|  |
| --- |
| Campylobacter |
| C difficile DNA |
| Plesiomonas shigelloides |
| Salmonella |
| Vibrio |
| Vibrio cholerae |
| Yersinia enterocolitica |
| Enteroaggregative E. coli |
| Enteropathogenic E. coli |
| Enterotoxigenic E. coli |
| Shiga-like toxin-producing E. coli |
| E. coli O157 |
| Shigella/Enteroinvasive E. coli |
| Cryptosporidium |
| Cyclospora cayetanensis |
| Entamoeba histolytica |
| Giardia lamblia |
| Adenovirus F 40/41 |
| Astrovirus |
| Norovirus GI/GII |
| Rotavirus A |
| Sapovirus (I, II, IV) |  |  |

**Table S1** Pathogens tested for by gastrointestinal multiplex laboratory testing at our institution

**Table S2** Antibiotics with anti-anaerobic activity administered to study patients

|  |
| --- |
| Amoxicillin-clavulanic acid |
| Ampicillin-sulbactam |
| Cefdinir  Cefoxitin  Cefotetan |
| Clindamycin |
| Delafloxacin |
| Doripenem |
| Eravacycline |
| Ertapenem |
| Imipenem |
| Meropenem |
| Metronidazole |
| Moxifloxacin |
| Omadacycline |
| Penicillin |
| Piperacillin-tazobactam |
| Tigecycline |

**Table S3** Common terminology Criteria for Adverse Events grading for diarrhea and colitis.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Grade | | | | |
| Adverse Events | 1 | 2 | 3 | 4 | 5 |
| Diarrhea | Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline | Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy output compared to baseline | Increase of ≥7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self-care ADL | Life-threatening consequences; urgent intervention indicated | Death |
| Colitis | Asymptomatic; clinical or diagnostic observations only; intervention not indicated | Abdominal pain; mucus or blood in stool | Severe abdominal pain; peritoneal signs | Life-threatening consequences; urgent intervention indicated | Death |

ADL: activity of daily living

**Table S4** Indications for antibiotic use.

|  |  |
| --- | --- |
| **Indication** | **No. of patients** |
| Upper respiratory infection | 21 (3.7) |
| Lower respiratory infection | 38 (6.7) |
| Gastrointestinal infection | 68 (12.0) |
| Urinary tract infection | 62 (10.9) |
| Skin/Soft tissue infection | 43 (7.6) |
| Sepsis and bacteremia | 11 (1.9) |
| Fever of unknown origin/empirical coverage | 166 (29.2) |
| Prophylaxis | 90 (15.8) |
| Multiple infections | 54 (9.5) |
| Not recorded | 16 (2.8) |

**Table S5.A.** Clinical features of patients who received anti-CTLA-4 therapy according to use of antibiotic therapy

|  |  |  |  |
| --- | --- | --- | --- |
| Feature | Antibiotic therapy  (*n* = 270) | No antibiotic therapy  (*n* = 130) | *p* |
| IMDC, *n* (%) | 146 (54.1) | 92 (70.8) | 0.002 |
| Mean duration of IMDC symptoms, days (SD) | 40 (198) | 13 (128) | 0.245 |
| Hospitalization, *n* (%) | 105 (71.9) | 44 (47.8) | < 0.001 |
| Mean duration of hospitalization, days (SD) | 8 (7) | 6 (4) | 0.070 |
| ICU admission, *n* (%) | 4 (2.7) | 1 (1.1) | 0.651 |
| Grade of colitis, *n* (%) |  |  | 0.011 |
| 1 | 22 (17.9) | 17 (25.0) |  |
| 2 | 45 (36.6) | 35 (51.5) |  |
| 3 | 53 (43.1) | 13 (19.1) |  |
| 4 | 3 (2.4) | 3 (4.4) |  |
| Grade of diarrhea, *n* (%) |  |  | 0.272 |
| 1 | 35 (24.0) | 25 (27.2) |  |
| 2 | 32 (21.9) | 26 (28.3) |  |
| 3 | 69 (47.3) | 39 (42.4) |  |
| 4 | 10 (6.8) | 2 (2.2) |  |
| Mean duration of steroid administration, days (SD) | 56 (45) | 66 (89) | 0.334 |
| Infliximab/vedolizumab administration, *n* (%) | 41 (28.1) | 21 (22.8) | 0.449 |
| Recurrence of IMDC, *n* (%) | 23 (15.8) | 20 (21.7) | 0.299 |

