Supplementary information

Intestinal *Akkermansia muciniphila* predicts clinical response to PD-1 blockade in patients with advanced non-small-cell lung cancer

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Supplementary Table 1. Prevalence of various substrains of *Akkermansia* in our cohort.

	SGB				
	9226 – MucT — no. (%)	9228 — no. (%)	9224 — no. (%)	9223 — no. (%)	None — no. (%)
Prevalence of SGBs in all patients (n=338)	131/338 (38.8)	26/338 (7.7)	5/338 (1.5)	1/338 (0.3)	175/338 (51.7)
Prevalence of SGBs in SGBs⁺ patients (n=163)	131/163 (80.4)	26/163 (15.9)	5/163 (3.1)	1/163 (0.6)	-

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SGBs: *Akkermansia* candidate species or species-level genome bins; MucT: *A. muciniphila.*

Table S A. Patient clinical characteristics

		Overall (n=338)	Akk$^+$ (n=131)	Akk$^-$ (n=207)	P-value
Age (year)	Median (range)	65 (24-92)	66 (44-85)	64 (24-92)	
	<65 yr	163 (48)	55 (42)	108 (52)	0.08*
	≥65 to <75 yr	118 (35)	57 (43)	61 (29)	
	≥75 yr	57 (17)	19 (15)	38 (18)	
Sex – no (%)	Male	226 (67)	86 (66)	140 (68)	0.72
	Female	112 (33)	45 (34)	67 (32)	
BMI, kg/cm^2 – no (%)	<25	204 (60)	74 (56)	130 (63)	
	[25-30[	94 (28)	40 (31)	54 (26)	0.66
	≥30	36 (11)	16 (12)	20 (10)	
	Unknown	4 (1)	1 (1)	3 (1)	
ECOG performance status – no (%)	0-1	268 (79)	111 (85)	157 (76)	
	2 or more	43 (13)	10 (8)	33 (16)	0.08
	Unknown	27 (8)	10 (8)	17 (8)	
Smoking status — no. (%)	Never smoked	26 (8)	12 (9)	14 (7)	
	Current/former smoker	309 (91)	118 (90)	191 (92)	0.73
	Unknown	3 (1)	1 (1)	2 (1)	
Tumor histology — no. (%)	Squamous	78 (23)	34 (26)	44 (21)	0.35
	Non-squamous	260 (77)	97 (74)	163 (79)	
Tumor staging — no. (%)	IIA-IIIB	37 (11)	15 (11)	22 (11)	0.97
	IV	294 (87)	114 (87)	180 (87)	

	Unknown	7 (2)	2 (2)	5 (2)	
TMB — no. (%)	≥ 10 mutations/megabase	3 (1)	1 (1)	2 (1)	
	< 10 mutations/megabase	13 (4)	9 (7)	4 (2)	*0.25*
	Unknown	322 (95)	121 (92)	201 (97)	
PD-L1 status — no. (%)	<1%	64 (19)	31 (24)	33 (16)	
	1–49%	53 (16)	22 (17)	33 (16)	*0.41*
	≥50%	118 (35)	42 (32)	76 (36)	
	Unknown	102 (30)	36 (27)	66 (32)	
Therapy line — no. (%)	Neoadjuvant	3 (1)	2 (2)	1 (1)	
	First line	92 (27)	31 (24)	61 (29)	*0.33*
	≥ Second line	243 (72)	98 (75)	145 (70)	
Therapy — no. (%)	Immunotherapy	327 (97)	129 (98)	198 (96)	
	Immunotherapy and Chemotherapy	11 (3)	2 (2)	9 (4)	*0.21*
Previous therapy — no. (%)	Chemotherapy	239 (71)	96 (73)	143 (69)	
	Tyrosine kinase inhibitors	11 (3)	4 (3)	7 (3)	*0.77*
	Others	20 (6)	6 (5)	15 (7)	
	Unknown	3 (1)	2 (2)	1 (0)	
Antibiotics — no. (%)	Yes	69 (20)	25 (19)	44 (21)	*0.68*
	No	269 (80)	106 (81)	163 (79)	

BMI, Body Mass Index; TMB, Tumor Mutational Burden.
Statistical analyses: Mann-Whitney*, Chi-Square or Fisher's exact tests are two-sided, with no adjustments for multiple comparisons.

