International Journal of Clinical Oncology (2022) 27:1321–1330
https://doi.org/10.1007/s10147-022-02188-8

ORIGINAL ARTICLE

Altered mucosal immunity in HIV-positive colon adenoma: decreased CD4$^+$ T cell infiltration is correlated with nadir but not current CD4$^+$ T cell blood counts

Yasuo Matsubara[1] · Yasunori Ota[2] · Yukihisa Tanaka[2] · Tamami Denda[2] · Yasuki Hijikata[3] · Narikazu Boku[1] · Lay Ahyoung Lim[4] · Yoshihiro Hirata[1] · Giichiro Tsurita[5] · Eisuke Adachi[6] · Hiroshi Yotsuyanagi[6]

Received: 24 February 2022 / Accepted: 6 May 2022 / Published online: 29 May 2022

Abstract

Background People living with HIV (PLWH) face greater risks of developing non-AIDS-defining cancers (NADCs) than the general population; however, the underlying mechanisms remain elusive. The tumor microenvironment plays a significant role in the carcinogenesis of colorectal cancer (CRC), an NADC. We studied this carcinogenesis in PLWH by determining inflammatory phenotypes and assessing PD-1/PD-L1 expression in premalignant CRC stages of colon adenomas in HIV-positive and HIV-negative patients.

Methods We obtained polyp specimens from 22 HIV-positive and 61 HIV-negative participants treated with colonoscopy and polyp excision. We analyzed adenomas from 33 HIV-positive and 99 HIV-negative patients by immunohistochemistry using anti-CD4, anti-CD8, anti-FoxP3, and anti-CD163 antibodies. Additionally, we analyzed the expression levels of immune checkpoint proteins. We also evaluated the correlation between cell infiltration and blood cell counts.

Results HIV-positive participants had fewer infiltrating CD4$^+$ T cells than HIV-negative participants ($p = 0.0016$). However, no statistical differences were observed in infiltrating CD8$^+$ and FoxP3$^+$ T cells and CD163$^+$ macrophages. Moreover, epithelial cells did not express PD-1 or PD-L1. Notably, CD4$^+$ T cell infiltration correlated with nadir blood CD4$^+$ T cell counts ($p < 0.05$) but not with current blood CD4$^+$ T cell counts.

Conclusion Immune surveillance dysfunction owing to decreased CD4$^+$ T cell infiltration in colon adenomas might be involved in colon carcinogenesis in HIV-positive individuals. Collectively, since the nadir blood CD4$^+$ T cell count is strongly correlated with CD4$^+$ T cell infiltration, it could facilitate efficient follow-up and enable treatment strategies for HIV-positive patients with colon adenomas.

Keywords Colon adenomas · Tumor microenvironment · HIV · CD4$^+$ T cells

✉ Yasuo Matsubara
ma-yasu@ims.u-tokyo.ac.jp

[1] Department of Oncology and General Medicine, IMSUT Hospital, Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

[2] Department of Diagnostic Pathology, IMSUT Hospital, Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

[3] Department of Palliative Medicine/Advanced Clinical Oncology, IMSUT Hospital, Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

[4] Department of Research, Kitasato Institute Hospital, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan

[5] Department of Surgery, IMSUT Hospital, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

[6] Department of Infectious Diseases and Applied Immunology, IMSUT Hospital of the Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

Introduction

Although antiretroviral therapy (ART) has considerably increased the overall survival of HIV-infected patients, people living with HIV (PLWH) still face a higher risk of developing several non-AIDS-defining cancers (NADCs) than the general population [1, 2]. Research studies have proposed several factors that cause high rates of NADC in PLWH. These include certain lifestyle elements (e.g., smoking and alcohol consumption), potential oncogenic virus co-infections, and other factors, such as chronic immunosuppression, impaired immune surveillance, inappropriate immune activation, pro-oncogenic HIV effects, inflammation and coagulation, and ART toxicity [3–5]. The degree of immunosuppression has a profound negative effect on carcinoma development [6]. However, mechanisms underlying increased NADC risk in HIV-infected individuals remain unelucidated.

Colorectal cancer (CRC) is an NADC and is the third most common malignancy [7]. Several studies have reported discordant results regarding the risk of developing CRC in PLWH compared with that in HIV-negative individuals [2, 8]. However, a recent report revealed that the risk of CRC is elevated in HIV-infected Asians [9]. In addition, CRC can be diagnosed at early onset, an advanced stage, and during rapid disease progression in PLWH [10]. Notably, PLWH have been reported to have a higher rate of colorectal adenomas than the general public [11].

The majority of CRCs develop based on the adenoma–carcinoma sequence [12, 13]. Moreover, the microenvironment of the tumor, which includes tumor-infiltrating cells, has a substantial role in the development of colorectal carcinogenesis. In fact, it supports the multistep progression of a normal epithelium to an adenoma and ultimately to an invasive colon carcinoma [14]. Multiple studies have observed increased inflammation within colon adenomas, suggesting its involvement in cancer progression [15–17]. To study colon carcinogenesis in PLWH at the level of the local immune microenvironment, we analyzed the inflammatory phenotypes of colonic adenomas in both HIV-infected and non-infected Japanese subjects. Furthermore, we examined the expression of immune checkpoint proteins, particularly programmed cell death protein 1/programmed cell death ligand 1 (PD-1/PD-L1), which play essential roles in regulating immune surveillance and tumor progression [18].

Patients and methods

Study participants

We obtained tissue samples from the colon adenomas of 22 HIV-positive and 61 HIV-negative individuals (control) with a similar age and sex distribution. HIV-positive status had been diagnosed by the detection of HIV antibodies via Western blot and HIV-RNA by polymerase chain reaction. Individuals in both these groups had undergone and endoscopic or surgical polyp excision between 2011 and 2021 at our hospital. These polyps had been histopathologically diagnosed as adenomatous polyps or adenomas. Patients with immune disorders other than HIV infection, such as hematologic and autoimmune diseases, and those taking aspirin, steroid, and/or other immunomodulatory drugs were excluded from this study. The adenoma samples were classified by their size (<10 mm and ≥10 mm), morphology (pedunculated and sessile), location (left and right colon), and histological grade (low-grade and high-grade dysplasia). Additionally, we obtained the demographic and laboratory data of the participants retrospectively through their hospital records. Nadir and current $CD4^+$ T cell counts were defined as the lowest $CD4^+$ T cell count and the $CD4^+$ T cell count at the time of polyp resection, respectively. This study was approved by the IMSUT research ethics committee (approval number: 2020-15-0618).

