Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Original Research

Immunological features of coronavirus disease 2019 in patients with cancer

Marion Thibaudin a,b,e,1, Jean-David Fumet a,b,c,d,e,1, Marjorie Bon a,b, Léa Hampe a,b, Emeric Limagne a,b,e, Francois Ghiringhelli a,b,c,d,e,*

a Cancer Biology Transfer Platform, Centre Georges-François Leclerc, F-21000, Dijon, France
b Centre de Recherche INSERM LNC-UMR1231, F-21000, Dijon, France
c Department of Medical Oncology, Centre Georges-François Leclerc, F-21000, Dijon, France
d University of Burgundy Franche-Comté, F-21000, Dijon, France
e Genetic and Immunology Medical Institute, Dijon, France

Received 30 June 2020; received in revised form 7 August 2020; accepted 18 August 2020
Available online 7 September 2020

KEYWORDS
COVID-19;
Cancer;
Immunomonitoring

Abstract  Background: Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2, has caused a major pandemic. Patients with cancer are at higher risk of severe COVID-19. We aimed to describe and compare the immunological features of cancer patients hospitalised for COVID-19 or other concomitant, cancer-related illness.

Methods: In this prospective study, the clinical and immunological characteristics of 11 cancer patients with COVID-19 and 11 non—COVID-19 cancer patients hospitalised in the same unit at the same period for other medical issues were analysed. We also used 10 healthy volunteers as controls. Peripheral immune parameters were analysed using multiparametric flow cytometry.

Results: The median age of COVID-19—positive cancer patients was 71.1 years, and 66.4 years for controls. Compared with non—COVID-19 cancer patients, COVID-19—positive cancer patients had more extensive lymphopenia and hypoalbuminemia, with higher levels of C-reactive protein. In COVID-19 patients, elevated procalcitonin was associated with a higher risk of death. By phenotypic analysis, COVID-19—positive patients presented CD3 lymphopenia, with inversion of the CD4/CD8 ratio and modification of monocyte activation, with accumulation of mMDSC (monocytic Myeloid-Derived Suppressor Cells) -like cells and a decrease in activated monocytes. Analysis of the T-cell compartment revealed a T-dependent inflammatory response with accumulation of Th17 cells and cytotoxic CD8 T cells producing TNFα, a decrease in HLA-DR (Human Leukocyte Antigen — DR isotype)-positive CD8 T cells and Treg/CD8 ratio.

* Corresponding author: Georges-Francois Leclere Cancer Center, 1 rue Professeur Marion, Dijon, 21000, France. Fax: +33 3 80 39 34 34.
E-mail address: fghiringhelli@cgl.fr (F. Ghiringhelli).
1 MT and JDF contributed equally.

https://doi.org/10.1016/j.ejca.2020.08.013
0959-8049/ 2020 Published by Elsevier Ltd.
1. Introduction

Since early 2020, the coronavirus disease 2019 (COVID-19) pandemic has affected millions of people worldwide. Although harmless for 85% of the population, COVID-19 can be life-threatening in vulnerable (e.g. older, immunosuppressed or comorbid) patients. Estimated mortality from COVID-19 is 1–3%, i.e. 10 to 30 times higher than the death rate from seasonal influenza. The causative agent of COVID-19 is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. The virus spreads via small droplets projected from the mouth and nose. Analysis of data on the propagation of SARS-CoV-2 in China suggests that close contact with an infected person is necessary. In fact, propagation is mainly limited to family members, health professionals and other persons in close contact with the infected individual [2]. This suggests that quarantine is the best means to contain the epidemic. To this end, lockdown was implemented in many countries around the world starting in March 2020.

Many comorbidities, such as older age, obesity, or diabetes, pathologies that affect the immune system, and cancer can all incur an increased risk of severe forms of COVID-19 infection [3]. Accordingly, emerging data from China, Italy and North America indicate that severe disease is more frequent and the death rate higher in patients with cancer who contract COVID-19 [4–7]. Recent data from the British National Health System (NHS) show that organ transplant, immunosuppression status, presence of cancer or haematological malignancy are associated with a high risk of death [8].

Lymphopenia, increased pro-inflammatory markers and cytokines, and potential blood hypercoagulability characterise severe COVID-19 cases, with features reminiscent of cytokine-release syndromes. Although immunosuppression and cancer could enhance the risk of severe COVID-19, one might also suspect that such patients would develop an unusual form of immune response after exposure to COVID-19. Therefore, we decided to explore this question by performing peripheral blood immunomonitoring on patients with cancer hospitalised in our cancer centre for mild to severe COVID-19 infection over a one-month period. We further compared them to cancer patients hospitalised for intercurrent non–COVID-19 medical problems during the same period and also to healthy volunteers (HVs).

