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Cytomegalovirus infection among patients with cancer receiving immune checkpoint inhibitors

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Abstract

Background Immune checkpoint inhibitors (ICIs), used for the treatment of solid and hematologic malignancies, come with the risk of immune-related adverse events (irAEs). Opportunistic infections (e.g., cytomegalovirus [CMV]) mimic irAE symptoms and are understudied in this population. We aimed to describe the incidence, characteristics, treatment and outcomes of CMV infection in ICI-treated patients.

Methods We conducted a single-center retrospective review of all adult patients who were CMV-positive after ICI therapy between 06/2011 and 05/2020. A CMV-positive non-ICI cohort was matched to the ICI group based on age, sex and cancer type. Variables of interest were collected through electronic medical records.

Results The study population comprised 192 patients overall. CMV infection incidence was 7.7% in ICI patients and 12.9% in non-ICI patients (P<0.001). Rates of infection clearance (83% vs. 50%, P=0.002) and recurrence (20% vs. 3%, P=0.037) were higher in ICI patients with hematologic vs. solid tumors, despite similar treatments. All-cause mortality was higher in solid rather than hematologic malignancies in ICI patients (83% vs. 54%, P=0.009); CMV-related mortality was low (3-4%) in both groups.

Conclusions CMV infection occurred in about 7.7% of the ICI-treated cancer population. The infection can be disseminated in multiple organs and has a wide spectrum of clinical symptoms. ICI-treated patients with a hematologic malignancy had higher viral clearance and recurrence than those with solid tumors. In this study, CMV itself did not lead to high mortality in cancer patients. Further study is needed to investigate the role of CMV infection in patients' irAEs and cancer outcome.

Keywords Cytomegalovirus, immune checkpoint inhibitor, immune-related adverse events, cancer

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Introduction

Immune checkpoint inhibitors (ICIs) are increasingly used to treat a variety of malignancies. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed cell death protein (PD-1) are expressed on the surface of T-cells, whereas the programmed cell death protein ligand (PD-L1) and protein B7 are expressed on tumor cells. Binding of CTLA-4 to B7 and PD-1 to PD-L1 prevents T-cells from killing tumor cells in the body [1]. Current approved immunotherapy agents block CTLA-4, PD-1 and PD-L1, leading to upregulation of the immune system. This may result in immune-related toxicities that can affect a variety of organs and are referred to as immune-related adverse events (irAEs).

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Conflict of Interest: None

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These irAEs can affect various organs, including the gastrointestinal (GI) tract, liver, skin, lungs and endocrine glands. CTLA-4 inhibitor-mediated toxicities tend to be more severe than PD-1 or PD-L1-mediated toxicities, probably because PD-1 signaling acts more peripherally than CTLA-4 signaling [2]. As one of the most common irAEs, GI toxicities (in the form of diarrhea and colitis) usually occur 6-7 weeks after initiation of ICI therapy and are more commonly associated with anti-CTLA-4 treatment [3,4]. Treatment for GI irAEs involves symptomatic management, but more moderate and severe symptoms require administration of corticosteroid and selective immunosuppressive therapy [5-8]. The diagnosis of GI irAEs requires the exclusion of infectious etiologies, such as cytomegalovirus (CMV).

CMV is a common herpes virus that can be cultured from multiple sites throughout the body and typically causes asymptomatic latent infections. Reactivation of a latent infection can occur during periods of immunosuppression, such as during treatment with corticosteroids, and may lead to a spectrum of diseases. The clinical presentation of CMV colitis and immune-related colitis may overlap with similar symptoms of diarrhea, hematochezia, abdominal pain, and ulcerative inflammation of the colon [9]. This underscores the importance of screening for CMV as part of the workup for GI irAEs.

Although the incidence, management and outcomes of irAEs have been well recognized and studied, data related to CMV infections among cancer patients receiving ICIs appear to be limited [5-8,10,11]. Several case reports have described CMV infections in patients with corticosteroid-refractory ICI-related colitis that improve with CMV treatment [13-17]. In a retrospective study by Franklin *et al* [18], of 370 patients treated with ICIs, 41 developed colitis. In 5 of these patients, colitis was refractory to corticosteroids and infliximab, and CMV was detected in all 5 patients (1 patient by stool alone and 4 by colonic biopsies). Those with positive colon biopsies were treated with ganciclovir and had resolution of their diarrhea [18]. In this retrospective study, we aimed to study the incidence of CMV infection among ICI-treated cancer patients and compare the clinical findings, risk factors leading to complications, treatments, and outcomes of CMV infection between ICI-treated vs. non-ICI-treated groups.

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Patients and methods

Patient population

This retrospective study was approved by the Institutional Review Board at The University of Texas MD Anderson Cancer Center. All adult cancer patients who received ICI therapy and tested positive for CMV infection between June 2011 and May 2020 were included. The ICI group and non-ICI control cohort were matched by age, sex, and cancer type. Patients with CMV infection before ICI use, or a cancer diagnosis but no recurrent infection afterward, were excluded. Cases with concurrent non-CMV infections were also excluded. Positive CMV infection was defined as CMV viremia, positive CMV polymerase chain reaction (PCR) in body fluids, including stool and bronchoalveolar lavage, or positive immunohistochemical staining on biopsy. Positive CMV serology alone was not considered as a sign of active CMV infection in our study.

Clinical characteristics

Patient-related demographic, clinical, and oncologic variables collected included age at the time of CMV diagnosis, sex, race/ethnicity, Charlson Comorbidity Index, cancer type and stage, as well as other reported irAEs.

CMV-related characteristics

Data pertaining to CMV infection analyzed included presenting symptoms, site of infection, time from ICI initiation to the onset of CMV infection, history of previous CMV infection, preceding antibiotic and immunosuppressant use, and peak CMV viral load. Data regarding treatment type (i.e., intravenous vs. oral antiviral) and duration were collected, as were data regarding stem cell transplant status for the patients with hematologic malignancy. Outcomes related to CMV infection among ICI vs. non-ICI treated groups were also recorded, including hospitalization, length of hospital stay, need for Intensive Care Unit (ICU) admission, clearance of infection, and recurrence. For both groups, the follow-up duration was defined as the time from the CMV diagnosis to the date of last clinical encounter or death.