Assessment of infiltrating immune cell phenotypes

We analyzed the infiltrating immune cell phenotypes by performing immunohistochemistry (IHC) on the colon adenomas. In this regard, we first stained the tissue by a polymer-based method (EnVision+^{TM} Dual Link System-HRP; Agilent Technologies, Santa Clara, CA, USA). The adenoma sections were deparaffinized; then, CD4, CD8, CD163, FoxP3, PD-1, and PD-L1 antigens were retrieved at 110 °C for 10 min with a citrate buffer and a tris-EDTA buffer. We used a 3% hydrogen peroxide solution for 5 min to inhibit endogenous peroxidase activity in the sections. We then incubated them with primary antibodies for 30 min at 37 °C. The supplier, clone, and dilutions of the

Table 1 Characteristics of antibodies used in immunohistochemistry

Cell type	Cell marker and antigen	Dilution	Supplier	Clone
T helper cell	CD4	1:250	Abcam, Cambridge, UK	EPR6855
Cytotoxic T cell	CD8	1:100	Abcam, Cambridge, UK	SP16
Regulatory T cell	FoxP3	1:50	Abcam, Cambridge, UK	SP97
M2-like macrophage	CD163	1:200	Leica Biosystems, Nussloch, Germany	10D6
	PD-1	1:100	Spring Bioscience, Fremont, CA, USA	Not applicable
	PD-L1	1:100	Cell Signaling Technology, Beverly, MA, USA	E1L3N

primary antibodies used in IHC are provided in Table 1. The sections were then washed in tris-buffered saline and treated with Envision+^{TM} Dual Link System-HRP reagent at 37 °C for 30 min. Thereafter, they were stained with diaminobenzidine for 3 min and counterstained with hematoxylin.

Using a high-power microscopic field (×400 magnification), we obtained digital images of five random independent areas in the top region of each adenoma sample. The number of antigen-positive cells was manually measured. Furthermore, we estimated this count in each tumor stroma and adenomatous epithelial area and determined the combined count. CD4 antigen-positive cells with lymphocytic morphology were counted as $CD4^+$ T cells because macrophages also express CD4. The total number of infiltrating immune cells in all five areas was used for statistical analysis.

Statistical analyses

GraphPad Prism version 9 for Windows (GraphPad Software, San Diego, California USA) was used to conduct statistical analyses. Descriptive statistics are indicated as the mean ± standard deviation. For comparisons between HIV-positive and HIV-negative subjects, the Mann–Whitney *U* test was applied. Additionally, to evaluate the relationship between infiltrating immune cells and blood cell counts, the Spearman correlation test was used. $p < 0.05$ were considered statistically significant.

Results

Samples characteristics

We included 83 participants (22 HIV positive and 61 HIV negative) in this study. Their demographic and clinical characteristics, including CRC risk elements (e.g., smoking, alcohol intake, obesity) [19], are shown in Table 2. All participants were male, and no significant differences were found in the characteristics of HIV-positive and HIV-negative participants (Table 2). All patients, except one in the HIV-positive group, were on ART, with the disease being controlled in 21 (95.5%) of them based on an endoscopic examination. The mean duration of the ART regimen was 9.3 years (range 0–26 years), and the mean time from CD4 nadir was 9.5 years (range 0–23 years). Only one HIV-positive participant had a history of AIDS (Table 3). The clinical and pathological features of the adenomas, such as their size, morphology, location, and histological grade, are summarized in Table 4. In total, 41.7%, 43.9%, and 55.3% of the adenomas comprised lesions ≥10 mm, had a pedunculated morphology, and included low-grade dysplasia, respectively. Additionally, 62.1% of the adenomas were found on the left side and 37.9% were on the right side of the colon.

Assessment of the immune cell phenotypes

The distributions of the infiltrating immunocytes, including $CD4^+$, $CD8^+$, and $FoxP3^+$ T cells and $CD163^+$ macrophages, in the top region of the adenomas examined by IHC are shown in Fig. 1. Notably, immune cells primarily infiltrated the tumor stroma. The numbers of infiltrating immune cells are presented in Fig. 2 and Table 5. A lower

Table 2 Demographic and clinical characteristics of study participants

	Total	HIV positive	HIV negative
N	83	22	61
Age, median (range), years	55.3 (30–78)	56 (32–78)	54.7 (30–78)
Sex, *n* (%)			
Male	84 (100%)	22 (100%)	61 (100%)
Female	0 (0%)	0 (0%)	0 (0%)
Smoker	45 (53.6%)	11 (50%)	33 (54.1%)
Alcohol drinker	42 (50%)	7 (31.8%)	34 (55.7%)
Obesity (BMI ≥ 25)	29 (34.5%)	6 (27.3%)	22 (36.1%)
Diabetes	5 (6.0%)	2 (9.1%)	3 (4.9%)

N number of participants; *BMI* body mass index

Table 3 Characteristics of HIV-positive subjects

N	22
On ART	21 (95.5%)
Untreated	1 (4.5%)
History of AIDS	1 (4.5%)
No history of AIDS	21 (95.5%)
Time on ART (years) median number of years (range)	9.3 (0–26)
Time from CD4 nadir (years) median number of years (range)	9.5 (0–23)
CD4 nadir (cells/μL) median number of cells (range)	192.0 (19–501)
CD8 at nadir of CD4 (cells/μL) median number of cells (range)	748.0 (240–1746)
CD4 at the time of polyp resection (cells/μL) median number of cells (range)	542.3 (215–1002)
CD8 at the time of polyp resection (cells/μL) median number of cells (range)	767.0 (242–2903)

AIDS acquired immunodeficiency syndrome; *ART* antiretroviral therapy; *N* number of subjects

number of infiltrating $CD4^+$ T cells was seen in HIV-positive participants than in HIV-negative patients ($p = 0.0014$ [stroma] and $p = 0.0016$ [combined]). However, there were no statistically significant differences in the number of $CD8^+$ and $FoxP3^+$ T cells and $CD163^+$ macrophages in adenomas between HIV-positive and HIV-negative patients.

