavirus disease 2019 (COVID-19) is a potentially fatal, pandemic infectious disease  
215 caused by severe respiratory syndrome coronavirus 2 (SARS-CoV-2)<sup>4</sup>. A body of compelling  
216 pathophysiological data suggests that interferons (IFNs) have a major role in disease control.  
217 These includes type I IFN (produced by plasmacytoid dendritic cells) and type III IFN (IFN- $\gamma$ )  
218 produced by T-cells from the adaptive immune system in the early and late phases of the  
219 disease, respectively<sup>5-8</sup>.

220 One can hypothesize that the MCs' ability to drive Th2 responses<sup>9-11</sup> (which counterbalance  
221 Th1 responses) might impair antiviral immunity in patients with cMCADs. Furthermore,  
222 histamine blocks the *in vitro* activity of plasmacytoid dendritic cells; *in vivo*, this might

223 weaken antiviral immune responses<sup>12</sup>. MCs might therefore (i) contribute to COVID-19-  
224 induced inflammation by releasing pro-inflammatory cytokines such as interleukin (IL-)1, IL-  
225 6, and tumor necrosis factor (TNF) and (ii) exacerbate COVID-19 lung damage *via*  
226 degranulation<sup>13,14</sup>. If so, this would render patients with cMCAD more susceptible to severe  
227 COVID-19.

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

## 233 **Methods**

### 234 *Patients*

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

243 Firstly, we sent a questionnaire to all adult patients (aged 18 or over) with mastocytosis or  
244 MMAS and for whom recent follow-up data were available in the CEREMAST national  
245 registry (n=828) and who were participating in a study sponsored by the *Association*  
246 *Française pour les Initiatives de Recherche sur le Mastocyte et les Mastocytose* (AFIRMM)

247 study. The questionnaire was designed to collect data on any signs of MC activation displayed  
248 during COVID-19, the patient's treatments, and the patient's specific signs and outcomes  
249 related to COVID-19. We then classified the patients as having had proven COVID-19 or not  
250 (Figure 1). Subsequently, we surveyed all members of the CEREMAST network (n=24) and  
251 thereby collected data on patients who had presented with COVID-19 but had not replied to  
252 the questionnaire. To check that we had not missed any severe cases (at least in the greater  
253 Paris region, as a proxy for national coverage), we also searched the French national hospital  
254 discharge database (*Programme de médicalisation des systèmes d'information*, PMSI) for  
255 cMCAD patients admitted for COVID-19 to hospitals in the *Assistance Publique - Hôpitaux*  
256 *de Paris* (the Paris Public Hospital Group, which treats 8.3 million patients a year). All  
257 patients with confirmed COVID-19 were assayed for anti-SARS-CoV-2 antibodies. In order  
258 to characterize the anti-SARS-CoV-2 immune response, patients included in the AFIRMM  
259 register were asked to provide a blood sample (for serology testing) during a follow-up  
260 consultation.

261 For the experiment on spontaneous IFN- $\gamma$  production, we incorporated a control group of  
262 patients with idiopathic mast cell activation syndrome (MCAS) and who had either  
263 experienced mild-to-moderate COVID-19 (n=2) or had no history of COVID-19 (n=9).  
264 MCAS was diagnosed according to the modified European Competence Network on  
265 Mastocytosis guidelines<sup>16</sup>. An increase in the serum total tryptase level by at least 20% above  
266 baseline plus 2 ng/mL during or within 4 hours of a symptomatic period was not investigated  
267 in the majority of patients.

268 To measure the spontaneous production of IFN $\gamma$  in the ELISpot assay, we studied PBMCs  
269 from patients with cMCADs and MCAS (with and without a history of COVID-19) and  
270 healthy donors with a history of COVID-19.

271 ***Ethic Statements***

272 All the patients with cMCADs were followed up in CEREMAST mastocytosis network  
273 reference centers and were enrolled in a prospective, nationwide, multicenter study sponsored  
274 by AFIRMM. The AFIRMM study was approved by the local investigational review board  
275 (*Comité de Protection des Personnes Ile-de-France*, France; reference: 93-00) and was  
276 carried out in compliance with the principles of the Declaration of Helsinki. The patients gave  
277 their written informed consent to participation. The patients' blood samples were obtained as  
278 part of routine follow-up care for their cMCADs. Data on the control cohorts were collected  
279 prospectively and analyzed as part of the COVID-HOP study (Clinicaltrials.gov:  
280 NCT04418375; other study identifier: APHP200609).