**Table S5.B.** Clinical features of patients who received anaerobic and aerobic antibiotic therapy among patients who received anti-CTLA-4 therapy. (No. of patients who received antibiotics = 270)

|  |  |  |  |
| --- | --- | --- | --- |
| Feature | Anaerobic  (*n* = 132) | Aerobic  (*n* = 138) | *p* |
| IMDC, *n* (%) | 81 (61.4) | 65 (47.1) | 0.021 |
| Immunosuppressive therapy for IMDC, *n* (%) | 65 (49.2) | 44 (31.9) | 0.014 |
| Mean time to IMDC onset, weeks (SD) | 9 (8) | 7 (5) | 0.135 |
| Mean duration of IMDC symptoms, days (SD) | 16 (15) | 71 (296) | 0.096 |
| Hospitalization, *n* (%) | 67 (82.7) | 38 (58.5) | 0.002 |
| Mean duration of hospitalization, days (SD) | 9 (7) | 8 (5) | 0.689 |
| ICU admission, *n* (%) | 4 (4.9) | 0 (0) | 0.129 |
| Grade of colitis, *n* (%) |  |  | 0.009 |
| 1 | 7 (9.6) | 15 (30) |  |
| 2 | 32 (43.8) | 13 (26.0) |  |
| 3 | 31 (42.5) | 22 (44.0) |  |
| 4 | 3 (4.1) | 0 (0) |  |
| Grade of diarrhea, *n* (%) |  |  | 0.571 |
| 1 | 19 (23.5) | 16 (24.6) |  |
| 2 | 15 (18.5) | 17 (26.2) |  |
| 3 | 40 (49.4) | 29 (44.6) |  |
| 4 | 7 (8.6) | 3 (4.6) |  |
| Mean calprotectin level (SD) | 563 (369) | 190 (158) | 0.010 |
| Mean duration of steroid administration, days (SD) | 54 (45) | 59 (46) | 0.641 |
| Infliximab/vedolizumab administration, *n* (%) | 20 (24.7) | 21 (32.3) | 0.356 |
| Recurrence of IMDC, *n* (%) | 12 (14.8) | 11 (16.9) | 0.820 |

**Table S6.A** Clinical features of patients who received anti-PD-1/L1 therapy according to use of antibiotic therapy

|  |  |  |  |
| --- | --- | --- | --- |
| Feature | Antibiotic therapy  (*n* = 299) | No antibiotic therapy  (*n* = 127) | *p* |
| IMDC, *n* (%) | 121 (40.5) | 75 (59.1) | 0.001 |
| Mean duration of IMDC symptoms, days (SD) | 21 (45) | 23 (55) | 0.782 |
| Hospitalization, *n* (%) | 63 (52.1) | 19 (25.3) | < 0.001 |
| Mean duration of hospitalization, days (SD) | 7 (8) | 7 (6) | 0.778 |
| ICU admission, *n* (%) | 6 (5.0) | 0 (0.0) | 0.084 |
| Grade of colitis, *n* (%) |  |  | 0.880 |
| 1 | 20 (25.0) | 15 (31.3) |  |
| 2 | 45 (56.3) | 25 (52.1) |  |
| 3 | 12 (15.0) | 6 (12.5) |  |
| 4 | 3 (3.8) | 2 (4.2) |  |
| Grade of diarrhea, *n* (%) |  |  | 0.397 |
| 1 | 42 (34.7) | 27 (36.0) |  |
| 2 | 37 (30.6) | 25 (33.3) |  |
| 3 | 34 (28.1) | 22 (29.3) |  |
| 4 | 8 (6.6) | 1 (1.3) |  |
| Mean duration of steroid administration, days (SD) | 45 (42) | 61 (63) | 0.143 |
| Infliximab/vedolizumab administration, *n* (%) | 11 (9.1) | 10 (13.3) | 0.354 |
| Recurrence of IMDC, *n* (%) | 19 (15.7) | 21 (28.0) | 0.045 |