Statistical analysis

Continuous variables were represented as means and standard deviations, or medians and interquartile ranges. Categorical variables were represented as frequencies and percentages. The Mann-Whitney *U* test was used to compare continuous variables. Fisher exact and χ^2 tests were used to compare categorical variables. Kaplan-Meier curves and log-rank tests were used to estimate and compare overall survival durations between subgroups, with CMV diagnosis as the starting point. All statistical tests were 2-sided. P-values of less than 0.05 were

considered statistically significant. Statistical analyses were performed using SPSS (version 24.0; IBM) software.

Results

Patient characteristics

Of the 9610 patients tested for CMV in the study period, 1059 received ICI and 8551 did not. Of the 1059 treated with ICI, 82 tested positive for CMV infection, and of those who did not receive ICI, 1107 tested positive (Supplementary Fig. 1). The estimated incidences of CMV among ICI patients and non-ICI patients were found to be 7.7% and 12.9%, respectively (P<0.001) (95% confidence interval 3.3-6.8%). The baseline clinical characteristics of the patient population were similar in both groups (Supplementary Table 1).

ICI group

The mean age of the 82 patients in the ICI group was 58.9 years; 65% were male and 61% were white. The median Charlson Comorbidity index was 6. A total of 45% of patients had solid tumors and 55% had a hematologic malignancy; 39 patients (87%) received stem cell transplants, the majority of whom also received ICI because of disease relapse after stem cell transplantation.

Non-ICI group

The mean age of the 110 patients in the non-ICI group was 57.9 years; 61% were male and 62% were white. The median Charlson Comorbidity index was 6. In this group, 41% of patients had solid tumors and 59% had a hematologic malignancy. The patient characteristics did not differ significantly between the ICI and non-ICI groups.

At the time of CMV diagnosis, the majority of patients in the ICI group (95%) had stage 3-4 disease, 65% had ECOG performance status of 0-2, almost half (48%) had cancer progression, and 33% of those who had CMV also had an irAE. The most common irAEs were pneumonitis (4/19, 21%), or endocrine in nature, including thyroiditis and hypophysitis (3/19, 16%). However, 32% of patients had multiple irAEs. The most common cause of death was cancer progression and cancer-related complications, with CMV-related mortality estimated to be only 4% of ICI-treated patients. The all-cause mortality rates were similar between the ICI-treated cohort (62%) and the non-ICI-treated cohort (67%) (P=0.541) (Table 1, Fig. 1). The median follow-up duration was about 4 months.

Comparison of CMV characteristics between ICI- and non-ICI-treated patients

The clinical characteristics of CMV infection were compared between the 2 groups (Table 1). A notable difference was found in terms of prior history of CMV, which was more prevalent in the ICI group (31% vs. 13%, P=0.004). The rest of the variables were similar between groups, including site distribution of CMV infection, CMV-related clinical presentation, peak viral load, nadir white blood cell (WBC) level, prior history of CMV and potential risk factors for CMV infection, such as hospitalization, ICU admission, sepsis, immunosuppressant use, and graft-versus-host disease (GVHD) prior to the onset of infection.

In ICI-treated patients, CMV location was mainly in the blood alone (51%), or in the blood and another location (37%). Only 9 patients (12%) had CMV in a location other than blood. The most common locations wherein CMV was found outside the blood were the lungs (38%) and GI tract (10%). The majority of patients presented with either constitutional symptoms (27%), multiple symptoms (27%), or respiratory symptoms alone (23%) (Table 1). Those who presented with multiple symptoms usually presented with a constitutional symptom and an organ-specific symptom. The median peak CMV viral load was 1329 IU/mL, and the nadir WBC level was 2 K/μL. For most patients, diagnoses were made with use of PCR (70%), or multiple modalities including PCR, immunohistochemical staining, culture or serology. A total of 34 patients (41%) were hospitalized prior to their CMV diagnosis, a possible trigger for CMV infection. Other possible triggers for CMV infection included ICU admission (9, 11%), mechanical ventilation (4, 5%), and severe sepsis (2, 3%); in addition, 26 patients (32%) had multiple triggering factors.

Comparison of CMV characteristics between patients with solid tumor and hematologic malignancy

The characteristics of CMV infection were compared between patients with solid tumors and those with hematologic malignancy (Table 2). In ICI-treated patients, the CMV location was similar between the 2 groups. The clinical presentations of CMV observed more frequently in solid tumor patients were multiple symptoms (47%) and respiratory symptoms (36%), whereas hematologic malignancy patients more frequently reported constitutional symptoms (41%) and multiple symptoms (30%) (P=0.002).

The peak viral load was similar between patients with solid tumors and those with hematologic malignancy, but the nadir WBC level was lower in the hematologic malignancy group (0.6 vs. 4 K/μL, P<0.001). The hematologic malignancy group also had a higher rate of prior CMV infection based on serology or prior positive CMV testing (41% vs. 17%, P=0.029). In both groups, most patients were treated with intravenous (IV) antivirals (58% in the solid tumor group and 78% in the hematologic malignancy group) over a similar duration of treatment (P=0.390). The rates of infection clearance (50% vs. 83%, P=0.002) and recurrence (3% vs. 20%, P=0.037) were significantly higher in the hematologic malignancy group; however, the all-cause mortality was higher in the solid tumor group (83% vs. 54%, P=0.009). The Kaplan-Meier analysis showed significantly better survival in the hematologic malignancy group (P<0.001, Fig. 1).

Table 1 Comparison of CMV characteristics between patients who received ICI and those who did not, N=192