Table S B. List of antibiotics (ATB) 60 days prior to ICB

		Overall (n=69)	Akk[+] (n=25)	Akk[-] (n=44)	P-value
Duration of ATB — no. (%)	≤ 7 days	23 (33)	10 (40)	13 (30)	
	> 7 days	19 (28)	6 (24)	13 (30)	0.73
	Unknown	27 (39)	9 (36)	18 (41)	
Type of ATB — no. (%)	β-lactams ± inhibitors (± combination with other ATB)	48 (70)	20 (80)	28 (64)	
	Quinolones (± combination with other ATB)	9 (13)	2 (8)	7 (16)	
	Macrolides	5 (7)	0 (0)	5 (11)	0.33
	Sulfonamides	8 (12)	4 (16)	4 (9)	
	Tetracyclines	1 (1)	0 (0)	1 (2)	
	Aminoglycosides	1 (1)	0 (0)	1 (2)	
	Unknown	4 (6)	1 (4)	3 (7)	
ECOG performance status — no. (%)	0-1	50 (72)	21 (84)	29 (66)	
	2 >	13 (19)	2 (8)	11 (25)	0.08
	Unknown	6 (9)	2 (8)	4 (9)	
Number of ATB courses — no. (%)	1	39 (57)	14 (56)	25 (57)	
	2	15 (22)	8 (32)	7 (16)	0.38
	3	6 (9)	1 (4)	5 (11)	

Indication — no. (%)					
	4	2 (3)	2 (8)	0 (0)	
	Unknown	6 (9)	0 (0)	7 (16)	
	Prophylactic ATB	3 (4)	0	3 (7)	
Indication — no. (%)	Therapeutic ATB	52 (75)	17 (68)	35 (79)	*0.23*
	Unknown	14 (21)	8 (32)	6 (14)	

ATB, Antibiotics.

Statistical analyses: Chi-Square or Fisher's exact tests. All tests were two-sided, with no adjustments for multiple comparisons.

Table S . Patient characteristics for mouse tumor models					
Human donors					**Mice**
ID	**Type of cancer**	***A.muciniphila SGB 9226***	**ORR**	**OS12**	**Type of cancer**
P1	Lung	0	NR	<12m	MCA205
P2	Lung	4.14373	NR	≥12m	MCA205
P3	Lung	5.29109	R	<12m	MCA205
P4	Lung	0	NR	<12m	MCA205
P5	Lung	0	NR	≥12m	MCA205
P6	Lung	0	NR	≥12m	MCA205
P8	Lung	0	NR	<12m	MCA205
P9	Lung	0	NR	<12m	MCA205
P10	Lung	3.09375	NR	≥12m	MCA205
P11	Lung	0	NR	<12m	MCA205
P13	Lung	0	NR	<12m	MCA205
P14	Lung	0	NR	<12m	MCA205
P15	Lung	0	NR	<12m	MCA205

P16	Lung	0.46892	NR	≥12m	MCA205
P17	Lung	0.24693	NR	≥12m	MCA205
P18	Lung	0.72853	NR	≥12m	MCA205
P19	Lung	0	NR	<12m	MCA205
P20	Lung	0	NR	≥12m	MCA205
P21	Lung	2.18628	NR	<12m	CT26, MC38, 4T1
P22	Lung	0	R	≥12m	B16F10, 4T1
P23	Lung	0	NR	<12m	MCA205
P24	Lung	0	NR	<12m	MC38, B16F10, 4T1
P25	Lung	0	NR	≥12m	MCA205
P26	Lung	0	NR	<12m	MCA205, 4T1
P27	Lung	0	NR	<12m	MCA205
P29	Lung	7.32764	R	≥12m	MCA205
P30	Lung	0	NR	<12m	MCA205
P31	Lung	13.30026	NR	<12m	MCA205
P32	Lung	22.53562	NR	<12m	MCA205

Table illustrating information presented in S1 C-E and Extended Figure 6.

