COVID-19 in a Multiple Myeloma Patient: Cellular and Humoral Immunity Against SARS-CoV-2 in a Short- and Long-term View

Ivana von Metzler (ivana.metzler@kgu.de)
Hospital of the Goethe University Frankfurt: Klinikum der Johann Wolfgang Goethe-Universitat Frankfurt
https://orcid.org/0000-0003-1699-4313

Julia Campe
Hospital of the Goethe University Frankfurt: Klinikum der Johann Wolfgang Goethe-Universitat Frankfurt

Sabine Huenecke
Hospital of the Goethe University Frankfurt: Klinikum der Johann Wolfgang Goethe-Universitat Frankfurt

Marc Raab
University Hospital Heidelberg Medical Clinic: Universitatsklinikum Heidelberg Medizinische Klinik

Hartmut Goldschmidt
University Hospital Heidelberg Medical Clinic: Universitatsklinikum Heidelberg Medizinische Klinik

Ralf Schubert
Hospital of the Goethe University Frankfurt: Klinikum der Johann Wolfgang Goethe-Universitat Frankfurt

Holger Rabenau
Hospital of the Goethe University Frankfurt: Klinikum der Johann Wolfgang Goethe-Universitat Frankfurt

Sandra Ciesek
Hospital of the Goethe University Frankfurt: Klinikum der Johann Wolfgang Goethe-Universitat Frankfurt

Hubert Serve
Hospital of the Goethe University Frankfurt: Klinikum der Johann Wolfgang Goethe-Universitat Frankfurt

Evelyn Ullrich
Hospital of the Goethe University Frankfurt: Klinikum der Johann Wolfgang Goethe-Universitat Frankfurt
https://orcid.org/0000-0001-8530-1192

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Abstract

Multiple myeloma patients are often treated with immunomodulatory drugs, proteasome inhibitors or monoclonal antibodies until disease progression. Chronic therapy in combination with the underlying disease frequently results in severe humoral and cellular immunodeficiency, which often manifests in recurrent infections. Here we report on the clinical management and immunological data of one multiple myeloma patient diagnosed with COVID-19. Despite severe hypogammaglobulinemia, deteriorated T cell counts and neutropenia, the patient unexpectedly combated COVID-19 by balanced response of innate immunity, strong CD8+ and CD4+ T cell activation and differentiation, development of specific T-cell memory subsets, as well as development of anti-SARS-CoV-2 type IgA and IgG antibodies. Even 6 months after re-introduction of lenalidomide maintenance therapy, specific T cell response and antibody levels remained detectable, indicating persisting immunity against SARS-CoV-2. We conclude that in MM patients who tested positive for SARS-CoV-2 and were receiving active MM treatment, immune response assessment could be a useful tool to help guide decision-making regarding the continuation of anti-tumor therapy and supportive therapy.

Introduction

Secondary immunodeficiency is a common feature in multiple myeloma patients. Hypogammaglobulinemia, neutropenia, reduced T and NK cell counts as well as impaired T and NK cell function are disease- and/ or therapy-induced factors that can contribute to the acquisition of severe bacterial and viral infections with adverse outcomes. From that point of view, we presumed that the SARS-CoV-2 pandemic [1] would place these patients at high risk for unfavorable outcome. Indeed, the first US study on 100 MM patients from NYC reported morality rates of almost 20% of patients, which was considerably higher than what had been reported in general population [2]. In contrast, the German multiple myeloma study group consortium reported no casualties among all 21 myeloma patients diagnosed with COVID-19 from March 1st to May 31st 2020 at secondary and tertiary comprehensive cancer centers in Germany [3]. So far, no myeloma-specific risk factors have been identified [2, 3]. Despite the lack of reliable data, few new guidelines and recommendations for treatment of MM during COVID-19 pandemic have been published [4]. Here we report on the clinical management and immunological data over a six month period of our first multiple myeloma patient diagnosed with COVID-19.

Methods

Patient's medical records, standard laboratory parameters including immune monitoring and chest CT imaging were collected and analyzed for this study. Written patient's consent was obtained for publication of this case report.

For further non-standard analyzes, blood sera were obtained from the here described patient, as well as from a positive control patient suffering from severe COVID-19, from another control patient with moderate COVID-19 symptoms, and from an age-matched healthy control. Blood sample collection was

in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University Hospital Frankfurt. All subjects provided written, informed consent.

SARS-CoV-2 IgG/IgA ELISA

Serum samples from the patient were collected at day 11, 21, 29, 44 and 56 respectively.