2. Material and methods

2.1. Study participants

From March 30th to May 9th, 2020, 11 consecutive patients with positive diagnosis of SARS-CoV-2 during the care of their cancer disease were hospitalised in Georges Francois Leclerc centre. Concomitantly 11 matched cancer patients without SARS-CoV-2 infection were hospitalised in Georges Francois Leclerc centre during the same period for another intercurrent complication during the care of their cancer disease. We used as control group of healthy blood donors from Etablissement Francais du Sang. The study was declared as an ancillary study of the NCT02281214 and NCT02840604. The study was approved by the CNIL (Commission Nationale de l’Informatique et des Libertés) (national commission for data privacy) and the local ethics committee, and was performed according to the Helsinki declaration and in compliance with the European reglementation.

We retrospectively obtained the medical history, physical examination, and haematological, biochemical, radiological, and microbiological data. The data collection forms were reviewed independently by two researchers.

2.2. Laboratory measurements

Real-time reverse transcription polymerase chain reaction (RT-PCR) assays were done for SARS-CoV-2. Respiratory specimens were collected by the local CDC (Centers for Disease Control) and then shipped to designated authoritative laboratories to detect SARS-CoV-2. The presence of SARS-CoV-2 in respiratory specimens was detected by real-time reverse transcription (RT-PCR) methods. The SARS-CoV-2 VIASURE Real-Time PCR Detection kit (supplied by CERTEST Biotec) containing the primers and probe targeting the CoV envelope gene were used. The use of this kit for the targeting of SARS-CoV-2 gene has been validated by the CNR (Centre National de Référence) virology respiratory laboratory in Lyon. Conditions for the amplifications were 45 °C for 15 min, 95 °C for 2 min, followed by 45 cycles of 95 °C for 10 s and 60 °C for 50 s.
2.3. Clinical laboratory measurements

Initial clinical laboratory investigations included a complete blood count, serum biochemical test (including liver and renal function, creatine kinase, low-density lipoprotein and electrolytes) and coagulation profile.

2.4. Evaluation of peripheral blood immunological indicators

2.4.1. Leucocyte population identification and numeration

2.4.1.1. Liquid reagents. Antibody clones CD127-Brilliant Violet 605 (clone A019D5), CCR7-Brilliant Violet 650 (clone G043H7) and CD45RA-Brilliant Violet 785 (cloneH1100) were purchased from BioLegend.

Dried format reagents: Using Beckman Coulter’s custom design service and its dry coating technology, custom tubes containing CD16-FITC (clone 3G8), CD56-PE (clone N901), CD25-APC (clone B1.49.9), HLA-DR-PE-Cyanine5.5 (Clone Immu-357), CD14-Cyanine7 (clone RMO52), CD4-APC (Clone 13B8.2), CD8-Alexa Fluor 700 (Clone B9.11), CD3-AA750 (Clone UCHT1), CD15-PacBlue (Clone 80H5) and CD45-Krome Orange (Clone J.33) were produced.

2.4.1.2. Staining protocol. 100 μL of total heparinized blood was added in DURAClone tube containing liquid antibodies, vortexed immediately for 15s and incubated for 15 min at room temperature in the dark. Two millilitre of red blood lysis solution (VersaLyse, Beckman Coulter) containing 50 lilitre of fixative agent (IOTest) for 15 min at room temperature in the dark. PBS 1X was added, and after centrifugation the pellet was resuspended in 25 μL of foetal bovine serum (FBS, Dutscher) and 300 μL of PerFix-NC R2 buffer was added. A 325 μL aliquot was transferred to a DURAClone tube containing the liquid antibody, vortexed immediately for 15s and incubated for 1 h at room temperature in the dark. PBS 1 × (3 mL) was added to the tubes, incubated for 5 min at room temperature in the dark before centrifugation for 5 min at 500g. After supernatant removal, the cells were resuspended in 3 mL of 1X PerFix-NC R3 buffer before another 5-min centrifugation at 500g. The pellet was dried and resuspended in 300 μL of 1X R3 buffer. Acquisition was done on CytoFLEX cytometer. The proportion of IFNγ+, TNFα+, IL-2+, IL-17A+ or IL-4+ expression by CD4+ T or CD8+ T cells and the proportion of CD25+ Foxp3+ CD4+ or CD8+ cells were studied with this labelling procedure. The gating strategy is described in Supplementary Fig. 7.

All analyses were done with Kaluza 1.3 software (Beckman Coulter).