### 281 ***Immunological assays***

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

290 The negative controls were PBMCs cultured in RPMI-1640 medium supplemented with L-  
291 glutamine, sodium bicarbonate (Sigma-Aldrich, Molsheim, France) and 10% human AB  
292 serum (Biowest, Nuaille, France), in the absence of stimulation. The positive controls were  
293 phytohemagglutinin (PHA) (Sigma-Aldrich) and the CEFX Ultra SuperStim Pool (JPT  
294 Peptide Technologies GmbH, BioNTech AG, Berlin, Germany). The tested SARS-CoV-2  
295 peptide pools were derived from a peptide scan through the SARS-CoV-2 spike glycoprotein

296 (two pools: S1 for the N-terminal fragment, and S2 for the C-terminal fragment), membrane  
297 protein (M), nucleoprotein (N), envelope small membrane protein (E), and ORF3a protein.  
298 We also tested peptide pools derived from the spike glycoprotein of the common human  
299 alpha-coronaviruses (HCoV-229E and HCoV-NL63) and beta-coronaviruses (HCoV-OC43  
300 and HCoV-HKU1).

301 The humoral immune response (including the production of anti-spike SARS-CoV-2 IgG, IgA  
302 antibodies and the neutralizing ability of anti-spike IgGs) was characterized using previously  
303 described S-flow and S-pseudotype neutralization assays<sup>17</sup>. Briefly, the S-flow assay used  
304 human embryonic kidney (HEK)293T cells transduced with the SARS-CoV-2 spike protein.  
305 Cells were incubated with sera from patients (dilution: 1:300) and stained with either anti-IgG  
306 or anti-IgA antibodies. The fluorescent signal was measured by flow cytometry. For the S-  
307 pseudotype neutralization assay, pseudotyped viruses carrying SARS-CoV-2 spike protein  
308 were used. The viral pseudotypes were incubated with the sera to be tested (dilution: 1:100),  
309 added to transduced HEK293T cells expressing ACE2, and incubated for 48 h at 37°C. The  
310 assay measures the anti-S antibodies' ability to neutralize an infection, as described  
311 elsewhere<sup>17</sup>.

### 312 *Non-cMCAD control groups*

313 For each group, the number of patients, the median age and the sex ratio are reported in  
314 Supplemental Table E1. Four control groups were used in this study: (i) non-MCAD patients  
315 convalescing from mild-to-moderate (n=17) and (ii) severe (n=15) forms of COVID-19 and  
316 who had already been evaluated with SARS-CoV-2 ELISpot and serology assays in Necker  
317 Hospital's immunology laboratory, (iii) idiopathic MCAS patients with or without a history of  
318 COVID-19, and (iv) non-MCAD patients with no history of COVID-19 (n=15). We compared  
319 the cMCAD patients with age-matched MCAS patients who presented symptoms of MC

320 activation and were being treated with antimediator agents; at the time of the SARS-CoV-2  
321 infection and the immunoassays, all 11 of the MCAS patients were being treated with H1  
322 antihistamines, 8 were being treated with H2 antihistamines, and 5 were being treated with  
323 montelukast. The cMCAD and MCAS patient subgroups did not differ significantly with  
324 regard to age (mean age: 45.6 vs. 49.2, respectively;  $p=0.35$ ).

### 325 *Statistics*

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

331

### 332 **Results**

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

338 The characteristics and outcomes of the 32 patients with cMCADs and COVID-19 are  
339 summarized in Table 1. The median (range) age was 49.7 years (range: 25.6–76.4), with  
340 female predominance (59.4%). The cMCAD subtype was cutaneous mastocytosis or  
341 mastocytosis in the skin in 14 patients, ISM in 15 patients, smoldering systemic mastocytosis  
342 (SSM) in one patient, and MMAS in 2 patients. Of the 21 patients having undergone genetic