PD-1/PD-L1 expression in the top region of the adenoma

PD-1 or PD-L1 expression was not detected in epithelial cells; however, notably fewer infiltrating immune cells in the adenomas expressed PD-1 or PD-L1 (Fig. 1). In addition, no statistically significant difference in the number of infiltrating cells expressing PD-1 and those expressing PD-L1 in adenomas was observed between HIV-positive and HIV-negative patients (Fig. 2, Table 5).

Table 4 Pathological characteristics of the colon adenomas analyzed in this study

	Total	HIV positive	HIV negative
n	132	33	99
Size, *n* (%)			
<10 mm	55 (41.7%)	10 (30.3%)	45 (45.5%)
≥10 mm	77 (58.3%)	23 (69.7%)	54 (54.5%)
Morphology peduncu-lated	58 (43.9%)	10 (30.3%)	48 (48.5%)
Sessile	74 (56.1%)	23 (69.7%)	51 (51.5%)
Location left colon	82 (62.1%)	25 (75.8%)	57 (56.6%)
Right colon	50 (37.9%)	8 (24.2%)	42 (43.4%)
Histological grade low	73 (55.3%)	20 (60.6%)	53 (53.5%)
High	59 (44.7%)	13 (39.4%)	46 (46.5%)

n number of adenomas analyzed

Correlation between immune cell infiltration and blood cell counts in HIV-positive participants

As depicted in Fig. 3, the number of infiltrating $CD4^+$ T cells was correlated with the nadir, but not the current, blood $CD4^+$ T cell counts ($p = 0.0054$ [stroma] and $p = 0.0043$ [combined]) in the HIV-positive participants. Interestingly, the number of infiltrating $CD8^+$ T cells was correlated with both the nadir and current blood $CD8^+$ T cell counts.

Discussion

The immune microenvironment is important for the establishment of sporadic CRC [14]. Thus, understanding the composition of immune cells in colon adenomas can determine the etiology of colon carcinogenesis in HIV-positive patients and help to develop future clinical interventions. This study assessed the number of infiltrating immunocytes and PD-1/PD-L1 expression in colon adenomas of HIV-positive and HIV-negative patients, which comprise the premalignant stage of CRC.

A study using an immunoediting model demonstrated that tumor-infiltrating lymphocytes substantially prevent tumor development in the elimination phase of the cancer immunoediting process [20]. Importantly, $CD4^+$ helper T cells, $CD8^+$ cytotoxic T cells, and natural killer (NK) cells play important roles in immune surveillance [20]. Of note, one study reported that $CD4^+$ and $CD8^+$ T cells are more numerous in colon adenomas than in normal tissue [17]. However, the HIV-induced dysfunction of NK cells can interrupt immune surveillance of malignant cells, thereby promoting cancer progression [4]. Nonetheless, very few NK cells were detected in adenomas in our preliminary examination using an anti-CD56 antibody; this observation was consistent with that of a recent report [15]. Therefore, in this study, we analyzed $CD4^+$ and $CD8^+$ T cells but not NK cells.

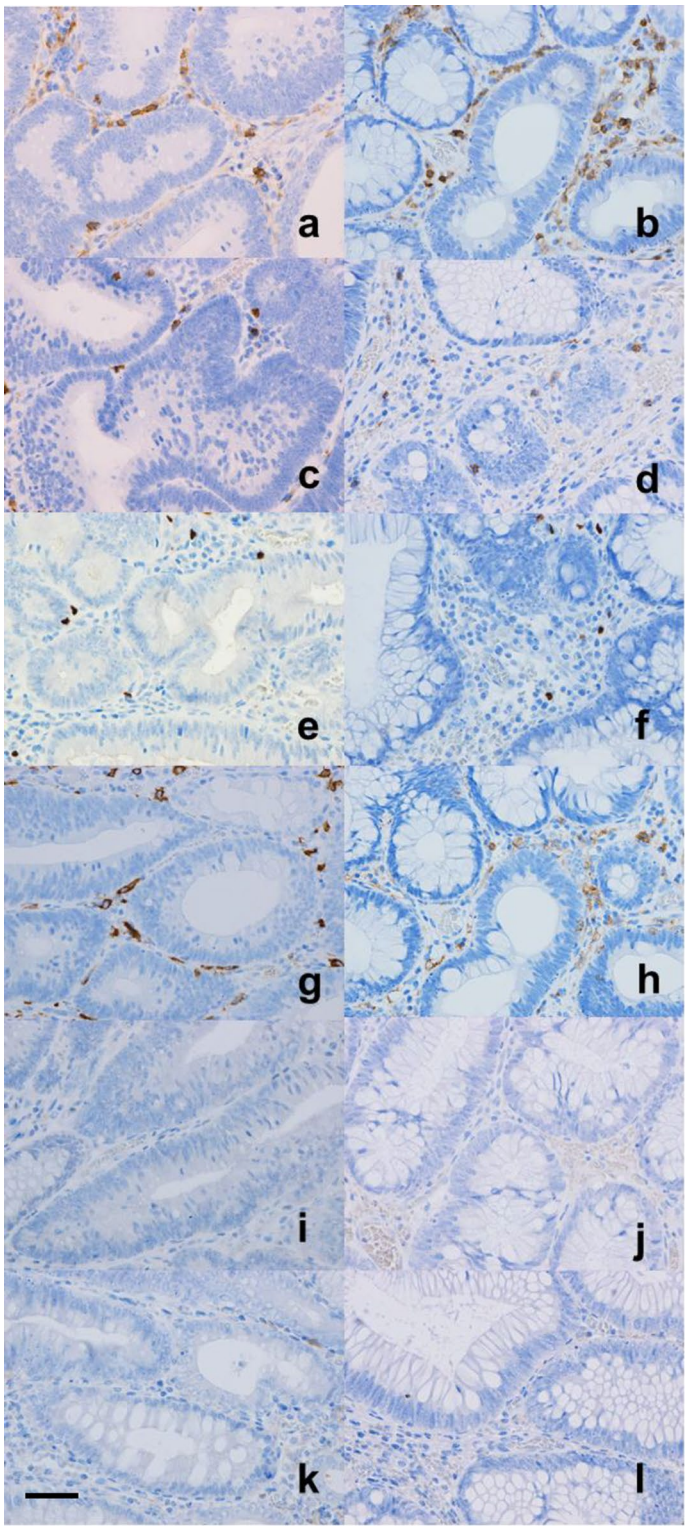

Fig. 1 Distribution of immunocytes infiltrating adenomas of HIV-positive and HIV-negative colon patients. $CD4^+$ T cells in **a** HIV-infected and **b** HIV-negative subjects. $CD8^+$ T cells in **c** HIV-infected and **d** HIV-negative subjects. $FoxP3^+$ T cells in **e** HIV-infected and **f** HIV-negative subjects. $CD163^+$ macrophages in **g** HIV-infected and **h** HIV-negative subjects. PD-1 expression in **i** HIV-infected and **j** HIV-negative subjects. PD-L1 expression in **k** HIV-infected and **l** HIV-negative subjects