2.4.2. Lymphocyte function analysis

2.4.2.1. Liquid reagents. Antibody clone IL-2-Brilliant Violet 605 (clone MQ1-17H12) was purchased from BioLegend.

2.4.2.2. Dried format reagents. Using Beckman Coulter’s custom design service and its dry coating technology, custom tubes containing IFNγ-FITC (Clone 45.15), CD25-PE (Clone B1.49.9), CD4-PE-Cyanine5.5 (Clone 13B8.2), IL-4-PE-Cyanine7 (Clone MP4-25D2), Foxp3-AF647 (Clone 259D), TNFα-AF700 (Clone IPM2 (188)), CD3-AA750 (Clone UCHT1), IL-17A-PacBlue (Clone B168) and CD8-KromeOrange (B9.11) were produced.

2.4.2.3. Staining protocol. 50 μL of total heparinized blood was added in DURActiv 1 dry tube (Beckman Coulter) containing Phorbol myristate acetate (PMA), ionomycin and brefeldin A for 3 h at 37 °C. After activation, 25 μL of PerFix-NC R1 buffer (PerFix-NC kit, Beckman Coulter) was added on vortex and incubated for 15 min at room temperature. Then, 2 mL of PBS 1X was added, and after centrifugation the pellet was resuspended in 25 μL of foetal bovine serum (FBS, Dutscher) and 300 μL of PerFix-NC R2 buffer was added. A 325 μL aliquot was transferred to a DURAClone tube containing the liquid antibody, vortexed immediately for 15s and incubated for 1 h at room temperature in the dark. PBS 1 × (3 mL) was added to the tubes, incubated for 5 min at room temperature in the dark before centrifugation for 5 min at 500g. After supernatant removal, the cells were resuspended in 3 mL of 1X PerFix-NC R3 buffer before another 5-min centrifugation at 500g. The pellet was dried and resuspended in 300 μL of 1X R3 buffer. Acquisition was done on CytoFLEX cytometer. The proportion of IFNγ+, TNFα+, IL-2+, IL-17A+ or IL-4+ expression by CD4+ T or CD8+ T cells and the proportion of CD25+ Foxp3+ CD4+ or CD8+ cells were studied with this labelling procedure. The gating strategy is described in Supplementary Fig. 7.

All analyses were done with Kaluza 1.3 software (Beckman Coulter).

2.5. Statistics

Continuous variables are expressed as median (IQR, InterQuartile Range) and compared with the Mann Whitney U test. Categorical variables are expressed as number (%) and compared by χ² test or Fisher’s exact test between COVID and non-COVID patients. A two-sided α of less than 0.05 was considered statistically significant. Statistical analyses were done using PRISM software.

3. Results

3.1. Patient demographics and baseline characteristics of COVID-19 and non—COVID-19 cancer hospitalised patients

Between March 30th and May 9th, 2020, a total of 22 cancer patients were admitted to our cancer center for suspicion of SARS-CoV-2 infection. The diagnosis was confirmed for 11 patients, including five with positive PCR, and six with negative PCR but compatible CT scan images and no other plausible aetiology. Among the 11 COVID-19–positive patients, median age was 71.1 years, and few comorbidities were reported. Nine of...
the 11 had metastatic disease, and four had received more than two lines of therapy. All 11 patients received antibiotic therapy, nine required oxygen therapy and eight had fever. At baseline, mean oxygen saturation (SpO₂) was 90% (58–95). Table 1 summarises the characteristics of the patients, and Supplementary Table 1 represents individual data for each patient.

Among the 11 non-COVID cancer patients, median age was 66.4 years. This group was comparable with the COVID-19–positive patients, with similar metastatic status, cancer type and comorbidity status (Table 1). As a second control data set, we also used data from 10 HVs with a mean age of 60.

Among the 11 COVID-19–positive patients, interstitial lung abnormalities were observed on chest image analysis. Typical findings were bilateral ground-glass opacities and subsegmental areas of high density (Fig. 1). At the time of admission, laboratory analysis showed a non-significant trend towards lymphopenia compared with the non-COVID cancer patients. Patients who died from COVID-19 were classified as severe COVID-19. Severe COVID-19 cases had increased WBC counts, but lower lymphocyte counts (Fig. 2). Further biological tests showed a significant increase in C-reactive protein in COVID-19–positive patients (153.5 versus 71.5, p = 0.0029). Procalcitonin elevation trended to be associated a higher risk of death (Fisher test p = 0.16). Albumin concentrations were significantly lower in COVID-19–positive patients than in non-COVID cancer patients (Fig. 2).