343 testing, 18 (85.7%) carried the D816V *KIT* mutation. Ten patients (31.3%) had experienced a  
344 severe anaphylactic reaction, and the median (range) basal serum tryptase level before the  
345 appearance of clinical or laboratory signs of COVID-19 was 13.0 µg/L (2.7–163.0 µg/L).  
346 Risk factors for severe COVID-19 were present in 13 of the 32 patients (40.6%), and four had  
347 at least two risk factors<sup>18</sup>: a body mass index >30 (n=4), age >65 (n= 4), ongoing  
348 cytoreductive therapy (midostaurin or cladribine) or recent (in the previous 12 months)  
349 administration of cladribine (n=3), cardiovascular conditions (including arterial hypertension  
350 and chronic heart failure) (n=7), and diabetes (n=2). At the time of the SARS-CoV-2  
351 infection, 23 of the 32 patients (71.9%) were taking symptomatic medications (H1  
352 antihistamines, H2 antihistamines, and/or montelukast), one patient with SSM was receiving  
353 midostaurin after failure to respond to cladribine, and one patient with ISM had recently  
354 received cladribine.

355 With regard to the diagnosis of COVID-19, 23 of the 32 patients (71.9%) had a positive  
356 SARS-CoV-2 PCR test on a nasal swab, and 9 of the 32 had a negative PCR test (or did not  
357 undergo a PCR test on a nasal swab, due to non-availability of the procedure at the time of  
358 infection) and positive serology test. The great majority of the patients (29 out of 32) became  
359 seropositive during their follow-up. As expected, patients with subsequently available serum  
360 samples (n=4) became seronegative after a median follow-up of 33.0 weeks. Regarding the  
361 symptoms of COVID-19, 22 of the 32 (68.8%) patients had fever (>38°C), and 18 (56.3%)  
362 had anosmia and/or ageusia. Only two patients were admitted to hospital for corticosteroid  
363 therapy and oxygen therapy (corresponding to stage 5 on the WHO COVID-19 clinical  
364 progression scale<sup>19</sup>) but neither required non-invasive or mechanical ventilation. Interestingly,  
365 8 of the 32 patients (25.0%) reported an increase in signs of MC activation during the episode  
366 of COVID-19, while 3 (9.4%) reported a decrease. No recurrences of the infection were  
367 reported.



368 The specific cellular and humoral anti-SARS-CoV-2 immune responses were studied in 21  
369 patients with cMCADs a median [IQR] of 24 weeks [7–36] from infection. Overall, 20 of the  
370 21 cMCAD patients developed a specific T-cell response against at least one of the SARS-  
371 CoV-2 peptide pools tested. The responses were usually moderately intense. The median  
372 [IQR] intensity was 37 [24–130] SFC/10<sup>3</sup> CD3 for the S1 pool, 108 [23–201] SFC/10<sup>3</sup> CD3  
373 for the S2 pool, 62 [21–146] SFC/10<sup>3</sup> CD3 for the M pool, 78 [48–256] SFC/10<sup>3</sup> CD3 for the  
374 N pool and 23 [10–66] SFC/10<sup>3</sup> CD3 for the AP3a pool. The only patient (#6) who did not  
375 develop a specific T-cell response was young (38), had presented mild symptoms of COVID-  
376 19 (confirmed by a positive PCR test on a nasal swab), and did not have a history of  
377 immunodeficiency or immunosuppressive therapy.

378 Interestingly, the frequencies and intensities of the S2, M, N and AP3a pool responses  
379 observed for cMCAD patients were similar to those observed for the control group of patients  
380 with mild-moderate COVID-19 (n=17) (Figure 2). However, the median [IQR] response for  
381 the spike glycoprotein N-terminal fragment pool was significantly lower for the patients with  
382 cMCADs (37 [24–130] SFC/10<sup>3</sup> CD3) than for the mild-to-moderate COVID-19 controls  
383 (114 [52–289] SFC/10<sup>3</sup> CD3; p=0.0288). The SARS-CoV-2 specific T-cell responses were  
384 significantly lower for cMCAD patients (p<0.001) than in severe COVID-19 controls (n=15),  
385 with the exception of the N pool (Figure 2). It is noteworthy that we did not detect (or  
386 detected very few) specific T-cell responses to the SARS-CoV-2 envelope small membrane  
387 protein in any of the groups. The anti-SARS-CoV-2 immune profiles of patient #20 (grade 5  
388 on the WHO COVID-19 clinical progression scale) and patient #30 (who had recently been  
389 given cladribine) did not appear to differ from those observed in the other patients.