Characteristic or outcome	ICI group, n=82	Non-ICI group, n=110	P-value[a]
CMV location, n (%)			0.058
Blood alone	43 (51)	67 (61)	
Blood and other location	30 (37)	40 (36)	
Other location only	9 (12)	3 (3)	
CMV location outside of blood,[b] n (%)			
GI tract	8 (10)	6 (5)	n/a
Respiratory tract	31 (38)	35 (32)	
Eye/retina	2 (2)	3 (3)	
Bone marrow	2 (2)	4 (4)	
Other[c]	0	1 (1)	
CMV-related clinical presentation, n (%)			0.106
None (found on surveillance)	10 (12)	22 (20)	
Constitutional	22 (27)	43 (39)	
Respiratory	19 (23)	17 (15)	
Gastrointestinal	9 (11)	8 (7)	
Other/multiple[c,d]	22 (27)	20 (18)	
Peak CMV viral load in blood (IU/mL), median (IQR), n=179	1329 (433-2875)	1069 (435-4379)	0.865
Nadir WBC level (K/μL),[e] median (IQR), n=189	2 (0.2-4.05)	0.9 (0.1-4)	0.121
Testing confirming diagnosis, n (%)			n/a
PCR[f]	57 (70)	82 (75)	
Immunohistochemical analysis[g]	5 (6)	0	
Multiple	20 (24)	28 (25)	
Prior history of CMV,[h] n (%)	25 (31)	14 (13)	0.004
Risk factors for CMV reactivation/disease, n (%)			n/a
None	9 (11)	10 (9)	
Hospitalization	34 (41)	61 (55)	
ICU admission	7 (9)	3 (3)	
Mechanical ventilation	3 (4)	0	
Severe sepsis	2 (2)	3 (3)	
Others[i]	1 (1)	2 (2)	
Multiple factors	26 (32)	31 (28)	
Immunosuppressant use,[j] n (%)	73 (89)	92 (84)	0.402
HSCT,[k] n (%)	39 (87)	45 (69)	0.041
Concomitant GVHD,[k] n (%)	21 (26)	24 (22)	0.606
Hospitalization for CMV, n (%)	19 (23)	14 (13)	0.081
Median days of hospitalization, (IQR) , n=33	12 (8-21)	14 (8-26)	0.704
ICU for CMV, n (%)	6 (7)	8 (7)	>0.99
Median days of ICU stay, (IQR), n=14	13 (4-26)	17 (11-25)	0.562
Treatment for CMV, n (%)			0.800
IV antiviral alone or in combination with PO	57 (70)	79 (72)	
PO antiviral	11 (13)	16 (15)	
Not treated	14 (17)	15 (14)	
Duration of treatment in days, median (IQR), n=163	17 (14-35)	21 (14-28)	0.865
Infection clearance, n (%)			>0.99
Cleared	56 (68)	76 (69)	
Not cleared or not tested for clearance	26 (32)	34 (31)	
Recurrence of CMV after clearance	10 (12)	16 (15)	0.676
Treatment of recurrent CMV infection	n=10	n=16	0.914
IV antiviral alone or in combination with PO	5 (50)	11 (69)	
PO antiviral	3 (30)	3 (19)	
Not treated	2 (20)	2 (13)	

(Contd...)

Table 1 (*Continued*)

Characteristic or outcome	ICI group, n=82	Non-ICI group, n=110	P-value[a]
Clearance of recurrent CMV infection	n=10	n=16	>0.99
Cleared	7 (70)	10 (63)	
Not cleared or not tested for clearance	3 (30)	6 (38)	
All-cause mortality, n (%)	51 (62)	74 (67)	0.541
Death due to CMV	2 (4)	5 (7)	0.700

[a]P-values of <0.05 were considered statistically significant. [b]32 patients in the ICI group and 28 patients in the non-ICI group had multiple locations of involvement. [c]Other location was the heart. [d]Other symptoms included hematologic, neurologic, and eye symptoms. [d]Those who had multiple presenting symptoms usually had a constitutional symptom such as fever or fatigue as well as an organ-specific symptom. [e]In the month before CMV infection. [f]Samples included blood, bronchoalveolar lavage, tracheal aspirate, pericardial fluid, and stool. [g]Immunohistochemical analysis was done on gastrointestinal biopsies and bronchoalveolar lavage fluid. [h]Based on serology (IgG) or prior history of positive test. [i]Other triggers included surgery and stem cell transplantation. [j]Immunosuppressant use within 3 months before CMV diagnosis; immunosuppressants included corticosteroids, chemotherapy regimens, and tacrolimus. [k]Refers only to patients with hematologic malignancies

CMV, cytomegalovirus; IQR, interquartile range; WBC, white blood cell; ICI, immune checkpoint inhibitor; ICU, intensive care unit; GVHD, graft-versus-host disease; IV, intravenous; PCR, polymerase chain reaction; PO, oral; HSCT, hematopoietic stem-cell transplant

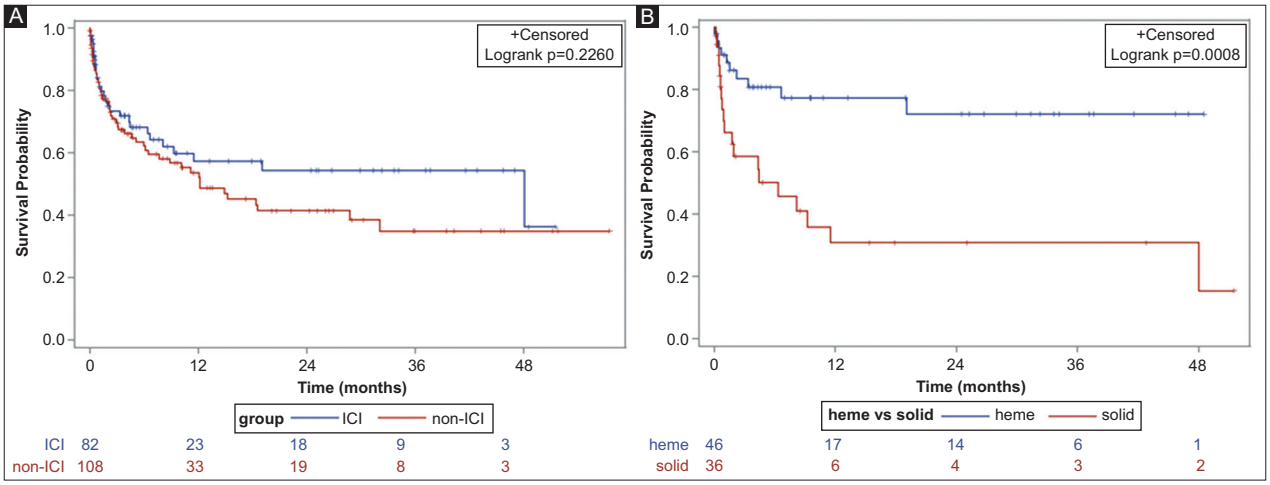

Figure 1 Kaplan-Meier survival curve for overall survival. (A) Comparison of ICI-treated patients and non-ICI-treated patients; (B) comparison of patients with hematologic and solid malignancy within the ICI-treated group. CMV diagnosis was the starting point for the calculation
ICI, immune checkpoint inhibitor; CMV, cytomegalovirus

When CMV characteristics were compared between solid tumor patients and hematologic malignancy patients among the entire cohort and in non-ICI patients, similar results were found (Supplementary Tables 2,3). Similarly, the nadir WBC level was significantly lower and the rates of infection clearance and recurrence were higher in the hematologic malignancy group.