At the date of database lockout (25 May 2020), six severe COVID-19–positive patients died (four men and two women), at a median age of 62.2 years (33.5–81.7). All had locally advanced or metastatic cancer, and all had pulmonary symptoms. Only two of the six had a comorbidty (high blood pressure).

### 3.2. Analysis of blood phenotypic immune parameters

We first examined the number and proportion of immune cells in peripheral blood [9]. Total leucocyte number did not vary between healthy donors, COVID-19–positive and non-COVID cancer patients (Fig. 3A). When looking at the proportion of myeloid cells, we observed no difference in the proportion of monocytes, but a marked increase in the proportion of neutrophils in cancer patients, with a higher proportion in COVID-19–positive cancer patients (Fig. 3A). No particular variation was observed for the frequency of CD15⁺CD16low eosinophils (Fig. SD1). For monocyte subsets, we observed a decrease in the absolute number of CD14⁺CD16⁻ monocytes in COVID-19–positive patients compared with both HVs and non–COVID-19 cancer patients. CD14high CD16⁺ monocytes accumulated in all cancer patients, but more marked extent in COVID-19–negative cancer patients (Fig. SD2).

Regarding maturation of monocytes, we observed that HLA-DRlow (Human Leukocyte Antigen – DR isotype) monocytes, which harbour a monocytic MDSC (Myeloid-Derived Suppressor Cells) phenotype, were increased in COVID-19–positive cancer patients, while

### Table 1

Baseline demographics.

| Characteristic                         | COVID-19 –positive | COVID-19 –negative |
|----------------------------------------|--------------------|--------------------|
| Median age                             | 77.3 (4)           | 66.4 (4)           |
| Caucasian                              | 11 (100%)          | 11 (100%)          |
| Cancer type                            |                    |                    |
| Lung cancer                            | 4 (36.4%)          | 5 (45.5%)          |
| Breast cancer                          | 1 (9.1%)           | 1 (9.1%)           |
| Sarcoma                                | 3 (27.3%)          | 0                  |
| Head and neck cancer                   | 1 (9.1%)           | 1 (9.1%)           |
| Biliary/pancreatic cancer              | 0                  | 2 (18.2%)          |
| Skin cancer                            | 0                  | 1 (9.1%)           |
| Stomach cancer                         | 0                  | 1 (9.1%)           |
| Endometrial cancer                     | 1 (9.1%)           | 0                  |
| Hepatocarcinoma                        | 1 (9.1%)           | 0                  |
| Stage                                   |                    |                    |
| Localised                              | 1 (9.1%)           | 1 (9.1%)           |
| Locally advanced/metastatic            | 10                 | 10                 |
| Cancer therapy                         |                    |                    |
| 1st line                               | 7 (63.6%)          | 4 (36.4%)          |
| 2nd line                               | 1 (9.1%)           | 5 (45.5%)          |
| >2nd line                              | 3 (27.3%)          | 2 (18.2%)          |
| ECOG                                   |                    |                    |
| 0                                      | 0                  | 1 (9.1%)           |
| 1                                      | 1 (9.1%)           | 2 (18.2%)          |
| 2                                      | 7 (63.6%)          | 7 (63.6%)          |
| 3                                      | 1 (9.1%)           | 0                  |
| 4                                      | 2 (18.2%)          | 0                  |
| Comorbidity                            |                    |                    |
| High blood pressure                    | 4 (36.4%)          | 6 (54.6%)          |
| Diabetes                               | 2 (18.2%)          | 2 (18.2%)          |
| Asthma                                 | 0                  | 2 (18.2%)          |
| Smoking                                | 6 (54.6%)          | 5 (45.5%)          |
| PCR                                    |                    |                    |
| Positive                               | 5 (45.5%)          | 0                  |
| Negative                               | 6 (54.6%)          | 11 (100%)          |
| CT scan                                |                    |                    |
| Positive                               | 7 (63.6%)          | 0                  |
| Negative                               | 0                  | 5 (45.5%)          |
| Unperformed                            | 4 (36.4%)          | 6 (54.6%)          |
| Symptoms                               |                    |                    |
| Fever                                  | 8 (72.8%)          | 6 (54.6%)          |
| Pulmonary symptoms                     | 10 (90.9%)         | 4 (36.4%)          |
| O₂ required                            | 9 (81.9%)          | 3 (27.3%)          |
| Antibiotherapy                         | 11 (100%)          | 5 (45.5%)          |
| Amox-ac clavulanic                     | 4 (36.4%)          | 3 (27.3%)          |
| C3G (Céphalosporines orales de 3ème)   | 1 (9.1%)           | 0                  |

CT, computed tomography; ECOG, Eastern Cooperative Oncology Group; PCR, polymerase chain reaction.
HLA-DR\textsuperscript{high}-activated monocytes were decreased (Fig. 3B).