The number of $CD4^+$ T cells in colon adenomas was significantly lower in the HIV-positive subjects than that in the HIV-negative patients. The blood count of $CD4^+$ T cells is a measure of HIV activity. In fact, there is accumulating evidence supporting a correlation between lower blood $CD4^+$ T cell counts and NADC risk. Thus, HIV-associated immunodeficiency affects cancer predisposition by hindering the immune surveillance of tumor cells [3, 4]. Furthermore, Bini et al. reported substantial associations between the blood $CD4^+$ T cell counts and distal colon neoplastic lesions [21].

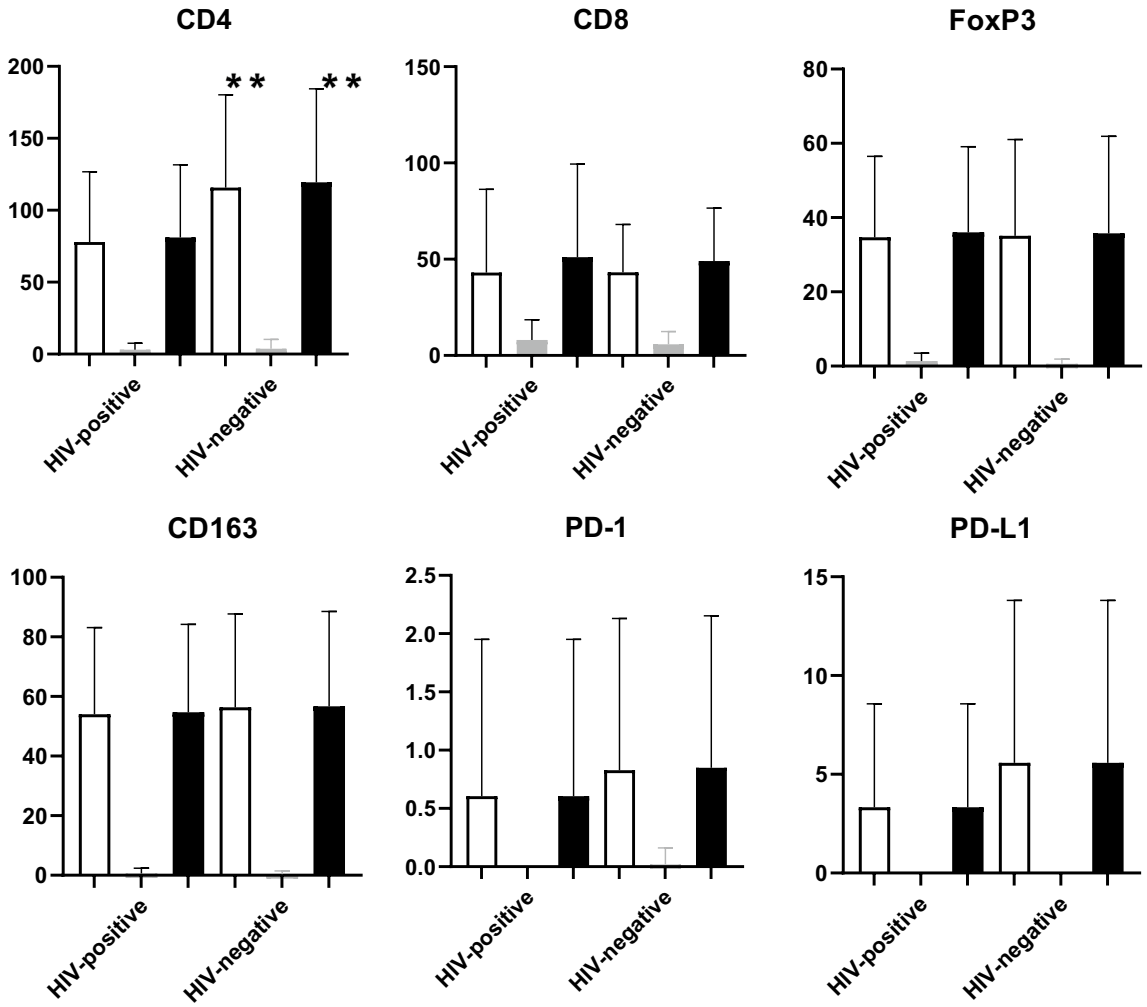

Fig. 2 Comparison of immune cell counts ($CD4^+$, $CD8^+$, and $FoxP3^+$ T cells, $CD163^+$ macrophages, and PD-1- and PD-L1-expressing infiltrating cells) between HIV-positive and HIV-negative subjects (each value represents the mean ± standard deviation. *$p<0.05$, **$p<0.01$). White: stroma, gray: epithelium, black: combined

However, no significant differences in infiltrating $CD8^+$ T cell density were observed between HIV-positive and negative subjects; this is concordant with other reports on other neoplasms in HIV-infected individuals [22, 23]. Our results indicate that decreased tumor infiltration by $CD4^+$ T cells promotes immune surveillance dysfunction, thereby contributing to colon carcinogenesis in HIV-positive subjects. Multiple studies have demonstrated that HIV-positive patients exhibit reduced $CD4^+$ T cell infiltration in tumors such as HIV-DLBCL [24] and cervical intraepithelial neoplasia [23]. However, most of these are AIDS-defining cancers or other infection-related malignancies [22, 23]. To our knowledge, ours is the first report to assess immune cells in precancerous colon lesions of HIV-positive subjects.