Comparison of treated and untreated patients

Of those who received ICI, 68 were treated with antivirals and 14 were not (Table 3). Both groups had similar peak CMV viral loads and similar rates of immunosuppressant use, GVHD, irAE and mortality. However, the group treated with antivirals had a significantly lower nadir WBC level (1.8 vs. 4.8 K/μL, P=0.006) and higher rates of infection clearance (75% vs. 36%, P=0.009) compared with the untreated group. The recurrence rates were similar between the 2 groups.

CMV characteristics in other groups

Within the ICI-treated patients, comparison of CMV characteristics between patients who received immunosuppression before developing CMV infection and those who did not, showed no significant differences. No differences were seen when comparing patients hospitalized for CMV infection with those who were not. Also, no differences were seen when comparing patients that developed irAEs to those who did not (Table 4).

The specific characteristics of CMV in patients with confirmed GI CMV infection are shown in Supplementary Table 4. Of 8 patients with GI CMV, 4 had irAEs (3 had GI irAEs and 1 had a pulmonary irAE). All of these patients had CMV infection within 30 days after irAE diagnosis, and the infection developed after the initiation of immunosuppressive treatment for the irAE. One death was attributed to CMV infection. This patient had concurrent pulmonary CMV infection and pulmonary irAE. The pathology in GI CMV showed typical "owl's eyes" nuclei on

Table 2 Comparison of CMV characteristics in ICI-treated patients between those with solid tumors and those with hematologic malignancy, N=82

Characteristic or outcome	Solid tumor, n=36	Hematologic malignancy, n=46	P-value[a]
CMV location, n (%)			0.045
Blood alone	14 (39)	29 (63)	
End organ involvementb	22 (62)	17 (37)	
CMV-related clinical presentation			0.002
None (found on surveillance)	3 (8)	7 (15)	
Constitutional	3 (8)	19 (41)	
Respiratory	13 (36)	6 (13)	
Other[c]/multiple	17 (47)	14 (30)	
Peak CMV viral load in blood (IU/mL), median (IQR), n=74	1951 (582-5123)	961 (298-2509)	0.332
Nadir WBC level (K/μL),[e]median (IQR), n=80	4 (2.9-6.9)	0.6 (0-2.0)	<0.001
Testing confirming diagnosis, n (%)			n/a
PCR[f]	20 (56)	37 (80)	
Immunohistochemical analysis[g]	5 (14)	0	
Multiple/Other	11 (31)	9 (20)	
Prior history of CMV,[h] n (%)	6 (17)	19 (41)	0.029
Immunosuppressant use,[i] n (%)	30 (83)	43 (93)	0.171
irAE, n (%)	15 (42)	6 (13)	0.005
Treatment for CMV, n (%)			0.134
IV antiviral alone or in combination with PO	21 (58)	36 (78)	
PO antiviral	6 (17)	5 (11)	
Not treated	9 (25)	5 (11)	
Duration of treatment in days, median (IQR), n=38	15 (14-28)	21 (14-37)	0.390
Infection clearance, n (%)			0.002
Cleared	18 (50)	38 (83)	
Not cleared or not tested for clearance	18 (50)	8 (17)	
Recurrence of CMV after clearance, n (%)	1 (3)	9 (20)	0.037
All-cause mortality, n (%)	30 (83)	25 (54)	0.009
Death due to CMV	1 (3)	1 (4)	>0.99

[a]P-values of <0.05 were considered statistically significant. [b]Other locations included the respiratory tract and GI tract. In some of these patients, CMV was found in the blood. [c]Other symptoms included gastrointestinal, hematologic, neurologic, and eye symptoms. [d]Those who had multiple presenting symptoms usually had a constitutional symptom such as fever or fatigue as well as an organ-specific symptom. eIn the month before CMV infection. [f]Samples included blood, bronchoalveolar lavage, tracheal aspirate, pericardial fluid, and stool. [g]Immunohistochemical analysis was performed on gastrointestinal biopsies and bronchoalveolar lavage fluid. [h]Based on serology (IgG) or prior history of positive test. iImmunosuppressants included corticosteroids, chemotherapy regimens, and tacrolimus

CMV, cytomegalovirus; IQR, interquartile range; WBC, white blood cell; ICI, immune checkpoint inhibitor; IV, intravenous; PCR, polymerase chain reaction; PO, oral; IrAE, immune-related adverse event

hematoxylin and eosin stain, and on immunohistochemical stain for colon biopsies (Fig. 2).

The locations of CMV infection in patients on CTLA-4, alone or in combination with PD-1/L1, and PD-1/L1 monotherapy are shown in Supplementary Table 5. There was no significant difference between the 2 groups in CMV location.

Discussion

CMV is a ubiquitous double-stranded DNA herpesvirus that may lie inactive in immunocompetent hosts and often causes infections in immunocompromised individuals. Although many studies have been performed on CMV infection in the transplant and leukemia populations, CMV infection in ICI-treated cancer patients has not been studied. Although symptoms of organ inflammation after exposure to ICI are often attributed to irAEs, CMV must remain among the differential diagnoses for these patients. Misdiagnosis of CMV infection and off-target management for presumed irAEs due to overlapping symptoms could lead to unfavorable outcomes [13,18,19]. This study represents the largest sample analysis examining the incidence and characteristics of CMV infection in the ICI-treated patient population.

In the non-transplant hematologic malignancy population, CMV infection and reactivation rates have ranged widely, from 2-67% [20]. One large prospective cohort study comparing patients with bone marrow transplants and patients with lung transplants showed incidences of 34.7% and 28.3%, respectively [21]. The incidence of CMV in the general population and in the cancer population has been difficult

Table 3 Comparison of CMV characteristics and outcomes in ICI-treated patients between those treated with antiviral therapy and those who were not