When looking at lymphoid cells, we did not observe any difference in the proportion of NK subsets, γδT cells or NKT cells between COVID-19–positive and non-COVID cancer patients (Fig. SD3). However, the frequency of these three cell types was lower in cancer patients than that in HVs (Fig. SD3). We observed a significant decrease in the proportion of CD3 T cells and a respective increase in the B cell proportion in cancer patients compared with HVs (Fig. 3C and not shown). We noted a modification of the CD4/CD8 ratio between COVID-19–positive and –negative patients, with an increase in the CD8 proportion and a decrease in the CD4 proportion in COVID-19–positive patients (Fig. 3D and E).

Together these data underline the induction of CD3 lymphopenia, with an inversion of the CD4/CD8 ratio, a change in monocyte activation, accumulation of mMDSC-like cells and a decrease in activated monocytes in COVID-19–positive cancer patients.

3.3. Analysis of T-cell phenotypic and secretion parameters

Looking at CD4 and CD8 subsets, we observed a decrease in effector memory cells in CD8 T cells and an increase in effector memory RA\textsuperscript{+} (EMRA\textsuperscript{+}) cells, suggesting an accumulation of terminally differentiated cells in COVID-19–positive patients (Fig. 4A). For the CD4 subset, we did not observe any difference for each subtype in COVID-19–positive patients with exception of a decrease of naïve CD4 T cells (Fig. 4B). The Treg proportion in CD4 T cells did not change in COVID-19–positive patients, but as for the overall increase in CD8 T cells, we observed a decrease in the Treg/CD8 ratio (Fig. 4C). Interestingly, we observed in the basal peripheral blood a marked decrease in HLA-DR expression in CD8 T cells in COVID-19–positive patients (Fig. 4D), which is marker of T-cell activation [10,11]. Concerning secretory function of T cells, we observed an increase in IL-17A and Th17 cells in COVID-19–positive patients (Fig. 4E) and an accumulation of cytotoxic (IFN\textsubscript{γ}\textsuperscript{+}) CD8 T cells producing TNF\textsubscript{α} (Fig. 4F). The study of CD8 T-cells secretion capacities showed no other differences between COVID-19–positive and –negative patients (Fig. SD4).

Together these data underline the induction of a T-dependent inflammatory response with an accumulation of Th17, TNF\textsubscript{α}-producing CD8 T cells and a concomitant decrease in CD8 HLA-DR–positive cells, suggesting a reduction in effector CD8 T cells in COVID-19–positive cancer patients.
During follow-up, the blood immune modifications persisted for 2 weeks. Interestingly, death from COVID-19 was associated with higher neutrophil and mMDSC levels and also with a lower number of inflammatory monocytes (IMs), CD3 T cells, CD8 HLA-DR$^+$ e cells and EMRA$^+$ CD8 T cells (Fig. SD5).

4. Discussion

To the best of our knowledge, this is the first preliminary study to describe the immunological characteristics of cancer patients with SARS-CoV-2 infection. A growing body of data is emerging regarding the clinical and epidemiological features of patients with COVID-19 [9]. In addition, there have been some reports on immune response during SARS-CoV-2 infection [12–14]. The results obtained in this study could be strengthened by a larger number of patients and by the addition of analyses on COVID-19–positive non-cancer patients.

With regard to the myeloid compartment, we observed an accumulation of HLA-DR$^{low}CD14^+$ cells, similar to mMDSC cells, as well as concomitant decrease in HLA-DR$^{high}CD14^+$ cells. Previous studies in non-cancer, symptomatic COVID-19 patients have shown a similar accumulation of HLA-DR$^{low}CD14^+$ IMs [15–17]. The expansion and activation of these cells frequently depends on the cytokines IL-1 and IL-6 [18,19]. This accumulation could be linked to an inflammatory signature found during COVID-19 [20]. Significantly elevated systemic levels of the pro-

Fig. 2. The box plot of routine laboratory biological analysis between COVID-19–positive and –negative patients. The data presented constitute the analyses performed on 11 COVID-19–positive patients and 11 COVID-19–negative patients. Statistical difference is determined by a Mann–Whitney test. ns = no significant, * = p < 0.05, ** = p < 0.01.
inflammatory cytokine IL-6 have been reported in several COVID-19 patient cohorts and shown to correlate with disease severity [21] and the presence of inflammatory cytokines in serum. As in non-cancer patients, SARS-CoV-2 infection appears to affect CD14 myeloid cell differentiation.