We also found a correlation between tumor-infiltrating cell count and nadir (and not current) blood count of $CD4^+$ T cells. This might explain why PLWH develop NADC frequently, despite having good immunity and a high $CD4^+$ T cell count [25]. Indeed, the nadir blood count of $CD4^+$ T cells has been associated with a few NADCs [26], atherosclerosis diseases (e.g., ischemic heart disease [27], and intracranial atherosclerosis [28]), and inflammatory markers in plasma, as in HIV-infected patients on stable ART with high levels of IL-6 [29]. Nadir CD4 might be associated with various complications in the chronic phase. Kobayashi et al. reported no distinct correlation between $CD4^+$ T cell density in HIV-positive cervical neoplasia and the nadir peripheral $CD4^+$ T cell count [23]. This discordance with our result could be because of the organ-specific nature of $CD4^+$ T cells. For example, during an acute HIV infection, the exhaustion of $CD4^+$ T cells occurs earlier in the gut mucosa than in the blood and other lymphatic tissues [30]. Moreover, studies have observed the considerably delayed recovery of intestinal $CD4^+$ T cell levels after ART initiation

Table 5 Mean number of infiltrating immune cells distributed in the colon adenomas

Stroma	HIV positive	HIV negative	*p*-value
$CD4^+$ infiltration	77.97±48.77	115.8±64.44	0.0014**
$CD8^+$ infiltration	43.00±43.31	43.16±24.94	0.3698
$FoxP3^+$ infiltration	34.67±21.85	35.09±25.94	0.5782
$CD163^+$ infiltration	54.06±29.08	56.38±31.31	0.7686
$PD\text{-}1^+$ infiltration	0.60±1.35	0.83±1.30	0.1115
$PD\text{-}L1^+$ infiltration	3.33±5.24	5.59±8.22	0.0662
Epithelium			
$CD4^+$ infiltration	3.12±4.4	3.72±6.60	0.8890
$CD8^+$ infiltration	8.06±10.52	5.848±6.59	0.7003
$FoxP3^+$ infiltration	1.33±2.18	0.66±1.24	0.1706
$CD163^+$ infiltration	0.64±1.73	0.34±1.15	0.1395
$PD\text{-}1^+$ infiltration	0.00±0.00	0.02±0.14	>0.9999
$PD\text{-}L1^+$ infiltration	0.00±0.00	0.00±0.00	>0.9999
Combined			
$CD4^+$ infiltration	81.09±50.58	119.5±64.93	0.0016**
$CD8^+$ infiltration	51.06±48.39	49.01±27.61	0.5138
$FoxP3^+$ infiltration	36.00±23.08	35.75±26.17	0.5154
$CD163^+$ infiltration	54.70±29.54	56.73±31.85	0.8210
$PD\text{-}1^+$ infiltration	0.60±1.35	0.85±1.30	0.0923
$PD\text{-}L1^+$ infiltration	3.33±5.24	5.59±8.22	0.0662

Statistical significance: *$p<0.05$, **$p<0.01$

in HIV-1-infected patients, despite patients having an undetectable plasma load of the virus and an increased peripheral blood $CD4^+$ T cell count [31, 32]. In our study, HIV-positive patients had been on ART for 0–26 years (median 9.4 years), which is a remarkably long period compared to that reported in previous reports on gut $CD4^+$ T cells. However, these patients still exhibited a significant reduction in $CD4^+$ T cells in their adenomas. The exact mechanism of this incomplete recovery is still unknown; nevertheless, insufficient cell recruitment to the gastrointestinal tract [32], susceptibility to HIV and depletion of gut-homing $CD4^+$ T cells [33], exhaustion [34], and insufficient differentiation [35] of $CD4^+$ T cells in gut mucosa, have been postulated to be a cause of the persistent mucosal depletion of $CD4^+$ T cells. Unfortunately, flow cytometry analysis of lymphocytes in peripheral blood and adenomas to determine lymphocyte subtype including gut-homing $CD4^+$ T cells could not be performed in the present study as described later. The reason of prolonged depletion of CD4 cell in colon adenomas could not be further assessed.

Thus, based on our results and previous reports, we speculate that the ART might not help HIV-positive patients to recover completely once colon mucosal $CD4^+$ cell numbers are lower, despite long-term treatment. Length of time on ART had no correlation with gastrointestinal $CD4^+$ T cells, and continuous depletion or irreparable damage of these cells were suggested [36]. Therefore, the ART should be provided during the earlier phases of HIV infection, such as before the reduction in gut-homing $CD4^+$ T cells, as this approach can potentially restore the gut mucosal immune system [37]. Furthermore, providing the ART with high $CD4^+$ T cell counts has been suggested to be useful in decreasing NADC risk [38].

Regulatory T cells (Tregs) and M2-like macrophages, which we assessed by FoxP3 and CD163 IHC, are representative inflammatory cells that are immunosuppressive [39, 40]. In fact, Tregs have been demonstrated to be increased in colon adenomas [17]. The relative frequency of Tregs increases as the disease progresses in HIV-infected subjects. It has also been shown that $CD4^+$ T cell depletion during HIV/SIV infection partially spares Tregs, wherein the latter are partially restored following the onset of ART [41]. Premalignant adenomas have an increased number of macrophages that comprise a mixed population of M1/M2 phenotypes [15, 41]. Although the role of polarized macrophages is largely uncertain in HIV, a shift towards M2 phenotypes is prevalent in the later stages of disease progression [42]. Owing to these observations, it can be deduced that these regulatory immune cells play distinct roles in colon adenomas of HIV-positive individuals. However, our results indicated no significant differences in $FoxP3^+$ or $CD163^+$ cell infiltration, suggesting that these cells might not have differing roles in HIV-positive and HIV-negative subjects. This might be because HIV has fewer adverse effects on Tregs and M2-like macrophages than it does on $CD4^+$ T cells. In fact, relative Treg resistance to HIV/SIV-mediated killing was reported [43]. Resistance of macrophages to cell death mediated by HIV infection was also shown [44]. Furthermore, the prevalence of HIV-infected macrophages in gastrointestinal mucosa is very low [45].