For semi-quantitative SARS-Cov-2 specific IgG/I gA analysis, ready-to-use test-kits (Euroimmun, Lübeck, Germany) were used according to manufacturer's recommendation. This ELISA uses SARS-CoV-2 recombinant antigen from spike glycoprotein (S protein).

Cytokine-Bead-Array for measurement of serum cytokine concentrations

For cytokine analysis patients sera were collected at the respective days and frozen at -80°C. Cytokine concentrations were examined using BD Cytometric Bead Array (CBA; BD Bioscience). The tests were performed according to the manufacturer's instructions. Data were acquired with the BD FACSVerse Bioanalyzer and were quantitated using the FCAP Array software (v3.0.1; BD Biosciences).

Results And Discussion

The patient, a 51-year-old male, presented with a three-day fever of 38.8°C, dry cough, and chills on March 25th. Other symptoms frequently reported in patients with COVID-19 were denied [5]. In January 2019, he was diagnosed with multiple myeloma (MM) type IgA kappa, R-ISS stage I, and 1/4-CRAB criteria. Initially, he was treated with an induction quadruplet consisting of an anti-CD38 antibody in combination with bortezomib, lenalidomide and dexamethasone, followed by high-dose chemotherapy (HDCT) with melphalan 200 mg/m^2 and autologous stem cell transplant (ASCT) in September 2019. He achieved complete remission after ASCT but remained MRD positive by flow cytometry (sensitivity 10^{-5}). From January 2020 onwards, he received continuous lenalidomide maintenance treatment.

During monthly follow-up examination, we noticed severe type IgA and IgM immunoparesis, CTC grade II neutropenia, and CTC grade II lymphocytopenia. In January 2020, we also noticed a CD4+ T cell deficiency with 109 CD4+ T cells/ µl (normal values: 300-1400/µL) of whole blood.

At admission, a CT chest scan indicated mild bilateral pulmonary infiltrates, and community acquired respiratory viruses (CARV)-PCR testing showed positivity for SARS-CoV-2. Lenalidomide maintenance treatment was paused. Laboratory examinations showed neutropenia, lymphocytopenia, as well as moderately increased C-reactive protein (CRP) and interleukin-6 (IL-6) levels (Supplementary Tab. I). However, viral RNA was not detectable in the peripheral blood. During hospitalization, clinical symptoms worsened, and the fever persisted with 39.2°C. Important laboratory parameters, as summarized in Supp.

Tab. I, showed lymphocytopenia (CTC grade IV), neutropenia (CTC grade I-III), a temporary decrease in the number of monocytes that persisted during hospitalization, and increased IL-6 levels with a maximum on day +6 (26.5 pg/ ml), accompanied by a slight increase in CRP, LDH and D-dimers. No alterations in pro-calcitonin, NT-proBNP, serum-creatinine or liver enzymes were detected. The clinical symptoms improved, and the temperature normalized from day +8, so that the patient could be discharged on April 2nd (day +9 from COVID-19 diagnosis, and day +12 from first symptoms).

Since then, he was regularly examined in our outpatient department. Neutrophil counts regenerated to CTC grade I neutropenia by April 24th (day +29), so we decided to resume lenalidomide maintenance.

Summarized, we observed that COVID-19 took a rather mild clinical course despite pulmonary affection in an immunocompromised patient with hematologic disease. We therefore raised the question on the immunological response that had combatted COVID-19, and we continued monitoring the immune response under re-treatment with lenalidomide. We quantified innate and adaptive immune cell subpopulations by multicolor flow cytometry, specific T-cell responses to SARS-CoV2, and cytokine serum levels during the course of disease and after re-introduction of lenalidomide until day +174. Despite overall lymphocytopenia with decreased CD3+ T cell numbers at COVID-19 diagnosis (Fig. 1a, Supplementary Tab. I), we observed a robust increase in cytotoxic CD3+CD8+ T cells with counts ranging from 233 cells/µl to 438 cells/µl of whole blood. The increased CD8+ T cell numbers were accompanied by an extraordinarily strong expression of HLA-DR on 86-95% of total CD8+ T-cell population, which is a marker for late activation (Fig. 1c), while CD69-expression as a marker of early activation remained constant (Fig. 1b).

CD19+ B-cell and CD3/CD56+ NK-cell levels were diminished at COVID-19 diagnosis, regenerated during the observation period, but started to decrease after lenalidomide re-introduction (Supplementary Tab. I).