T cells play a fundamental role in SARS-CoV-2 infection. A major marker associated with this disease is lymphopenia, with drastically reduced numbers of both CD4 and CD8 T cells in moderate and severe COVID-19 cases [9,22–25]. Previous reports have shown predominant CD8 T-cell depletion, which seems
Fig. 4. Leucocyte populations and lymphocyte function analysis between COVID-19-positive and -negative patients and healthy volunteers.

A–D: Whole blood of healthy volunteers or COVID-19—positive and —negative patients was stained with anti-CD16, anti-CD56, anti-CD25, anti-HLA-DR, anti-CD14, anti-CD4, anti-CD8, anti-CD3, anti-CD15, anti-CD45, anti-CD127, anti-CCR7 and anti-CD45RA antibodies and analysed by flow cytometry. A: The frequency of naïve (N: CD45RA⁺ CCR7⁺), central memory (CM: CD45RA⁻ CCR7⁺), effector memory (EM: CD45RA⁻ CCR7⁻) and effector memory RA⁺ (EMRA: CD45RA⁺ CCR7⁻) among CD8⁺ T cells is depicted. B: The frequency of naïve (N: CD45RA⁺ CCR7⁺), central memory (CM: CD45RA⁻ CCR7⁺), effector memory (EM: CD45RA⁻ CCR7⁻) and effector memory RA⁺ (EMRA: CD45RA⁺ CCR7⁻) among CD4⁺ T cells is depicted. C: The ratio of CD8 to regulatory T cells (TREG) is depicted. D: The frequency of HLA-DR⁺ CD8⁺ T cells is depicted. E–F: Whole blood of healthy volunteers or COVID-19—positive and —negative patients was activated and then stained with anti-IFNγ, anti-CD25, anti-CD4, anti-IL-4, anti-Foxp3, anti-TNFα, anti-IL-17A, anti-CD8 and anti-IL-2 antibodies and analysed by flow cytometry. E: The frequency of IL-17A—positive CD4⁺ T cells (Th17 cells) among CD4⁺ T cells is depicted. F: The frequency of TNFα—positive CD8⁺ T cells among IFNγ⁺ CD8⁺ T cells is depicted. The data presented constitute the analyses performed on 11 COVID-19—positive patients, 11 COVID-19—negative patients and 10 healthy volunteers. Statistical difference is determined by a Mann–Whitney test. ns = no significant, * = p<0.05 and ** = p<0.01.
accumulation of IFN-γ + TNFα + cells. Surprisingly, we observed a substantial reduction in HLA-DR expression on CD8 T cells. HLA-DR is recognised as a marker of T-cell activation [10,11] and has been shown to be increased in cytotoxic T lymphocytes in autoimmune diseases [38] and in patients with HIV infection [39]. Thus, CD8 immune response is ambivalent, with on the one hand, a decrease in the T-cell count and activated HLA-DR CD8 T cells, and, on the other hand, a concomitant decrease in the Treg/CD8 ratio and an accumulation of polyfunction CD8 T cells, suggesting a marked CD8-dependent inflammatory response that might be deleterious.

5. Conclusions

This manuscript underlines the particular immune response in cancer patients with COVID-19. As in other reports, we observed marked T-cell lymphopenia and a decrease in HLA-DR + CD8 T cells. However, we observed some particularities, with an inversion of the CD4/CD8 ratio, and an induction of an inflammatory response, with accumulation of MDSC, Th17 and TNFα + inflammatory CD8 T cells. This information could be important in broadening our understanding of the complications that occurs in cancer patients infected with SARS-CoV-2 virus.

Financial support

None.

Author contributions

M.T., J.D.F. and F.G. contributed to literature search, data collection, data analysis, data interpretation and writing of the manuscript. M.T., J.D.F., E.L. and F.G. contributed to the study design. M.T., J.D.F., M.B. and L.H. made contributions to collection of the data and the data analysis. M.T., J.D.F., E.L. and F.G. participated in drafting the article and revising it critically for important intellectual content. All authors reviewed the manuscript and gave final approval of the submitted version.

Conflict of interest statement

F. Ghiringhelli reports receiving honoraria for oral communications from Lilly, Sanofi, Bristol-Myers Squibb, Astra Zeneca and Amgen; receiving funding for clinical trials from Astra Zeneca; receiving travel grants from Roche France, Amgen and Servier; and being an advisory board member for Merck Serano, Amgen, Roche France and Sanofi.