Increased expression of PD-1/PD-L1 is one of the most important mechanisms of immune escape utilized by tumors [18]. For example, one study reported PD-1/PD-L1 expression in intraepithelial lymphocytes and increased PD-L1 expression in epithelial cells of colon sessile serrated adenomas [46]. Whereas PD-1/PD-L1 expression was not found in epithelial cells of colon adenomas in our study, it was observed in a small number of the infiltrates. No significant differences were revealed in the number of PD-1-positive infiltrates or PD-L1-expressing infiltrates in adenomas between HIV-positive and HIV-negative patients. Most of these cells had lymphocyte and macrophage and/or dendritic cell morphologies, respectively. Notably, HIV-specific T cells showed increased PD-1 receptor expression. This decreased following ART initiation but not to pre-infection levels [47]. Furthermore, PD-L1 expression has been detected in HIV-infected lymph node cells, which show a myeloid/macrophage morphology [48]. Although these reports indicate that PD-1/PD-L1 has key roles in the

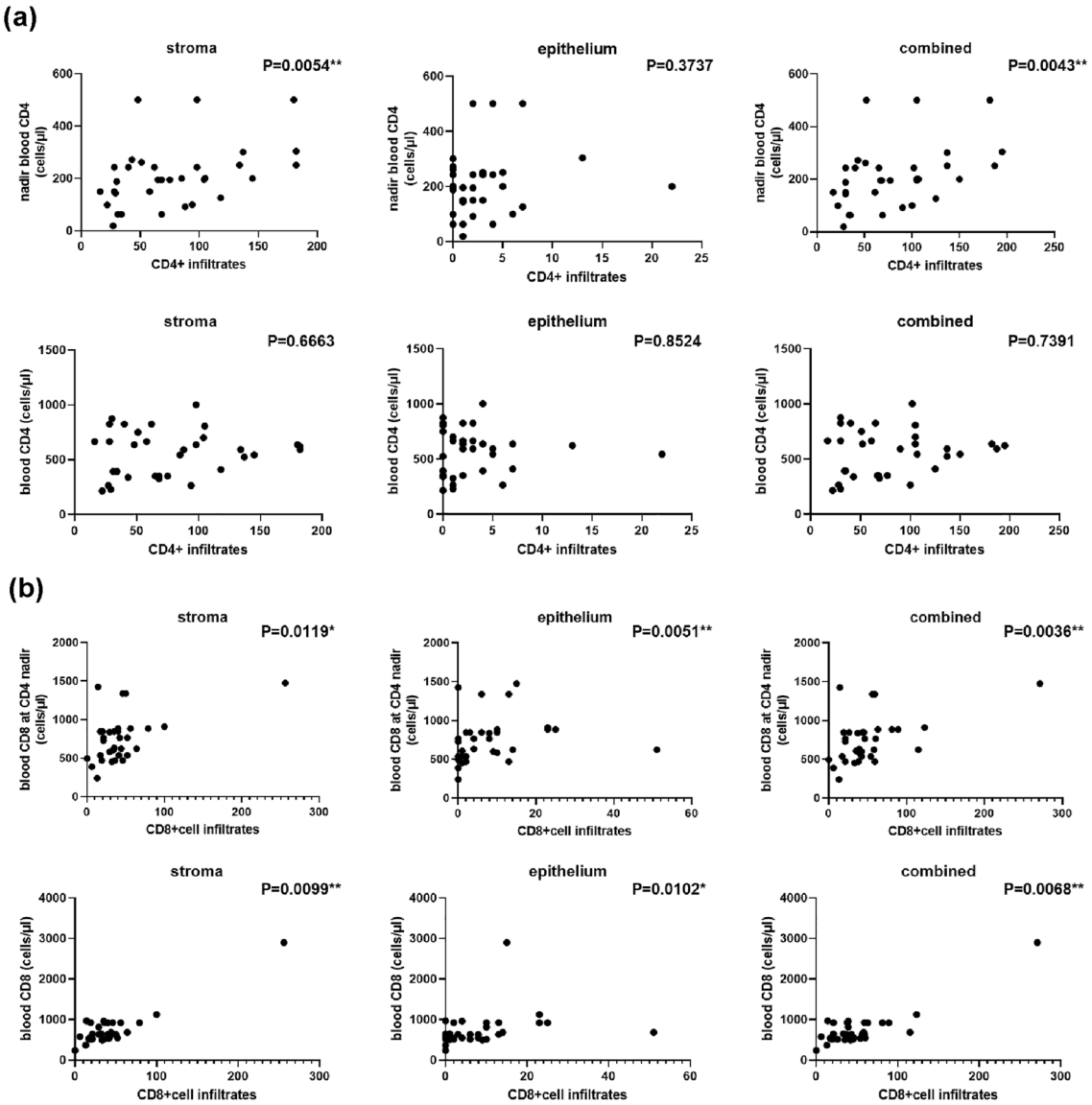

Fig. 3 **a** Correlation between **a** nadir and **b** current blood counts of $CD4^+$ T cells and number of infiltrating $CD4^+$ T cells in HIV-positive adenomas. **b** Correlation between **c** nadir and **d** current blood counts of $CD8^+$ T cells and number of infiltrating $CD8^+$ T cells in adenomas of HIV-positive patients.*$p<0.05$, **$p<0.01$, ***$p<0.001$

immune cells of HIV-positive subjects, our results indicate that the PD-1/PD-L1 interaction is insignificant in colon adenomas, regardless of patient HIV infection status.

Whereas we considered the common risk factors of colon adenomas, such as obesity, smoking, and alcohol intake, the retrospective study design and relatively low number of HIV-positive (and exclusively male) subjects are the limitations of this study. Additionally, $CD4^+$ T cells have different immune functions in anti-tumor responses. Indeed, infiltrating T cells do not always elicit immune responses without activating co-stimulatory signals [49]. However, we did not evaluate the functional role of the infiltrates as our study comprised a cross-sectional nature and was dependent on a histological analysis. Flow cytometric analysis was not applicable because the polyp samples were embedded in paraffin, and blood samples were not usable because of retrospective study design. Another limitation is that there is no standardized method to evaluate the local immune environment of colorectal adenomas. We analyzed immune cells in the top region of the adenomas because most adenomas tend to have more dysplasia in that region. Moreover, cell dysplasia is commonly located at the surface area of crypts [50].

In summary, our study is the first of its kind to demonstrate decreased $CD4^+$ T cell infiltration in colon adenomas of HIV-positive subjects. We also determined its

correlation with the nadir blood count of $CD4^+$ T cells. Cancer screening is critical, as PLWH frequently develop NADC despite having good antiviral immunity with high $CD4^+$ T cell numbers and undetectable viremia [25]. Thus, the nadir blood count of $CD4^+$ T cells could be a possible guide for efficient follow-up and treatment strategies for colon adenomas in HIV-positive patients. Nonetheless, further investigations are required to assess the functional roles of the immune microenvironment in colon carcinogenesis and determine an efficient treatment for colon adenomas in HIV-positive subjects.