Table S .	Patient characteristics for tumor biopsies used in RNA sequencing.				
		Overall (n=44)	Akk- (n=22)	Akk+ (n=22)	*P-value*
Stage — no. (%)	Limited	17 (39)	9 (41)	8 (36)	
	Advanced	27 (61)	13 (59)	14 (64)	*1.00*
Age (year)	Median (range)	69 (55-82)	68 (58-78)	73 (55-82)	
	<65 yr	12 (27)	6 (28)	6 (28)	
	≥65 to <75 yr	19 (43)	11 (50)	8 (36)	*0.55*
	≥75 yr	13 (30)	5 (22)	8 (36)	
Sex — no. (%)	Male	22 (50)	9 (41)	13 (59)	
	Female	22 (50)	13 (59)	9 (41)	*0.37*
ECOG performance status — no. (%)	0-1	39 (89)	20 (91)	19 (86)	
	2 or more	5 (11)	2 (9)	3 (14)	*1.00*
Smoking status — no. (%)	Never smoked	2 (5)	0 (0)	2 (9)	
	Current/former smoker	42 (95)	22 (100)	20 (91)	*0.48*
Tumor histology — no. (%)	Squamous	12 (27)	6 (27)	6 (28)	
	Non-squamous	28 (64)	14 (64)	14 (63)	*1.00*
	Others	4 (9)	2 (9)	2 (9)	
PD-L1 status — no. (%)	<1%	6 (14)	1 (5)	5 (23)	
	1-49%	6 (14)	4 (18)	2 (9)	
	≥50%	21 (48)	10 (45)	11 (50)	*0.60*
	Unknown	11 (24)	7 (32)	4 (18)	

Statistical analyses: Chi-Square or Fisher's exact tests. All tests were two-sided, with no adjustments for multiple comparisons.

Table S5A. Patient clinical characteristics						
		Overall (n=338)	**Akk^{low} (n=101)**	**Akk^{high} (n=30)**	**Akk^{-} (n=207)**	*P-value*
Age (year)	Median (range)	65 (24-92)	66 (45-85)	67 (44-83)	64 (24-92)	
	<65 yr	163 (48)	44 (44)	11 (37)	108 (52)	
	≥65 to <75 yr	118 (35)	46 (45)	11 (37)	61 (29)	*0.15**
	≥75 yr	57 (17)	11 (11)	8 (26)	38 (18)	
Sex – no (%)	Male	226 (67)	66 (65)	20 (67)	140 (68)	
	Female	112 (33)	35 (35)	10 (33)	67 (32)	*0.93*
BMI, kg/cm^2 – no (%)	<25	204 (60)	56 (55)	18 (60)	130 (63)	
	[25-30[	94 (28)	33 (33)	7 (23)	54 (26)	*0.54*
	≥30	36 (11)	11 (11)	5 (17)	20 (10)	
	Unknown	4 (1)	1 (1)	0	3 (1)	
ECOG performance status – no (%)	0-1	268 (79)	86 (85)	25 (84)	157 (75)	
	2 or more	43 (13)	6 (6)	4 (13)	33 (16)	*0.05*
	Unknown	27 (8)	9 (9)	1 (3)	17 (8)	
Smoking status — no. (%)	Never smoked	26 (8)	11 (11)	1 (3)	14 (7)	
	Current/former smoker	309 (91)	89 (88)	29 (97)	191 (92)	*0.28*
	Unknown	3 (1)	1 (1)	0 (0)	2 (1)	