The other authors have no potential conflict of interest to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2020.08.013.

References

[1] Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579:270–3. https://doi.org/10.1038/s41586-020-2012-7.
[2] Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Song Z-G, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;579:265–9. https://doi.org/10.1038/s41586-020-2008-3.
[3] Liu M, Gao Y, Shi S, Chen Y, Yang K, Tian J. Drinking no-links to the severity of COVID-19: a systematic review and meta-analysis. J Infect 2020. https://doi.org/10.1016/j.jinf.2020.05.042.
[4] Guan W-J, Ni Z-Y, Hu Y, Liang W-H, Ou C-Q, He J-X, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020. https://doi.org/10.1056/NEJMoa2002032.
[5] Zhang L, Zhu F, Xie L, Wang C, Wang J, Chen R, et al. Clinical characteristics of COVID-19-infected cancer patients: a retrospective case study in three hospitals within Wuhan, China. Ann Oncol 2020. https://doi.org/10.1016/j.annonc.2020.03.296.
[6] Liang W, Guan W, Chen R, Wang W, Li J, Xu K, et al. Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. Lancet Oncol 2020;21:333–7. https://doi.org/10.1016/S1470-2045(20)30096-6.
[7] Miyashita H, Mikami T, Chopra N, Yamada T, Chernyavsky S, Rizk D, et al. Do patients with cancer have a poorer prognosis of COVID-19? An experience in New York city. Ann Oncol Off J Eur Soc Med Oncol 2020. https://doi.org/10.1016/j.annonc.2020.04.006.

[8] The OpenSAFELY Collaborative, Williamson E, Walker AJ, Bhaskaran KJ, Bacon S, Bates C, et al. OpenSAFELY: factors associated with COVID-19-related hospital death in the linked electronic health records of 17 million adult NHS patients. Epidemiology 2020. https://doi.org/10.1016/j.epidem.2020.06.009.2999.

[9] Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest 2020;130:2620–9. https://doi.org/10.1172/JCI137244.

[10] Baecher-Allan C, Wolf E, Hafler DA. MHC class II expression identifies functionally distinct human regulatory T cells. J Immunol 2006;176:4622–31. https://doi.org/10.4049/jimmunol.176.8.4622.

[11] Viallard J-F, Blanco P, André M, Etienne G, Liferman F, Thibaudin M. Thibaudin et al. / European Journal of Cancer 139 (2020) 70–89

[12] Chan JF-W, Yuan S, Kok K-H, To KK-W, Chu H, Yang J, et al. Epidemiology of coronavirus disease 2019 (COVID-19) in China. JAMA 2020;323:524–33. https://doi.org/10.1001/jama.2020.1585.

[13] Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet Lond Engl 2020;395:507–13. https://doi.org/10.1016/S0140-6736(20)30111-7.

[14] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet Lond Engl 2020;395:497–506. https://doi.org/10.1016/S0140-6736(20)30183-5.

[15] Giamarellos-Bourboulis EJ, Netea MG, Rovina N, Akinosoglou K, Antoniadou A, Antonakos N, et al. Complex immune dysregulation in COVID-19 patients with severe respiratory failure. Cell Host Microbe 2020. https://doi.org/10.1016/j.chom.2020.04.009. S193131282030217-7.

[16] Zhang D, Guo R, Lei L, Liu H, Wang Y, Wang Y, et al. COVID-19 infection induces readily detectable morphological and inflammation-related phenotypic changes in peripheral blood monocytes, the severity of which correlate with patient outcome. MedRxiv 2020. https://doi.org/10.1101/2020.03.24.20042655.

[17] Zhou Y, Fu B, Zheng X, Wang D, Zhao C, Qi Y, et al. Aberrant pathogenic GM-CSF+ T cells and inflammatory CD14+ CD16+ monocytes in severe pulmonary syndrome patients of a new coronavirus. Immunology 2020. https://doi.org/10.1111/imm.13455.7.

[18] Ren W, Su W, Tang H, Le W, Zhang X, Zheng Y, et al. Immune cell profiling of COVID-19 patients in the recovery stage by single-cell sequencing. Cell Discov 2020;6:1–18. https://doi.org/10.1038/s41421-020-0168-9.

[19] Guo C, Li B, Ma H, Wang X, Cai P, Yu Q, et al. Tocilizumab treatment in severe COVID-19 patients attenuates the inflammatory storm incited by monocyte centric immune interactions revealed by single-cell analysis. https://doi.org/10.1101/2020.04.08.029769; 2020.