Moreover, we followed up on innate immunity-derived cytokine response by monitoring Interleukin-1β (IL-1β), IL-6, Interleukin-8 (IL-8), Interleukin-10 (IL-10) and IP-10 (CXCL10, marker for interferon- γ) levels (Fig. 2a). To compare and rank the measured cytokine values into the COVID-19 landscape, we co-evaluated cytokine profiles from an age-matched patient with severe (= WHO scale 6) and with moderate COVID-19 symptoms (< WHO scale 4; Fig. 2b). As indicated by moderately elevated IP-10, the innate immune response was reflected by moderate induction of interferon-γ (Fig. 2b) [7]. Although our patient had significantly elevated IL-6 levels during the hospitalization period, the levels normalized as the symptoms improved (Supplementary Tab. I, Fig. 2a). This was unlike to a critically ill control patient, whose IL-6, IL-1β and IP-10 levels persisted at high levels even 3 weeks after the COVID-19 diagnosis. In contrast, IL-10, known as anti-inflammatory regulator of immunity to infection [8], was strongly elevated in the myeloma patient but low in a critically ill COVID-19 patient.

Next, we performed an IFN-γ ELISPOT assay with patient´s peripheral blood mononuclear cells (PBMCs) that were collected at day+44 and day +174, to examine the specific effector memory T-cell response to SARS–CoV-2 (Fig. 2 e-f). Despite low initial naïve T-cell counts, the patient was able to develop specific T-

cell memory subsets that showed a type II interferon reactivity to peptides from SARS-CoV-2 membrane, nucleocapsid and spike protein to the time points tested. This is in line with increasing central memory and effector memory cell counts in the patient's blood from day +29 to +44, especially in the CD3+ CD8+ T cell compartment. Additionally, this specific T-cell dependent immunity appeared to be long lasting as reactive T cells persisted at day+174.

The next finding was the detection of anti-SARS-CoV-2 type IgA and IgG antibodies in the patient's serum at quite remarkable levels (Fig. 2 c-d). Qualitative anti-SARS-CoV-2 type IgG antibody detection was in parallel conducted in our virology department and remained positive even on day +174 (Table 1, Method: ELISA, Euroimmun, Lübeck, Germany). This is notable regarding pre-existing deficiency of CD4+ T cells and reduced IgA und IgM levels due to induction chemotherapy long before SARS-CoV-2 diagnosis. It reflects that not the absolute CD4+ T cells values, but their capacity to get activated and to differentiate into memory CD4+ T cells, could be crucial for completion of effective immunological response to SARS-CoV-2.

After re-introduction of lenalidomide, the activation status and distribution of cell populations remained unchanged despite an absolute decrease in lymphocyte subpopulations. In addition, specific IgG antibody levels and anti-SARS-CoV-2 reactive T-cells were also not affected by lenalidomide re-introduction, indicating persisting immunity despite the known immune modifying effects of this drug, at least in a 6 month view.

Furthermore, at every time point after day +11 the patient was tested for SARS-CoV-2, and the results remained negative (data not shown).

Conclusion

We summarize, that the immune response seen in this immunocompromised patient was modest, but specific and sufficient for virus eradication. Hematologic malignancies per se might not be the crucial factor that is affecting the course of COVID-19.

Declarations

Funding

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Conflicts of interest / Competing interests

All authors declare that they have no conflict of interest or competing interests.

Availability of data and material

The data and material will be made available upon request.

Code availability

Not applicable

Author contributions

von Metzler and Ullrich contributed to the study conception and design. Material preparation, data collection and analysis were performed by Campe, Huenecke, Raab, Goldschmidt, Schubert and Rabenau. Data interpretation has been discussed by Campe, Huenecke, Raab, Goldschmidt, Schubert, Rabenau, Ciesek, Serve, von Metzler, Ullrich. The first draft of the manuscript was written by von Metzler, Ullrich and Campe and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

Blood sample collection was in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University Hospital Frankfurt.

Consent to participate

All subjects provided written, informed consent for participation at this study.

Consent for publication

All authors approved the final manuscript and agree with publication.