Tumor histology — no. (%)	Squamous	78 (23)	22 (22)	12 (40)	44 (21)	
	Non-squamous	260 (77)	79 (88)	18 (60)	163 (79)	0.07
Tumor staging — no. (%)	IIA-IIIB	37 (11)	14 (14)	1 (3)	22 (11)	
	IV	294 (87)	85 (84)	29 (97)	180 (87)	0.26
	Unknown	7 (2)	2 (2)	0	5 (2)	
TMB — no. (%)	≥ 10 mutations/megabase	3 (1)	1 (1)	0	2 (1)	
	< 10 mutations/megabase	13 (4)	5 (5)	4 (13)	4 (2)	0.41
	Unknown	322 (95)	95 (94)	26 (87)	201 (97)	
PD-L1 status — no. (%)	<1%	64 (19)	21 (21)	10 (33)	33 (16)	
	1–49%	53 (16)	18 (18)	4 (13)	33 (16)	
	≥50%	118 (35)	34 (36)	8 (27)	76 (36)	0.26
	Unknown	102 (30)	28 (27)	8 (27)	66 (32)	
Therapy line — no. (%)	Neoadjuvant	3 (1)	2 (2)	0	1 (1)	
	First line	92 (27)	28 (28)	3 (10)	61 (29)	0.13
	≥ Second line	243 (72)	71 (72)	27 (90)	145 (70)	
Therapy — no. (%)	Immunotherapy	327 (97)	99 (98)	30 (100)	198 (96)	
	Immunotherapy and Chemotherapy	11 (3)	2 (2)	0 (0)	9 (4)	0.31
Previous therapy — no. (%)	Chemotherapy	239 (71)	77 (76)	24 (80)	143 (69)	
	Tyrosine kinase inhibitors	11 (3)	3 (3)	1 (3)	7 (3)	0.84

	Others	20 (6)	5 (5)	0 (0)	15 (7)
	Unknown	4 (1)	1 (1)	2 (7)	1 (0)
Antibiotics — no. (%)	Yes	69 (20)	15 (15)	10 (33)	44 (21)
	No	269 (80)	86 (85)	20 (67)	163 (79)	0.07

BMI, Body Mass Index; TMB, Tumor Mutational Burden.

Statistical analyses: Mann-Whitney*, Chi-Square or Fisher's exact tests. All tests were two-sided, with no adjustments for multiple comparisons.

Table S5B.   List of antibiotics (ATB) 60 days prior to ICI

		Overall (n=69)	Akk^low (n=15)	Akk ^high (n=10)	Akk -(n=44)	P-value
Duration of ATB — no. (%)	≤ 7 days	23 (33)	6 (43)	4 (36)	13 (30)	
	> 7 days	19 (28)	2 (14)	4 (36)	13 (30)	0.80
	Unknown	27 (39)	6 (43)	3 (27)	18 (41)	
Type of ATB — no. (%)	β-lactams ± inhibitors (± combination with other ATB)	48 (70)	9 (64)	11 (100)	28 (64)	
	Quinolones (± combination with other ATB)	9 (13)	1 (7)	1 (9)	7 (16)	
	Macrolides	5 (7)	0	0	5 (11)	
	Sulfonamides	8 (12)	1 (7)	0	4 (9)	0.95
	Tetracyclines	1 (1)	0	0	1 (2)	
	Aminoglycosides	1 (1)	0	0	1 (2)	
	Unknown	4 (6)	1 (7)	0	3 (7)	
ECOG performance status — no. (%)	0-1	50 (72)	13 (87)	8 (80)	29 (66)	
	2 >	13 (19)	0	2 (20)	11 (25)	0.11
	Unknown	6 (9)	2 (13)	0	4 (9)	
Number of ATB courses — no. (%)	1	39 (57)	11 (79)	3 (27)	25 (57)	
	2	15 (22)	3 (21)	5 (45)	7 (16)	0.45
	3	6 (9)	0	1 (9)	5 (11)	

	4	2 (3)	0	2 (18)	0 (0)	
	Unknown	6 (9)	0	0	7 (16)	
	Prophylactic ATB	3 (4)	0	0	3 (7)	
Indication — no. (%)	Therapeutic ATB	52 (75)	14 (93)	3 (30)	35 (79)	*0.49*
	Unknown	14 (21)	1 (7)	7 (70)	6 (14)	

ATB, Antibiotics.

Statistical analyses: Chi-Square or Fisher's exact tests. All tests were two-sided, with no adjustments for multiple comparisons.