Characteristic or outcome	Treated with antivirals, n=68	Not treated with antivirals, n=14	P-value[a]
CMV location, n (%)			0.860
Blood alone	35 (51)	8 (57)	
Blood and other location	25 (37)	5 (36)	
Other location only	8 (12)	1 (7)	
CMV-related clinical presentation			n/a
None (found on surveillance)	6 (9)	4 (29)	
Constitutional	21 (31)	1 (7)	
Respiratory	15 (22)	4 (29)	
Gastrointestinal	7 (10)	2 (14)	
Other[b]	4 (6)	0	
Multiple[c]	15 (22)	3 (21)	
Peak CMV viral load in blood (IU/mL), median (IQR) , n=74	1393 (668-3430)	432 (286-1545)	0.119
Nadir WBC level (K/μL),[d] median (IQR) , n=80	1.8 (0.1-3.5)	4.8 (1.7-6.8)	0.006
Prior history of CMV,[e] n (%)	21 (31)	4 (29)	>0.99
Immunosuppressant use,[f] n (%)	6 (9)	3 (21)	0.178
Concomitant GVHD,[g] n (%)	20 (29)	1 (7)	0.102
irAE, n (%)	18 (26)	3 (21)	>0.99
Infection clearance, n (%)			0.009
Cleared	51 (75)	5 (36)	
Not cleared or not tested for clearance	17 (25)	9 (64)	
Recurrence of CMV after clearance, n (%), n=56	9 (13)	1 (7)	>0.99
Mortality, n (%)	41 (60)	10 (71)	0.552
CMV related mortality	2 (3)	0	n/a

[a]P-value of <0.05 was considered statistically significant. [b]Other symptoms included hematologic, neurologic, and eye symptoms. [c]Those who had multiple presenting symptoms usually had a constitutional symptom such as fever or fatigue as well as an organ-specific symptom. [d]In the month before CMV infection. [e]Based on serology (IgG) or prior history of positive test. [f]Immunosuppressant use within 3 months before CMV diagnosis; immunosuppressants included corticosteroids, chemotherapy regimens, and tacrolimus. [g]Refers only to patients with hematologic malignancies

CMV, cytomegalovirus; IQR, interquartile range; WBC, white blood cell; ICI, immune checkpoint inhibitor; GVHD, graft-versus-host disease

to establish, given the non-specific symptoms, highly varied cancer types, immunosuppression, and comorbidities. In our study, among the patients tested, the incidence of CMV infection in ICI-treated patients was 7.7% and in non-ICI treated patients 12.9% (P<0.001). This suggests that ICI treatment itself, compared with non-ICI treatment, may not increase the risk of CMV infection in the cancer population. The recurrence rate of CMV infection was similar between the groups (12, 15%). The majority of patients in both groups were treated with IV antivirals, and the clearance rates of recurrent infection were also similar between the 2 groups (70, 63%). However, within the ICI group, we observed a higher rate of CMV infection among patients with hematologic malignancy (56%) compared to those with a solid tumor (44%). The high rate (87%) of stem cell transplantation or bone marrow transplantation among hematologic malignancy patients could have contributed to this finding. Despite the fact that ICI is less frequently used for hematologic malignancies in current routine practice, hematologic malignancy and chemotherapy itself could be a risk factor for CMV infection, given its association with bone marrow malfunction and frequent episodes of neutropenia [22]. In our ICI-treated population, a higher percentage of patients with liquid tumors had a prior history of CMV or were seropositive for CMV. This could be related to the high percentage of patients in our hematologic malignancy cohort (87%) who had received bone marrow transplants or stem cell transplants and required intense immunosuppressant use after transplantation to prevent rejection; this finding could also be due to the patients' neutropenic status (nadir WBC of 0.6). In contrast, the solid tumor group had a higher incidence of irAEs (42%) compared to the hematologic malignancy group (13%), and a higher incidence of end-organ involvement in CMV infection (62% vs. 37%, P=0.045). The patients with solid tumors had a significantly higher median WBC level (4 K/μL) than the hematologic malignancy group (0.6 K/μL). Despite similar immunosuppressant use among the 2 groups, the lower WBC level in the hematologic malignancy group may be secondary to selected immunosuppressant use that may cause bone marrow suppression. Valganciclovir, a commonly used prophylactic agent for CMV infection, is known to cause neutropenia [21,23].

It has been postulated that CMV infection can be a risk factor for severe irAEs [17,18]. A recent retrospective analysis found a strong association between ICI-related pneumonitis and pulmonary CMV infection and suggested that ICI pneumonitis may have a component of an immune reconstitution syndrome

Table 4 Comparison of CMV characteristics of patients within the ICI group: patients who developed irAEs vs. those who did not[a]

Characteristic or outcome	irAE group, n=21	No irAE group, n=61	P-value[b]
CMV location, n (%)			0.964
Blood alone	11 (52)	32 (52)	
Blood and other location	8 (38)	22 (36)	
Other location only	2 (10)	7 (11)	
CMV viral load in blood (IU/mL), median (IQR) , n=74	2960 (960-16592)	1073 (432-2388)	0.535
Nadir WBC level (K/μL),[c] median (IQR) , n=80	3.7 (2.6-5.2)	1.6 (0.2-3.3)	0.032
Prior history of CMV,[d] n (%)	5 (24)	20 (33)	0.003
Immunosuppressant use,[e] n (%)	17 (81)	56 (92)f	0.170
Concomitant GVHD,[g] n (%)	3 (14)	18 (28)	0.168
Hospitalization for CMV, n (%)	8 (38)	11 (18)	0.060
Duration of hospitalization in days, median (IQR), n=19	12 (7-21)	12 (9-19)	0.741
ICU for CMV, n (%)	2	4	n/a
Duration of ICU stay in days, median (IQR), n=6	8 (5-12)	21 (9-35)	n/a
Treatment for CMV, n (%)			n/a
IV antiviral	7 (33)	27 (44)	
PO antiviral	3 (14)	8 (13)	
Both IV and PO	8 (38)	15 (25)	
Not treated	3 (14)	11 (18)	
Duration of treatment in days, median (IQR), n=68	21 (15-33)	14 (14-34)	0.562
Infection clearance, n (%)			0.415
Cleared	14 (67)	42 (69)	
Not cleared or not tested for clearance	7 (33)	32 (31)	
Mortality, n (%)	14 (67)	37 (61)	0.624

[a]All patients with irAEs had CMV diagnosed after irAE diagnosis. [b]P-values of <0.05 were considered statistically significant. [c]In the month before CMV infection. [d]Based on serology (IgG) or prior history of positive test. eImmunosuppressant use within 3 months before CMV diagnosis; immunosuppressants included corticosteroids, chemotherapy regimens, and tacrolimus. [f]The main reason that the non-irAE group received immunosuppressants was for cancer treatment in all patients and stem cell transplantation in the hematologic malignancy group. [g]Refers only to patients with hematologic malignancies

CMV, cytomegalovirus; IQR, interquartile range; WBC, white blood cell; ICI, immune checkpoint inhibitor; IV, intravenous; PO, oral; irAE, immune-related adverse event; GVHD, graft-versus-host disease