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Figures

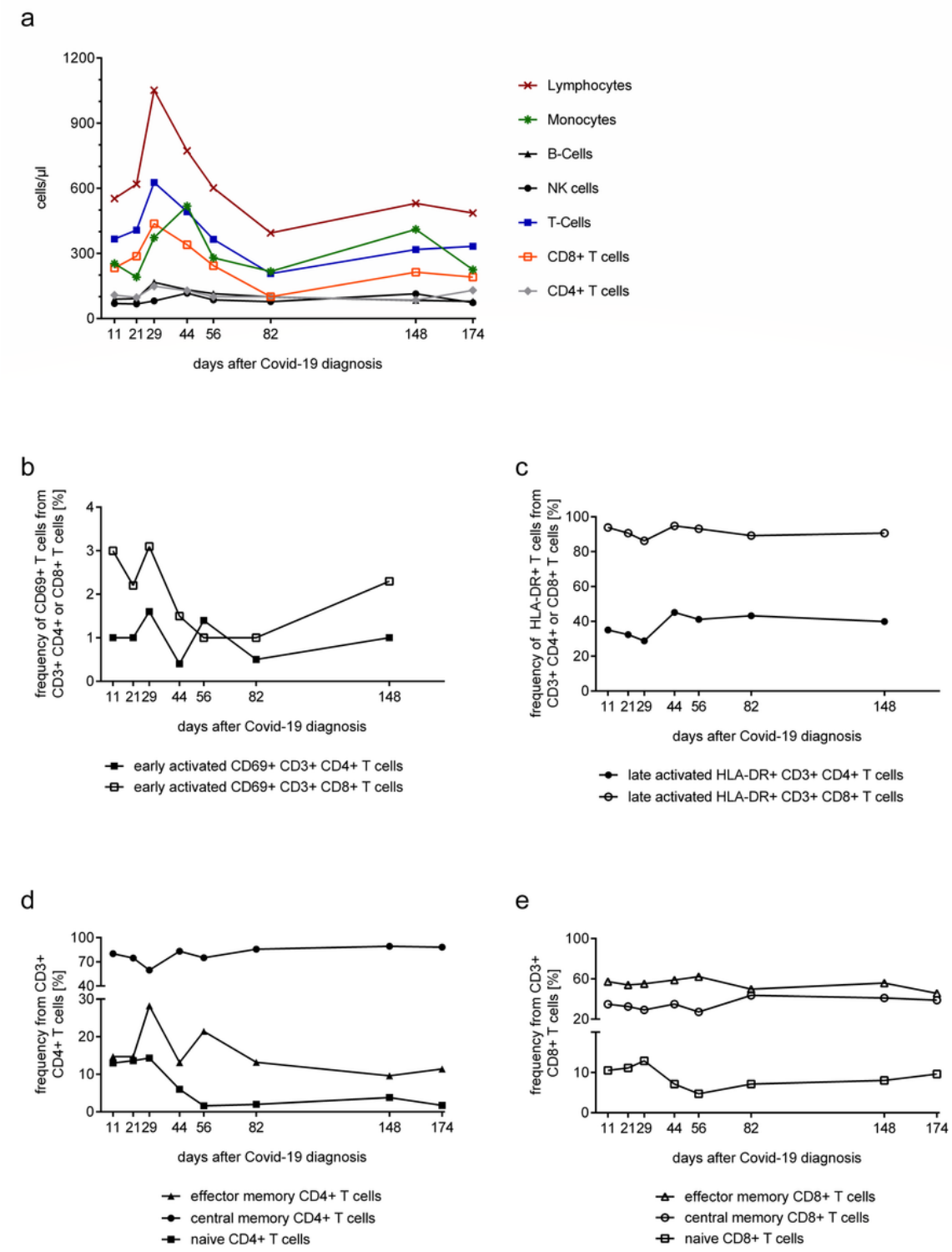

Figure 1

Temporal assessment of Immune cell populations, T-cell activation and T-cell subpopulations following COVID-19 diagnosis. (a) Number of immune cell populations/µL whole blood (b) Frequency of early activated CD4+ and CD8+ T cells defined by CD69-expression. (c) Frequency of late activated CD4+ and CD8+ T cells defined by HLA-DR-expression. (d, e) Frequencies of naïve, effector memory, central memory CD4+ (d) and CD8+ (e) T cells.