390 To evaluate the overall anticoronavirus immune response in 18 patients with cMCADs, we  
391 studied T-cell specific responses against the spike glycoprotein of human alpha- and beta-  
392 coronaviruses HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1. Two peptide

393 pools (S1 and S2) were tested, as had been done for the SARS-CoV-2 spike glycoprotein  
394 (Supplemental figure E1). The samples from cMCAD patients and non-cMCAD controls gave  
395 very similar responses. We did not evidence any defects in the anti-endemic coronavirus  
396 response in patients with cMCADs; the frequencies and intensities were similar to those  
397 observed for non-cMCAD controls. The same was true when comparing IFN- $\gamma$  production  
398 responses to the ELISpot positive control CEFX Ultra SuperStim Pool (containing 176 known  
399 peptide epitopes derived from a broad range of infectious agents) in cMCAD vs. non-cMCAD  
400 patients (Supplemental figure E2).

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

409 Interestingly, inspection of the ELISpot plates showed that the background signal was  
410 significantly higher in cMCAD wells than in non-cMCAD control wells. In wells containing  
411 non-stimulated PBMCs in culture medium but with no peptide pools, we counted more than  
412 10 small spots per  $2 \times 10^5$  CD3<sup>+</sup> cells in 10 of the 24 cMCAD patients (with or without a  
413 history of COVID-19), 3 of the 31 non-cMCAD controls ( $p=0.009$  in Fisher's exact test), and  
414 2 of the 11 controls with idiopathic MCAS (Figure 3A).

415 It should be noted that the SARS-CoV-2-specific spots were much larger and more intense  
416 than the background spots (Figure 3B-E). Thus, adjustment the ELISpot reader's settings  
417 made it possible to count the SARS-CoV-2-specific spots accurately and objectively. This

418 phenomenon resulted from spontaneous IFN- $\gamma$  release in the absence of stimulation. Given  
419 that we had tested the total PBMC fraction, we were not able to identify the specific subset of  
420 IFN- $\gamma$  producing cells. PBMCs include T-cells, B-cells, natural killer (NK) cells, monocytes,  
421 and other myeloid cells (such as dendritic cells). Spontaneous IFN- $\gamma$  release can be associated  
422 with elevated levels of basal T-cell activation. In a study of HIV-1-seronegative people, Liu et  
423 al.<sup>20</sup> reported that the frequency of activated CD4+ T-cells (CD4+CD38+HLA-DR+) and  
424 CD8+ T-cells (CD8+CD38+HLA-DR+) was greater in individuals with a high background  
425 than in individuals with a low background. Another hypothesis involves NK cells, which  
426 might contribute to the maintenance of an elevated baseline IFN- $\gamma$  level. It has been reported  
427 that NK cells from polyallergic patients spontaneously released greater amounts of IFN- $\gamma$ , IL-  
428 4, IL-5 and IL-13 than NK cells from healthy individuals did<sup>21</sup> – highlighting the *in vivo*  
429 activation of NK cells in atopic patients and suggesting that NK cells might be involved in an  
430 unbalanced cytokine network in allergic inflammation.

431 Accordingly, we sought to determine whether the spontaneous release of IFN- $\gamma$  by PBMCs  
432 from cMCAD patients was related to NK cell activity, as has been reported for polyallergic  
433 patients. Human NK cells can be divided into NK1 and NK2 subsets on the basis of their  
434 ability to secrete IFN- $\gamma$ <sup>22</sup>. NK1 secrete IFN- $\gamma$  and inhibits IgE synthesis in allergy<sup>21</sup>. Levels of  
435 total IgE might therefore constitute an indirect marker of NK1 activation. Thus, we  
436 determined the total IgE titer in sera from 17 patients with cMCADs. The cohort's total IgE  
437 levels were generally low (median [IQR]: 20 IU/ml [IQR 12.8 – 36.5]), and were not  
438 correlated with spontaneous IFN- $\gamma$  release. Lastly, we sought to determine whether the  
439 spontaneous IFN- $\gamma$  release was correlated with the patients' characteristics. Although there  
440 was no correlation between spontaneous IFN- $\gamma$  release and age, current symptomatic  
441 medications, a history of anaphylaxis, or the presence of the *KIT* D816V mutation, the basal

442 serum tryptase level was indeed correlated in patients with cutaneous mastocytosis, MIS, or  
443 ISM (Figure 4,  $r^2=0.61$ ,  $p=0.0004$ ).