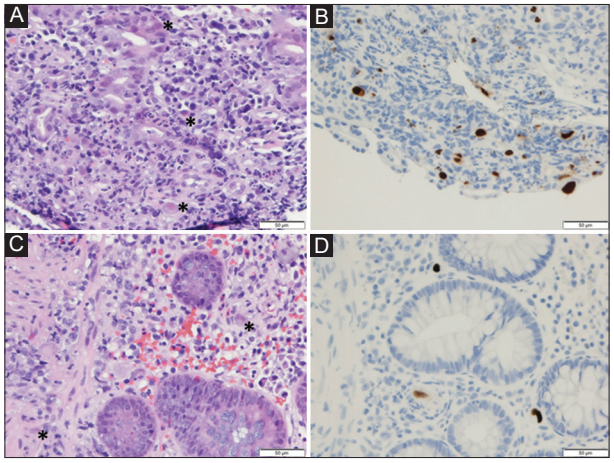

Figure 2 (A) and (C) are hematoxylin and eosin stain with typical "owl's eyes" or basophilic intranuclear inclusion bodies; (B) and (D) are immunohistochemical stain (magnification, 20×)

associated with CMV pneumonitis [24]. In addition, several case reports have shown GI CMV infection to be associated with immune-mediated diarrhea and colitis (IMDC) refractory to therapy [17,18,25]. The proposed mechanism for this is that steroid treatment for irAEs lowers T-cell–mediated immunity and therefore increases the chances of CMV infection [17]. However, no large-scale analyses have been performed to determine the association of CMV with ICI treatment itself and with irAEs. In our cohort, of the 82 patients with CMV in the ICI-treated group, 21 (26%) had irAEs. All 21 had received a diagnosis of CMV infection after their irAE diagnosis, and 15 (71%) of the 21 patients had received immunosuppressive agents for their irAE before their CMV diagnosis.

Among all ICI-treated patients, 8 developed GI irAEs (7 had IMDC and 1 had duodenitis). All of these patients developed CMV infection after undergoing immunosuppressive treatment with corticosteroids for their GI irAE. Seven of 8 of these patients developed GI CMV infection within 3 months of ICI initiation. Five of these patients also received infliximab and 2 received vedolizumab. Of these 8 patients, 6 had concurrent toxicity in another organ; 5 underwent endoscopic evaluation for CMV, 3 of whom had CMV confirmed on their GI pathology samples.

Since symptoms of CMV are difficult to distinguish from those of IMDC, it was difficult to determine whether

the patients who had concurrent GI irAEs presented with symptomatic CMV infection in the GI tract, or if they presented with GI irAE symptoms with incidental CMV infection. It is probable that CMV infection may simply play the role of a bystander in the context of GI irAEs and does not necessarily benefit from antiviral treatment. ICI treatment itself does not appear to confer a higher risk for CMV infection in the GI tract compared to non-ICI treatment. However, the high degree of inflammation associated with GI irAEs could predispose to GI infections such as CMV. Although the GI CMV infection rate in patients with GI irAEs is estimated to be as high as 38%, we do not observe serious clinical consequences in the vast majority of patients.

The mortality rates were comparable between the ICI-treated cohort (62%) and the non-ICI-treated cohort (67%). Seven patients had CMV-related deaths, with 2 in the ICI-treated cohort; both of these patients had widespread CMV infection in 2 or more locations, and both had lung involvement. Of the 5 patients in the non-ICI-treated group, 3 had lung involvement and 2 had only viremia. This suggests that the significance of CMV infection and its worse prognosis may be organ-dependent. CMV viremia with pulmonary involvement appeared to be the most hazardous infection compared with other organ infections.

CMV reactivation has been observed in the setting of decreased host defenses, such as ICU admission and sepsis. One study showed that CMV incidence in seronegative non-immunocompromised ICU patients can be 15-20%, and up to 20-40% in seropositive patients [26]. In our cohort, 89% of patients in the ICI group and 90% of patients in the non-ICI group had precipitating factors, including hospitalization, ICU admission, mechanical ventilation, severe sepsis and surgery, frequently encountered among cancer populations. CMV reactivation can lead to widespread cytokine-release syndromes that result in significant morbidity and mortality in these patients. The percentages of patients with a history of CMV infection in the ICI and non-ICI groups were 31% and 13%, respectively. Although the immunosuppressive treatment for either ICI-related toxicities or as part of chemotherapy regimens in these patients did not differ between these 2 groups in the 3 months preceding CMV infection, this may be related to the higher percentage of end-organ involvement without viremia in the ICI group.

Although this study is the largest of its kind to examine CMV in the ICI population, it is important to acknowledge its limitations. First, given the retrospective nature of the study, the data collection was limited by the subjective nature of documentation in the patients' electronic medical records. Second, the treatment of CMV infection was left to the discretion of the treating physicians and therefore was not consistent across the group. The small number of patients who did not receive antiviral treatment made it unlikely that a clear benefit of treatment could be delineated. Third, the lack of a control group without CMV infection in our study limited our ability to measure the overall impact of CMV infection among cancer patients. In addition, the classification of patients into ICI and non-ICI groups did not account for the coadministration of chemotherapy in the ICI group, which could potentially affect CMV incidence and characteristics. Finally, the small sample size of patients with irAEs prevented our investigation of the association between CMV and irAEs.

In conclusion, CMV infection occurs in less than 8% of patients treated with ICIs, which is less than the infection rate in patients treated with non-ICI chemotherapeutic agents. ICIs do not appear to be an independent risk factor for CMV infection. Among patients treated with ICIs, those with hematologic malignancies appear to have a higher rate of CMV infection and fewer irAEs than do those with solid tumors. This same group with hematologic malignancies is also more likely to achieve clearance of CMV infection, have higher rates of recurrence, and lower overall mortality. Mortality rates associated with CMV infection are low in patients with advanced cancer. The role of antiviral treatment for CMV infections and the effects of these infections on cancer outcomes require further investigation.