**Table S6.B** Clinical features of patients who received anaerobic and aerobic antibiotic therapy among patients who received anti-PD-(L)1 therapy. (No. of patients who received antibiotics = 299)

|  |  |  |  |
| --- | --- | --- | --- |
| Feature | Anaerobic  (*n* = 156) | Aerobic  (*n* = 143) | *p* |
| IMDC, *n* (%) | 64 (41.0) | 57 (39.9) | 0.906 |
| Immunosuppressive therapy for IMDC, *n* (%) | 37 (23.7) | 27 (18.9) | 0.520 |
| Mean time to IMDC onset, weeks (SD) | 21 (26) | 15 (15) | 0.119 |
| Mean duration of IMDC symptoms, days (SD) | 22 (55) | 21 (32) | 0.848 |
| Hospitalization, *n* (%) | 39 (60.9) | 24 (42.1) | 0.046 |
| Mean duration of hospitalization, days (SD) | 9 (10) | 5 (3) | 0.063 |
| ICU admission, *n* (%) | 6 (9.4) | 0 (0) | 0.029 |
| Grade of colitis, *n* (%) |  |  | 0.172 |
| 1 | 9 (18.8) | 11 (34.4) |  |
| 2 | 27 (56.3) | 18 (56.3) |  |
| 3 | 9 (18.8) | 3 (9.4) |  |
| 4 | 3 (6.3) | 0 (0) |  |
| Grade of diarrhea, *n* (%) |  |  | 0.134 |
| 1 | 19 (29.7) | 23 (40.4) |  |
| 2 | 18 (28.1) | 19 (33.3) |  |
| 3 | 20 (31.3) | 14 (24.6) |  |
| 4 | 7 (10.9) | 1 (1.8) |  |
| Mean duration of steroid administration, days (SD) | 40 (41) | 52 (43) | 0.284 |
| Intravenous steroid administration, *n* (%) | 20 (60.6) | 7 (26.9) | 0.017 |
| Infliximab/vedolizumab administration, *n* (%) | 8 (12.5) | 3 (5.3) | 0.214 |
| Recurrence of IMDC, *n* (%) | 13 (20.3) | 6 (10.5) | 0.210 |
| Colon perforation, *n* (%) | 2 (3.1) | 0 (0) | 0.498 |

**Table S7** Multivariate logistic regression analysis of risk of IMDC

|  |  |  |
| --- | --- | --- |
| **Characteristic** | **OR (95% CI)** | ***p*** |
| ICI type |  |  |
| Anti-PD-1/L1 | Reference |  |
| Anti-CTLA-4 | 2.16 (1.56-2.99) | < 0.001 |
| Duration of ICI therapy | 1.00 (0.99-1.00) | 0.519 |
| Antibiotic therapy |  |  |
| Anaerobic | 0.44 (0.29-0.66) | < 0.001 |
| Aerobic | 0.34 (0.23-0.51) | < 0.001 |
| None | Reference |  |

*Abbreviations*: *OR* Odds ratio. *CI* Confidence interval

**Table S8** Univariate Cox regression analysis of overall survival in the study population

|  |  |  |
| --- | --- | --- |
| **Characteristic** | **HR (95% CI)** | ***p*** |
| Age | 1.01 (1.00-1.02) | 0.007 |
| ICI type |  |  |
| Anti-PD-1/L1 | Reference |  |
| Anti-CTLA-4 | 0.72 (0.57-0.91) | 0.005 |
| Combination | 0.65 (0.46-0.91) | 0.012 |
| Stage IV cancer | 1.57 (1.04-2.37) | 0.031 |
| Time to IMDC onset | 0.97 (0.95-0.99) | 0.001 |
| IMDC | 0.44 (0.36-0.55) | <0.001 |
| Duration of IMDCsymptoms | 0.99 (0.99-0.99) | 0.007 |
| Calprotectin level | 1.00 (1.00-1.01) | 0.006 |
| Antibiotic therapy | 2.21 (1.70-2.86) | <0.001 |
| Anaerobic | 1.54 (1.22-1.96) | <0.001 |
| Timing of antibiotic therapy |  |  |
| Before ICI therapy | Reference |  |
| After ICI therapy | 1.64 (1.11-2.38) | 0.013 |

*Abbreviations*: *HR* Hazard ratio. *CI* Confidence interval

**Fig. S1.** Kaplan-Meier curves for overall survival of patients who did and did not receive antibiotic therapy.



**Fig. S2.** Kaplan-Meier curves for overall survival of patients who did and did not receive antibiotic therapy with antianaerobic activity.