444

## 445 **Discussion**

446 To the best of our knowledge, the present study is the first to have investigated the antiviral  
447 immune response in patients with cMCADs. Given that MCs are key players in the Th2  
448 immune response, one could reasonably fear that patients with cMCADs might produce  
449 abnormally poor immune responses to infections by viruses like SARS-CoV-2. To address  
450 this hypothesis, we studied a comprehensive range of responses and outcomes in cMCAD  
451 patients having developed PCR- or serology-confirmed COVID-19 during a 12-month period  
452 in France. Overall, no cases of severe COVID-19 were observed in this comprehensive series  
453 of patients – despite the high prevalence of risk factors (obesity, advanced age, cardiovascular  
454 conditions, immunosuppressive treatments, etc.). Strikingly, only two patients (with one and  
455 three risk factors, respectively) had to be hospitalized for low-flow oxygen therapy, and the  
456 outcomes were favorable in both cases. These findings are in line with recently published data  
457 from an international study<sup>23</sup>. However, the present study extends our knowledge of cMCADs  
458 and COVID-19 because of the exhaustive nature of our inclusion process for patients with  
459 cMCADs through the nationwide CEREMAST rare disease network. Indeed, whenever a  
460 patient with mastocytosis not referenced in the CEREMAST network was hospitalized for the  
461 treatment of COVID-19 in an intensive care unit, the local and national reference centers were  
462 systematically contacted for an expert opinion on potential drug contraindications (due to the  
463 mandatory precautions needed for anesthesia). The exhaustive recruitment of patients with  
464 cMCADs and severe or life-threatening COVID-19 was confirmed by consulting PMSI  
465 hospital discharge records for the greater Paris region; we did not retrieve any inpatients who

466 had not been detected through the CEREMAST network. For obvious reasons, the only source  
467 of study bias was related to patients with asymptomatic, mild or moderate forms of COVID-  
468 19 who did not require hospitalization nor request advice from their referring physicians.

469 To characterize the antiviral immune responses in patients with cMCADs, we have  
470 investigated, using ELISpot assay, the T-cells specific responses against SARS-CoV-2,  
471 endemic coronavirus and the CEFX Ultra SuperStim Pool containing 176 known peptide  
472 epitopes derived from a broad range infectious agent. Furthermore, we studied the specific  
473 humoral anti-SARS-CoV-2 response by assaying circulating levels of specific IgG and IgA  
474 antibodies and neutralizing antibodies.

475 We observed that patients with cMCADs and non-cMCAD controls with a history of mild or  
476 moderate COVID-19 had very similar T-cell profiles in response to SARS-CoV-2, endemic  
477 coronaviruses, and the CEFX Ultra SuperStim Pool. Considering these observations as a  
478 whole and in contrast to initial expectations, we believe that patients with mastocytosis were  
479 indeed able to develop an effective, protective Th1 cell response against SARS-CoV-2. Thus,  
480 MCs from patients with cMCADs do not appear to worsen the Th1 response. However, given  
481 that less intense responses against SARS-CoV-2 spike glycoprotein were observed in cMCAD  
482 patients, we cannot rule out an impact of MCs on the amplitude of the Th1 response; this  
483 would raise concerns about the post-immunization cellular response. Furthermore, recent  
484 research has shown that MCs are involved in the Th1 response in general and the Th1  
485 response to viruses in particular<sup>24-26</sup>. MCs might have a role in the Th1/Th2 balance that is  
486 potentially important for preventing the development of severe forms of COVID-19.

487 Unexpectedly, our ELISpot assay results revealed that spontaneous IFN- $\gamma$  release from  
488 PBMCs (i.e. release in the absence of any stimulation) was more frequent in cMCAD patients  
489 than in controls. We found that spontaneous IFN- $\gamma$  release was positively correlated with the  
490 cMCAD patients' basal tryptase levels; this was the only correlated clinical and laboratory

491 characteristic, in fact. Although tryptase is very unlikely to be directly involved in this  
492 phenotype (especially since patients with advanced mastocytosis have very high tryptase  
493 levels and are not known to be especially protected against infection), we believe that the  
494 clonal MC burden is linked to IFN- $\gamma$  release in patients with non-advanced mastocytosis. To  
495 the best of our knowledge, this finding has not previously been reported in the literature and  
496 may suggest a degree of additional protection against severe viral diseases. Our laboratory is  
497 now working to determine whether this observation is related to either a specific cytokine  
498 profile in a patient's plasma or a direct cellular interaction between MCs and T-cells. If  
499 confirmed, this specific phenotype in cMCAD patients might lead to therapeutic implications  
500 in the field of infectious diseases.