Acknowledgment

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Summary Box

What is already known:

- Immune checkpoint inhibitors (ICIs) can lead to immune-related adverse events, particularly in the gastrointestinal tract
- Cytomegalovirus (CMV) colitis in the immunosuppressed can present with a similar clinical picture to immune-related colitis
- Several studies have found that CMV can be detected in specific cases of steroid-refractory immune-mediated colitis, and treatment with antiretroviral medications led to symptom resolution

What the new findings are:

- CMV infections occurred less frequently in cancer patients on ICIs (7.7%) compared to those on non-ICI treatment (12.9%)
- Rates of CMV infection clearance (83% vs. 50%) and recurrence (20% vs. 3%) were higher in ICI patients with hematological malignancies as opposed to those with solid tumors
- All-cause mortality was higher in ICI patients with solid tumors (83%) compared to those with hematologic malignancies (554); overall CMV-related mortality was low, around 3-4% in both groups

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Supplementary material

Supplementary Table 1 Patient characteristics, n=192

Characteristic or outcome	ICI group, n=82	Non-ICI group, n=110	P-value
Age at CMV diagnosis (y), mean (SD), n=192	58.9 (14.6)	57.9 (13.6)	0.612
Male, n (%)	53 (65)	67 (61)	0.652
Race, n (%)			0.782
White	50 (61)	68 (62)	
Black	9 (11)	15 (14)	
Other	23 (28)	27 (25)	
Charlson comorbidity index, median (IQR)	6 (3-9)	6 (3-7)	0.159
Cancer type, n (%)			0.658
Hematologic	45 (55)	65 (59)	
Solid tumor[a]	37 (45)	45 (41)	
Cancer stage,[b] n (%)	n=37	n=41	0.678
Stage 1-2	2 (5)	4 (10)	
Stage 3-4	35 (95)	37 (90)	
Cancer status at time of CMV, n (%)			0.302
Stable cancer, or partial/complete remission	43 (52)	67 (61)	
Cancer progression	39 (48)	43 (39)	
Surgical excision of solid tumor, n=82	3 (8)	9 (20)	0.209
ECOG[c] performance status at time of CMV, n (%)			0.440
0-2	53 (65)	77 (70)	
3-4	29 (35)	33 (30)	
Patients with ICI colitis, n (%)	8 (10)	n/a	n/a
CMV timing in relation to IMDC, n (%), n=8			n/a
Concurrent [d]	6 (75)	n/a	
Before	0 (0)	n/a	
After	2 (25)	n/a	
CMV without irAEs	63 (77)	n/a	
Patients with irAEs other than colitis, n=19, n (%)			n/a
Liver	2 (11)	n/a	
Endocrine[e]	3 (16)	n/a	
Pulmonary	4 (21)	n/a	
Skin	1 (5)	n/a	
Muscle	1 (5)	n/a	
Multiple	6 (32)	n/a	
Other[f]	2 (11)	n/a	
Cause of death, n (%)	n=51	n=74	n/a
Due to CMV[g]	2 (4)	5 (7)	
Due to irAE	3 (6)	n/a	
Due to malignancy	24 (47)	46 (62)	
Cancer-related complications	21 (41)	20 (27)	
Other[h]	1 (2)	3 (4)	

[a]Solid tumor types included lung cancer, genitourinary cancers, melanoma, gastrointestinal cancers, head and neck cancer, sarcoma, and thymoma. [b]Cancer staging based on American Joint Committee on Cancer, Cancer staging manual 8th edition for all solid malignancies. [c]Developed by the Eastern Cooperative Oncology Group. [d]Defined as CMV diagnosis made within 30 days of IMDC diagnosis. [e]Endocrine adverse events included thyroiditis and hypophysitis. [f]Other organs with adverse events included the heart, liver, kidney, and duodenum. [g]Death was determined to be due to CMV if the patient died due to organ dysfunction where they had CMV infection. hMyocardial infarction

SD, standard deviation; IQR, interquartile range; CMV, cytomegalovirus; GI, gastrointestinal; GU, genitourinary; ICI, immune checkpoint inhibitor; irAE, immune-related adverse events; IMDC, immune checkpoint inhibitor-mediated diarrhea and colitis

Supplementary Table 2 Comparison of CMV characteristics in all patients between those with solid tumors and those with hematologic malignancy

Characteristic or outcome	Solid tumor, n=81	Hematologic malignancy, n=111	P-value[a]
CMV location, n (%)			0.091
Blood alone	39 (48)	71 (64)	
Blood and other location	36 (44)	34 (31)	
Other location only[b]	6 (7)	6 (5)	
CMV related clinical presentation			0.109
None (found on surveillance)	10 (12)	22 (20)	
Constitutional	25 (31)	40 (36)	
Respiratory	22 (27)	14 (13)	
Gastrointestinal	6 (7)	11 (10)	
Other[c] /multiple[d]	18 (22)	24 (22)	
Peak CMV viral load in blood (IU/mL), median (IQR)	1428 (432-5014)	1066 (415-2735)	0.441
Nadir WBC level (K/μL),[e] median (IQR)	3.85 (1.65-5.98)	0.2 (0-1.95)	<0.001
Testing confirming diagnosis, n (%)			n/a
PCR[f]	50 (62)	89 (80)	
Immunohistochemical analysis[g]	5 (6)	0	
Multiple	26 (32)	22 (20)	
Prior history of CMV,[h] n (%)	10 (12)	29 (26)	0.028
Immunosuppressant use,[i] n (%)	66 (81)	99 (89)	0.145
Treatment for CMV, n (%)			0.003
IV antiviral alone or in combination with PO	51 (63)	86 (77)	
PO antiviral	10 (12)	17 (15)	
Not treated	20 (25)	8 (7)	
Duration of treatment in days, median (IQR), n=164	14 (9-21)	21 (14-35)	<0.001
Infection clearance, n (%)			
Cleared	36 (44)	96 (86)	
Not cleared or not tested for clearance	45 (55)	15 (14)	<0.001
Recurrence of CMV after clearance, n (%)	3 (4)	23 (21)	0.001
All-cause mortality, n (%)	64 (79)	61 (55)	0.001

[a]P-values of <0.05 were considered statistically significant. [b]Other locations included the respiratory tract and GI tract. [c]Other symptoms included gastrointestinal, hematologic, neurologic, and eye symptoms. [d]Those who had multiple presenting symptoms usually had a constitutional symptom such as fever or fatigue as well as an organ-specific symptom. [e]In the month before CMV infection. [f]Samples included blood, bronchoalveolar lavage, tracheal aspirate, pericardial fluid, and stool. [g]Immunohistochemical analysis was performed on gastrointestinal biopsies and bronchoalveolar lavage fluid. [h]Based on serology (IgG) or prior history of positive test. iImmunosuppressants included corticosteroids, chemotherapy regimens, and tacrolimus

SD, standard deviation; IQR, interquartile range; CMV, cytomegalovirus; ICI, immune checkpoint inhibitor; IV, intravenous; PO, oral