Acknowledgements We would like to thank Tomoe Senkoji for managing the clinical data.

Author contributions All authors contributed to the study's conception and design. Data collection and analysis were performed by YM. Histological sample preparation and analysis were performed by YO, YT, TD, and YM. The first draft of the manuscript was written by YM, and all authors provided their input on previous versions of the manuscript. All authors read and approved the final manuscript.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

1. Hernández-Ramírez RU, Shiels MS, Dubrow R et al (2017) Cancer risk in HIV-infected people in the USA from 1996 to 2012: a population-based, registry-linkage study. Lancet HIV 4:e495–e504. https://doi.org/10.1016/S2352-3018(17)30125-X
2. Chiao EY, Coghill A, Kizub D et al (2021) The effect of non-AIDS-defining cancers on people living with HIV. Lancet Oncol 22:e240–e253. https://doi.org/10.1016/S1470-2045(21)00137-6
3. Dubrow R, Silverberg MJ, Park LS et al (2012) HIV infection, aging, and immune function: implications for cancer risk and prevention. Curr Opin Oncol 24:506–516. https://doi.org/10.1097/CCO.0b013e328355e131
4. da Silva Neto MM, Brites C, Borges ÁH (2020) Cancer during HIV infection. APMIS 128:121–128. https://doi.org/10.1111/apm.13020
5. Shiels MS, Engels EA (2017) Evolving epidemiology of HIV-associated malignancies. Curr Opin HIV AIDS 12:6–11. https://doi.org/10.1097/COH.0000000000000327
6. Coghill AE, Shiels MS, Suneja G et al (2015) Elevated cancer-specific mortality among HIV-infected patients in the United States. J Clin Oncol 33:2376–2383. https://doi.org/10.1200/JCO.2014.59.5967
7. Araghi M, Soerjomataram I, Jenkins M et al (2019) Global trends in colorectal cancer mortality: projections to the year 2035. Int J Cancer 144:2992–3000. https://doi.org/10.1002/ijc.32055
8. Jensen BE, Oette M, Haes J et al (2017) HIV-associated gastrointestinal cancer. Oncol Res Treat 40:115–118
9. Nagata N, Nishijima T, Niikura R et al (2018) Increased risk of non-AIDS-defining cancers in Asian HIV-infected patients: a long-term cohort study. BMC Cancer 18:1066. https://doi.org/10.1186/s12885-018-4963-8
10. Berretta M, Cappellani A, Di Benedetto F et al (2009) Clinical presentation and outcome of colorectal cancer in HIV-positive patients: a clinical case–control study. Onkologie 32:319–324. https://doi.org/10.1159/000215719
11. Gutkin E, Hussain SA, Mehta P et al (2012) Prevalence of adenomas found on colonoscopy in patients with HIV. Gastroenterol Res 5:52–56. https://doi.org/10.4021/gr433w
12. Fearon ER, Vogelstein B (1990) A genetic model for colorectal tumorigenesis. Cell 61:759–767. https://doi.org/10.1016/0092-8674(90)90186-I
13. Cross W, Kovac M, Mustonen V et al (2018) The evolutionary landscape of colorectal tumorigenesis. Nat Ecol Evol 2:1661–1672. https://doi.org/10.1038/s41559-018-0642-z
14. Peddareddigari VG, Wang D, Dubois RN (2010) The tumor microenvironment in colorectal carcinogenesis. Cancer Microenviron 3:149–166. https://doi.org/10.1007/s12307-010-0038-3
15. McLean MH, Murray GI, Stewart KN et al (2011) The inflammatory microenvironment in colorectal neoplasia. PLOS ONE 6:e15366. https://doi.org/10.1371/journal.pone.0015366
16. Mansouri D, McSorley ST, Park JH et al (2021) The inflammatory microenvironment in screen-detected premaligant adenomatous polyps: early results from the integrated technologies for improved polyp surveillance (INCISE) project. Eur J Gastroenterol Hepatol 33:983–989. https://doi.org/10.1097/MEG.0000000000002202
17. Cui G, Shi Y, Cui J et al (2012) Immune microenvironmental shift along human colorectal adenoma-carcinoma sequence: is it relevant to tumor development, biomarkers and biotherapeutic targets? Scand J Gastroenterol 47:367–377. https://doi.org/10.3109/00365521.2011.648950
18. Chang E, Pelosof L, Lemery S et al (2021) Systematic review of PD-1/PD-L1 inhibitors in oncology: from personalized medicine to public health. Oncologist 26:e1786–e1799. https://doi.org/10.1002/onco.13887
19. Sawicki T, Ruszkowska M, Danielewicz A et al (2021) A review of colorectal cancer in terms of epidemiology, risk factors, development, symptoms and diagnosis. Cancers 13:2025. https://doi.org/10.3390/cancers13092025
20. Dunn GP, Bruce AT, Ikeda H et al (2002) Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 3:991–998. https://doi.org/10.1038/ni1102-991
21. Bini EJ, Green B, Poles MA (2009) Screening colonoscopy for the detection of neoplastic lesions in asymptomatic HIV-infected subjects. Gut 58:1129–1134. https://doi.org/10.1136/gut.2008.165985
22. Dandachi D, Morón F (2020) Effects of HIV on the tumor microenvironment. Adv Exp Med Biol 1263:45–54. https://doi.org/10.1007/978-3-030-44518-8_4
23. Kobayashi A, Greenblatt RM, Anastos K et al (2004) Functional attributes of mucosal immunity in cervical intraepithelial neoplasia and effects of HIV infection. Cancer Res 64:6766–6774. https://doi.org/10.1158/0008-5472.CAN-04-1091
24. Taylor JG, Liapis K, Gribben JG (2015) The role of the tumor microenvironment in HIV-associated lymphomas. Biomark Med 9:473–482. https://doi.org/10.2217/bmm.15.13
25. Ceccarelli M, Venanzi Rullo E, Marino MA et al (2020) Non-AIDS defining cancers: a comprehensive update on diagnosis and management. Eur Rev Med Pharmacol Sci 24:3849–3875. https://doi.org/10.26355/eurrev_202004_20852
26. Powles T, Robinson D, Stebbing J et al (2009) Highly active antiretroviral therapy and the incidence of non-AIDS-defining cancers in people with HIV infection. J Clin Oncol 27:884–890. https://doi.org/10.1200/JCO.2008.19.6626
27. Silverberg MJ, Leyden W, Quesenberry CP Jr et al (2009) Race/ethnicity and risk of AIDS and death among HIV-infected patients with access to care. J Gen Intern Med 24:1065–1072. https://doi.org/10.1007/s11606-009-1049-y