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

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

511

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#	Sex	Age	BMI	cMCADs	<i>KIT</i> 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-19<sup>18</sup>. NA: not available. Cytoreductive therapy: midostaurin or ongoing/recent (previous 12 months) administration of cladribine. COVID-19 scale: WHO COVID-19 clinical progression scale<sup>19</sup>. \*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.**

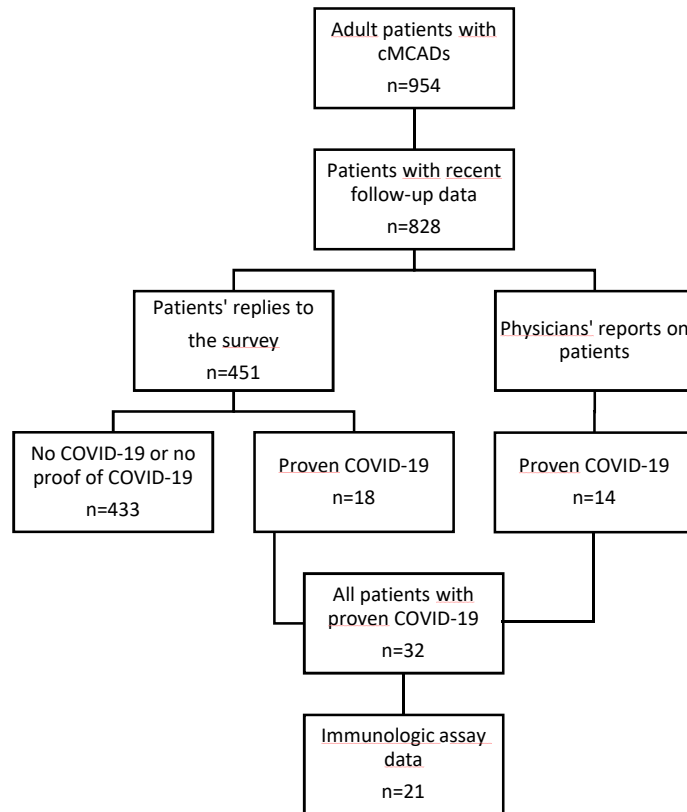
The tested SARS-CoV-2 peptide pools were derived from a peptide scan through the SARS-CoV-2 spike glycoprotein (S1: N-terminal fragment, S2: C-terminal fragment), membrane protein (M), nucleoprotein (N), and ORF3a protein (AP3a). The negative controls were PBMCs in culture medium alone, and the positive controls were PHA and the CEFX Ultra SuperStim Pool. Results were expressed as the number of spot-forming cells (SFCs)/10<sup>6</sup> CD3<sup>+</sup> 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- $\gamma$ 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

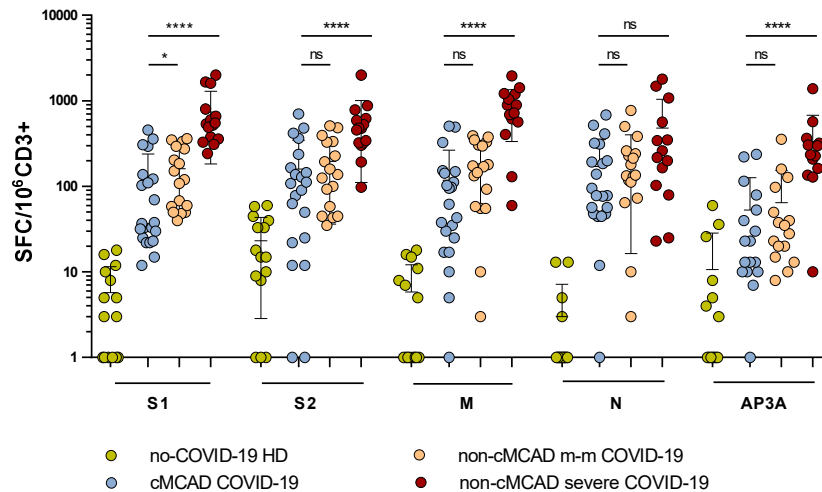
activation syndrome; CTR: non-cMCAD/non-MCAS control patients convalescing from COVID-19 (n=32). Empty circles: no history of COVID-19. Filled circles: history of COVID-19. **Pictures of ELISpot assays. B:** A well with non-stimulated PBMCs from a COVID-19 non-cMCAD control. **C:** A well with non-stimulated 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- $\gamma$  production (spot-forming cells (SFCs)/ $2 \times 10^5$  CD3), as observed in ELISpot assays. n=24 patients with CM, MIS and ISM. Linear regression:  $r^2=0.44$  ( $p<0.0004$ ).