Supplementary Table 3 Comparison of CMV characteristics in non-ICI patients between those with solid tumors and those with hematologic malignancy

Characteristic or outcome	Solid tumor, n=45	Hematologic malignancy, n=65	P-value[a]
CMV location, n (%)			0.002
Blood alone	25 (56)	42 (65)	
Blood and other location or other location only[b]	20 (44)	23 (35)	
CMV-related clinical presentation, n (%)			0.087
None (found on surveillance)	7 (16)	15 (23)	
Constitutional	22 (49)	21 (32)	
Respiratory	9 (20)	8 (12)	
Other[c] /multiple[d]	7 (16)	21 (32)	
Peak CMV viral load in blood (IU/mL), median (IQR)	1038 (392-4574)	1095 (471-4357)	0.897
Nadir WBC level (K/μL),[e] median (IQR)	3.7 (0.9-5.7)	0.1 (0-1.8)	<0.001
Testing confirming diagnosis, n (%)			0.126
PCR[f]	30 (67)	52 (80)	
Multiple/other	15 (33)	13 (20)	
Prior history of CMV,[h] n (%)	4 (9)	10 (15)	0.392
Immunosuppressant use,[i] n (%)	36 (80)	56 (86)	0.438
Treatment for CMV, n (%)			0.006
IV antiviral alone or in combination with PO	30 (67)	50 (77)	
PO antiviral	4 (9)	12 (18)	
Not treated	11 (24)	3 (5)	
Duration of treatment in days, median (IQR)	13 (5-21)	21 (20-30)	<0.001
Infection clearance, n (%)	18 (40)	58 (89)	<0.001
Cleared	27 (60)	7 (11)	
Not cleared or not tested for clearance	2 (4)	14 (22)	
Recurrence of CMV after clearance, n (%)			0.013
All-cause mortality, n (%)	34 (76)	40 (62)	0.150

[a]P-values of <0.05 were considered statistically significant. [b]Other locations included the respiratory tract and GI tract. [c]Other symptoms included gastrointestinal, hematologic, neurologic, and eye symptoms. [d]Those who had multiple presenting symptoms usually had a constitutional symptom such as fever or fatigue as well as an organ-specific symptom. [e]In the month before CMV infection. [f]Samples included blood, bronchoalveolar lavage, tracheal aspirate, pericardial fluid, and stool. gImmunohistochemical analysis was done on gastrointestinal biopsies and bronchoalveolar lavage fluid. [h]Based on serology (IgG) or prior history of positive test. iImmunosuppressants included corticosteroids, chemotherapy regimens, and tacrolimus.

IQR, interquartile range; CMV, cytomegalovirus; ICI, immune checkpoint inhibitor; IV, intravenous; PO, oral

Supplementary Table 4 CMV characteristics of patients with confirmed GI CMV infection

ID	Age (y)	Sex	CMV location in addition to GI	Immuno-suppressant use before CMV diagnosis[a]	CMV Treatment	Treatment duration, days	Infection clearance[b]	Time from ICI initiation to GI CMV (days)	irAE-related to ICI	CMV relation to irAE[c,d]	Death
1	71	M	Respiratory tract	Chemotherapy regimen	IV	14	No	998	No	n/a	Malignancy
2	65	M	None	None	Not treated	0	No	75	No	n/a	Malignancy
3	63	M	Blood	Corticosteroid	IV/PO	15	No	63	GI	Concurrent after irAE treatment	Malignancy
4	80	F	Blood, respiratory tract, eye	Corticosteroid	IV/PO	27	No	80	Pulm	Concurrent after irAE treatment	CMV
5	39	F	Blood	Chemotherapy regimen and corticosteroid	IV/PO	37	Yes	39	GI, liver, skin	Concurrent after irAE treatment	Alive
6	53	M	Blood	Chemotherapy regimen	IV	24	Yes	53	No	n/a	Alive
7	50	F	None	Corticosteroid	IV/PO	15	Yes	50	GI	Concurrent after irAE treatment	Malignancy
8	54	M	None	None	PO	10	No	54	No	n/a	Alive

[a]Within 30 days prior to CMV diagnosis. [b]Clearance was defined as viral load decreasing to undetectable levels or repeat stool studies or colonoscopy with biopsy becoming negative. [c]Concurrent infection means CMV diagnosis within 30 days of irAE diagnosis. [d]Of the 3 patients with GI irAE, one had corticosteroid treatment only, one had corticosteroid and infliximab, and one had corticosteroid, infliximab, and vedolizumab

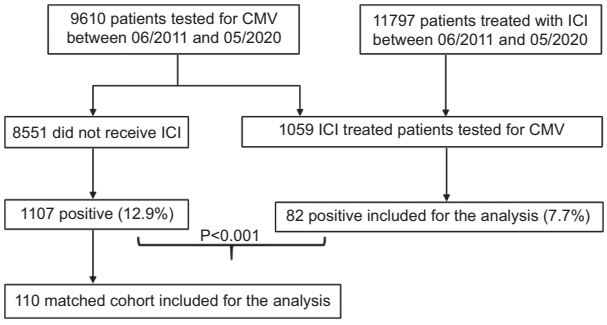
n/a, not available; PO, per os; IV, intravenous; CMV, cytomegalovirus; GI, gastrointestinal; ICI, immune checkpoint inhibitor; irAE, immune-related adverse events; M, male; F, female

Supplementary Table 5 Comparison of CMV infection location between different classes of immunotherapy agents

CMV location	CTLA-4 monotherapy or combination with PD-1/L1, N=11	PD-1/L1 monotherapy, N=71	P-value[a]
CMV infection location, n (%)			P=0.051
Blood alone	9 (82)	34 (48)	
Blood + end organ or end organ alone	2 (18)	37 (52)	
GI CMV infection, n (%)	1 (9)	7 (10)	n/a

[a]P-values <0.05 were considered statistically significant

n/a, not applicable; CMV, cytomegalovirus; GI, gastrointestinal; CTLA-4, cytotoxic T-lymphocyte antigen-4; PD-1, programmed cell death protein-1



Supplementary Figure 1 Patient selection flowchart. Tests confirming diagnosis for the ICI group were PCR (57 patients), immunohistochemical analysis (5 patients), and multiple methods (20 patients); for the non-ICI group, tests were PCR (82 patients) and multiple methods (28 patients)
CMV, cytomegalovirus; ICI, immune checkpoint inhibitor; PCR, polymerase chain reaction