Table S6. Uni-Multi-ariate analyses for OS			Univariate			Multivariate	
		N	HR (CI 95%)	P-value	HR (CI 95%)	P-value	
A. muciniphila	No	207	Reference		Reference		
	Low	101	0.56 (0.39 – 0.8)	**0.002**	0.63 (0.44 –0.92)	**0.015**	
	High	30	1.58 (1.00 – 2.5)	**0.049**	1.67 (1.03 – 2.71)	**0.038**	
Age	(continuous)	338	1.00 (0.98 – 1.0)	*0.656*	0.99 (0.98 – 1.01)	*0.379*	
Sex	Female	112	Reference		Reference		
	Male	226	0.88 (0.65 – 1.2)	*0.437*	0.88 (0.63 – 1.21)	*0.419*	
ECOG Performance status	0	122	Reference		Reference		
	1	146	2.00 (1.41 – 2.9)	**0.000114**	1.78 (1.23 – 2.57)	**0.002**	
	>2	43	2.49 (1.59 – 3.9)	**7.43e-05**	2.27 (1.40 – 3.67)	**0.0005**	
	Unknown	27	0.87 (0.39 – 1.9)	*0.7*	0.84 (0.37 – 1.89)	*0.676*	
ATB	No	269	Reference		Reference		
	Yes	69	1.6 (1.1 – 2.3)	**0.009**	1.36 (0.95 – 1.94)	*0.088*	
Histology	Non-Squamous	260	Reference		Reference		
	Squamous	78	1.1 (0.81 – 1.6)	*0.467*	1.11 (0.78 – 1.58)	*0.556*	
PD-L1	<1%	64	Reference		Reference		
	1-49%	53	1.10 (0.65 – 1.9)	*0.733*	1.11 (0.65 – 1.91)	*0.708*	

	≥50%	118	0.99 (0.63 – 1.5)	*0.957*	0.90 (0.53 – 1.54)	*0.713*
	Unknown	102	1.40 (0.92 – 2.1)	*0.117*	1.22 (0.78 – 1.89)	*0.382*
Immunotherapy Line	1	98	Reference		Reference	
	2	240	1.1 (0.8 – 1.6)	*0.485*	0.87 (0.54 – 1.4)	*0.575*

ATB, Antibiotics. *P*-values were calculated using the Wald test including each covariate separately in a Cox Proportional Hazards Regression Model. Significant *P*-value were marked in bold.

Table S7. _A.muciniphila_-associated intestinal commensalism potentially relevant to predict overall survival.

	MaAsLin2				PKM trichotomic distribution	PKM Binary with Akk
adjP	LogFC	CI.UP	CI.L	Taxa		
5.578e-08	2.1311	2.4577	1.80457	_Akkermansia_muciniphila_		
0.0012	1.8706	2.2980	1.44327	_Intestinimonas_butyriciproducens_	0.19	Not done
0.0012	1.6276	2.0011	1.2542	_Ruminococcaceae_bacterium_D5_	0. 1217	Not done
0.0019	1.4563	1.8048	1.1078	_Eubacterium_hallii_	0.0180	0.0265
0.0237	1.2512	1.6163	0.8860	_Roseburia_hominis_	Not done	Not done
0.0237	1.5531	2.0039	1.1023	_Ruminococcaceae_bacterium_D16_	Not done	Not done
0.0295	1.2471	1.6226	0.8716	_Coprobacter_fastidiosus_	0.0344	0.1290
0.0311	0.8399	1.0967	0.5830	_Bacteroides_plebeius_	Not done	Not done
0.0343	1.0605	1.3916	0.7294	_Anaerostipes_hadrus_	0.0059	0.06
0.0356	0.9916	1.3102	0.6731	_Bacteroides_ovatus_	Not done	Not done
0.0356	1.2895	1.7081	0.8710	_Alistipes_indistinctus_	0.23	Not done
0.0356	0.8481	1.1229	0.5734	_Eubacterium_eligens_	Not done	Not done
0.0356	-1.3844	-0.9363	-1.8324	_Clostridium_innocuum_	<0.0001	0.1190
0.0379	1.1870	1.5777	0.79632	_Parasutterella_excrementihominis_	0.0063	Not done
0.0432	-1.1987	-0.7960	-1.6014	_Dielma_fastidiosa_	Not done	Not done
0.0466	0.9117	1.2226	0.6008	_Bifidobacterium_adolescentis_	0.0181	0.0391
0.0487	1.1409	1.5344	0.7474	_Harryflintia_acetispora_	Not done	Not done

adjP, Adjusted P-value using MaAslin2 multivariate analysis; LogFC, Log Fold Change; CI, Confidence Interval; UP, upper; L, Lower; PKM, P-value Kaplan-Meier.

Table illustrating information presented in Figure 3.