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

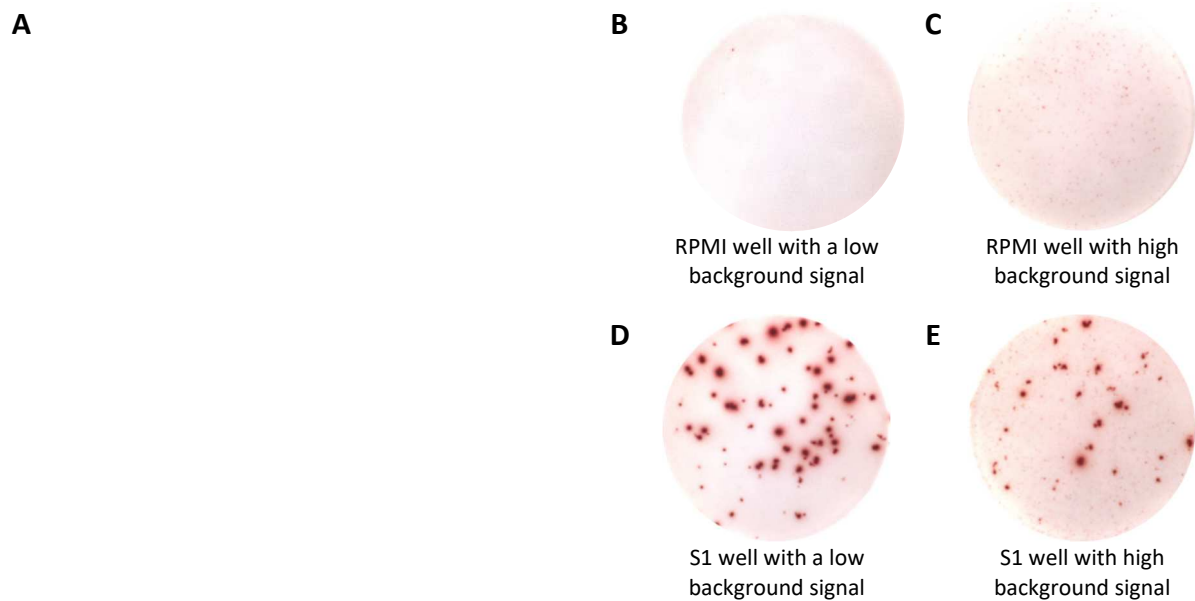
cMCAD: clonal mast cell activation disorder.



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

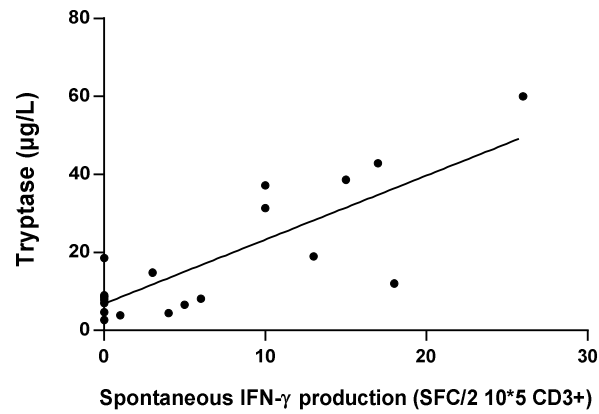
The tested SARS-CoV-2 peptide pools were derived from a peptide scan through the SARS-CoV-2 spike glycoprotein (S1: N-terminal fragment, S2: C-terminal fragment), membrane protein (M), nucleoprotein (N), and ORF3a protein (AP3a). The negative controls were PBMCs in culture medium alone, and the positive controls were PHA and the CEFX Ultra SuperStim Pool. Results were expressed as the number of spot-forming cells (SFCs)/10<sup>6</sup> CD3<sup>+</sup> 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- $\gamma$  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)/2x10<sup>5</sup> CD3), as observed in ELISpot assays. n=24 patients with CM, MIS and ISM. Linear regression:  $r^2=0.44$  ( $p=0.0004$ ).