[20] Ong EZ, Chan YFZ, Leong WY, Lee NMY, Kalimuddin S, Haja Mohideen SM, et al. A dynamic immune response shapes COVID-19 progression. Cell Host Microbe 2020. https://doi.org/10.1016/j.chom.2020.03.021.

[21] Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet 2020;395:1033–4. https://doi.org/10.1016/S0140-6736(20)30628-0.

[22] Wang F, Nie J, Wang H, Zhao Q, Xiong Y, Deng L, et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. J Infect Dis 2020. https://doi.org/10.1093/jid/jiaa15.8.

[23] Nie S, Zhao X, Zhao K, Zhang Z, Zhang Z, Zhang Z. Metabolic disturbances and inflammatory dysfunction predict severity of coronavirus disease 2019 (COVID-19): a retrospective study. https://doi.org/10.1101/2020.03.24.20042283; 2020.

[24] Zeng Q, Li Y, Huang G, Wu W, Dong S, Xu Y. Mortality of COVID-19 is associated with cellular immune function compared to immune function in Chinese han population. Infectious Diseases (except HIV/AIDS) 2020. https://doi.org/10.1016/j.2020.03.08.20031229.

[25] Zheng M, Gao Y, Wang G, Song G, Liu S, Sun D, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. Cell Mol Immunol 2020;17:533–5. https://doi.org/10.1038/s41423-020-0402-2.

[26] Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L, et al. Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). Front Immunol 2020:11. https://doi.org/10.3389/fimmu.2020.00622.

[27] Liu J, Li S, Liu J, Liang B, Wang X, Wang H, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. EBioMedicine 2020. https://doi.org/10.1016/j.ebiom.2020.102763.

[28] Tan L, Wang Q, Zhang D, Ding J, Huang Q, Tang Y-Q, et al. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. Signal Transduct Target Ther 2020;5. https://doi.org/10.1038/s41392-020-0148-4.

[29] Zhou Y, Fu B, Zheng X, Wang D, Zhao C, Qi Y, et al. Pathogenic T cells and inflammatory monocytes incite inflammatory storm in severe COVID-19 patients. Natl Sci Rev 2020. https://doi.org/10.1093/nsr/nwaa041.

[30] Braun J, Loyal L, Frensch M, Wendisch D, Georg P, Kurth F, et al. Presence of SARS-CoV-2 reactive T cells in COVID-19 patients and healthy donors. https://doi.org/10.1016/j.2020.04.17. 20061440; 2020.

[31] Dong C, Ni L, Ye F, Chen M-L, Feng Y, Deng Y-Q, et al. Characterization of anti-viral immunity in recovered individuals infected by SARS-CoV-2. https://doi.org/10.1016/j.2020.03.17. 20036640; 2020.

[32] Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nat Med 2020;1–3. https://doi.org/10.1038/s41591-020-09019-9.

[33] Thevarajan I, Nguyen THO, Koutsakos M, Druce J, Caley L, van de Sandt CE, et al. Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. Nat Med 2020. https://doi.org/10.1038/s41591-020-0819-2. 1–3.

[34] Yang X, Dai T, Zhou X, Qian H, Guo R, Lei L, et al. Analysis of adaptive immune cell populations and phenotypes in the patients infected by SARS-CoV-2. MedRxiv 2020. https://doi.org/10.1101/2020.03.23.20040675. 2020.03.23.20040675.

[35] Zheng H-Y, Zhang M, Yang C-X, Zhang N, Wang X-C, Yang X-P, et al. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. Cell Mol Immunol 2020;17:541–3. https://doi.org/10.1038/s41423-020-0401-3.

[36] Martin F, Apetoh L, Chirgihelli F. Controversies on the role of Th17 in cancer: a TGF-β-dependent immunosuppressive activity? Trends Mol Med 2012;18:742–9. https://doi.org/10.1038/trends.2012.09.007.

[37] Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenfer F, et al. Pulmonary vascular endotheliitis, thrombosis, and angiogenesis in covid-19. N Eng J Med 2020. https://doi.org/10.1056/NEJMoa2015432. 0: null.
[38] Viallard JF, Bloch-Michel C, Neau-Cransac M, Taupin JL, Garrigue S, Miossec V, et al. HLA-DR expression on lymphocyte subsets as a marker of disease activity in patients with systemic lupus erythematosus. Clin Exp Immunol 2001;125:485–91. https://doi.org/10.1046/j.1365-2249.2001.01623.x.

[39] Sáez-Cirión A, Lacabaratz C, Lambotte O, Versmisse P, Urrutia A, Boufassa F, et al. HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. Proc Natl Acad Sci U S A 2007;104:6776–81. https://doi.org/10.1073/pnas.0611244104.