28. Spagnolo-Allende A, Gutierrez J (2021) Role of brain arterial remodeling in HIV-associated cerebrovascular outcomes. Front Neurol 12:593605. https://doi.org/10.3389/fneur.2021.593605
29. Borges ÁH, O'Connor JL, Phillips AN et al (2015) Factors associated with plasma IL-6 levels during HIV infection. J Infect Dis 212:585–595. https://doi.org/10.1093/infdis/jiv123
30. Wang Y, Lu X, Wu H et al (2018) Gut-homing $\alpha 4\beta 7$ CD4+ T cells: potential key players in both acute HIV infection and HIV-associated cancers. Cell Mol Immunol 15:190–192. https://doi.org/10.1038/cmi.2017.104
31. Briceño O, Pinto-Cardoso S, Rodríguez-Bernabe N et al (2016) Gut homing CD4+ and CD8+ T-cell frequencies in HIV infected individuals on antiretroviral treatment. PLOS ONE 11:e0166496. https://doi.org/10.1371/journal.pone.0166496
32. Mehandru S, Poles MA, Tenner-Racz K et al (2006) Lack of mucosal immune reconstitution during prolonged treatment of acute and early HIV-1 infection. PLOS Med 3:e484. https://doi.org/10.1371/journal.pmed.0030484
33. Arthos J, Cicala C, Martinelli E et al (2008) HIV-1 envelope protein binds to and signals through integrin alpha4beta7, the gut mucosal homing receptor for peripheral T cells. Nat Immunol 9:301–309. https://doi.org/10.1038/ni1566
34. Rueda CM, Velilla PA, Chougnet CA et al (2012) HIV-induced T-cell activation/exhaustion in rectal mucosa is controlled only partially by antiretroviral treatment. PLOS ONE 7:e30307. https://doi.org/10.1371/journal.pone.0030307
35. Yukl SA, Shergill AK, Girling V et al (2015) Site-specific differences in T cell frequencies and phenotypes in the blood and gut of HIV-uninfected and ART-treated HIV+ adults. PLOS ONE 10:e0121290. https://doi.org/10.1371/journal.pone.0121290
36. Asowata OE, Singh A, Ngoepe A et al (2021) Irreversible depletion of intestinal CD4+ T cells is associated with T cell activation during chronic HIV infection. JCI Insight 6:e146162. https://doi.org/10.1172/jci.insight.146162
37. Guadalupe M, Reay E, Sankaran S et al (2003) Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. J Virol 77:11708–11717. https://doi.org/10.1128/jvi.77.21.11708-11717.2003
38. Borges AH, Dubrow R, Silverberg MJ (2014) Factors contributing to risk for cancer among HIV-infected individuals, and evidence that earlier combination antiretroviral therapy will alter this risk. Curr Opin HIV AIDS 9:34–40. https://doi.org/10.1097/COH.0000000000000025
39. Whiteside TL (2014) Regulatory T cell subsets in human cancer: are they regulating for or against tumor progression? Cancer Immunol Immunother 63:67–72. https://doi.org/10.1007/s00262-013-1490-y
40. Mantovani A, Sica A (2010) Macrophages, innate immunity and cancer: balance, tolerance, and diversity. Curr Opin Immunol 22:231–237. https://doi.org/10.1016/j.coi.2010.01.009
41. Shi Y, Li Z, Zheng W et al (2015) Changes of immunocytic phenotypes and functions from human colorectal adenomatous stage to cancerous stage: update. Immunobiology 220:1186–1196. https://doi.org/10.1016/j.imbio.2015.06.003
42. Herbein G, Varin A (2010) The macrophage in HIV-1 infection: from activation to deactivation? Retrovirology 7:33. https://doi.org/10.1186/1742-4690-7-33
43. Kleinman AJ, Sivanandham R, Pandrea I et al (2018) Regulatory T cells as potential targets for HIV cure research. Front Immunol 9:734. https://doi.org/10.3389/fimmu.2018.00734
44. Kruize Z, Kootstra NA (2019) The role of macrophages in HIV-1 persistence and pathogenesis. Front Microbiol 10:2828. https://doi.org/10.3389/fmicb.2019.02828
45. Smith PD, Meng G, Salazar-Gonzalez JF et al (2003) Macrophage HIV-1 infection and the gastrointestinal tract reservoir. J Leukoc Biol 74:642–649. https://doi.org/10.1189/jlb.0503219
46. Acosta-Gonzalez G, Ouseph M, Lombardo K et al (2019) Immune environment in serrated lesions of the colon: intraepithelial lymphocyte density, PD-1, and PD-L1 expression correlate with serrated neoplasia pathway progression. Hum Pathol 83:115–123. https://doi.org/10.1016/j.humpath.2018.08.020
47. Fenwick C, Joo V, Jacquier P et al (2019) T-cell exhaustion in HIV infection. Immunol Rev 292:149–163. https://doi.org/10.1111/imr.12823
48. Gill AL, Green SA, Abdullah S et al (2016) Programed death-1/programed death-ligand 1 expression in lymph nodes of HIV infected patients: results of a pilot safety study in rhesus macaques using anti-programed death-ligand 1 (avelumab). AIDS 30:2487–2493. https://doi.org/10.1097/QAD.0000000000001217
49. Scarpa M, Bortolami M, Cecchetto A et al (2011) Mucosal immune environment in colonic carcinogenesis: CD80 up-regulation in colonic dysplasia in ulcerative colitis. Eur J Cancer 47:611–619. https://doi.org/10.1016/j.ejca.2010.10.010
50. Shih IM, Wang TL, Traverso G et al (2001) Top-down morphogenesis of colorectal tumors. Proc Natl Acad Sci 98:2640–2645. https://doi.org/10.1073/pnas.051629398