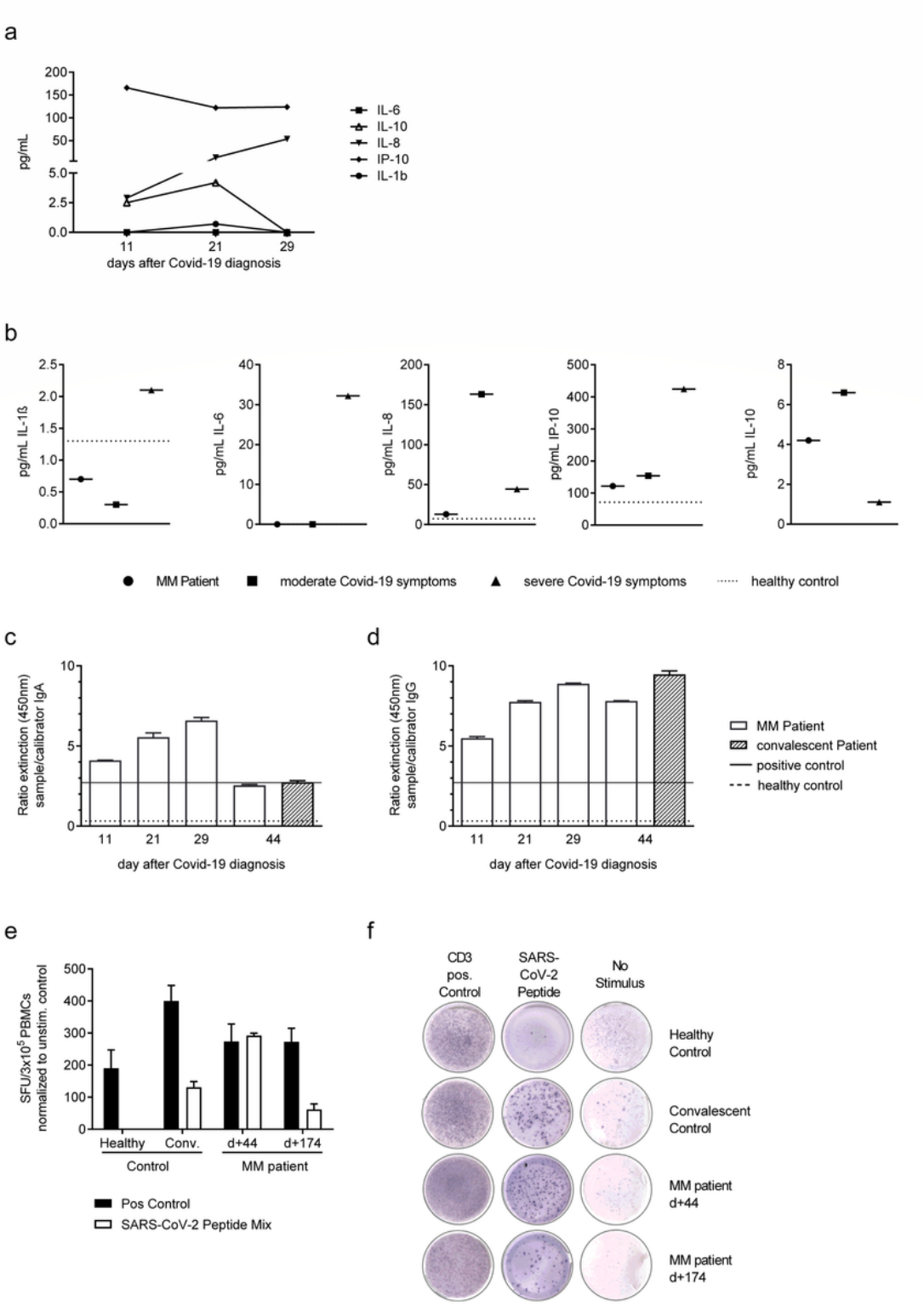

Figure 2

Profile of predominant cytokines, antibody, and memory T cell response (a) Development of detectable cytokines in serum of the multiple myeloma (MM) patient over time. (b) Comparison of detected cytokine concentrations between SARS-CoV-2 infected MM patient and male COVID-19 patients with severe (n=1, 52 years old) and moderate (n=1, 65 years old) symptoms at day 21 +/-3 days after diagnosis of SARS-CoV-2 infection. The dotted line indicates the values measured in a male healthy control (n=1, <50 years

old). (c-d) Qualitative detection of SARS-CoV-2 specific IgA (c) and IgG (d) in the MM patient's serum to different time points after COVID-19 diagnosis in comparison to an age matched male convalescent patient (n=1, 52 years old, mild COVID-19 symptoms, day +44 after diagnosis). (e-f) IFN-γ response of cryopreserved, thawed and overnight rested PBMCs to SARS-CoV-2 peptide mix measured by ELISpot assay. Comparison of PBMCs from MM Patient isolated at day +44 and +174 with an age matched, male convalescent Patient (n=1, 52 years old) at day +44 after COVID-19 diagnosis and the same healthy male (n=1, <50 years) used for the other analyses. 25ng/mL purified anti-CD3 (clone OKT-3) served as positive control. For the measurement of a SARS-CoV-2 specific response, a mix of 1.25µg/mL S-Protein, M-Protein and N-Protein Peptivator (Miltenyi) respectively was used. 3x105 PBMCs were seeded in triplicates per condition with a total volume of 100µL per well and incubated with stimuli for 24h. Quantification of spot forming units (SFU)/3x105 PBMCs normalized to the unspecific response (SFU/3x105 PBMCs without stimulus) (e) and representative Images of the wells (f) are displayed.

Supplementary Files

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- Finalv.MetzlerCOVID19SupplemenatryTable1.docx