Online Resource Material

Cancer Immunology, Immunotherapy

Kathrin Bauer et al.
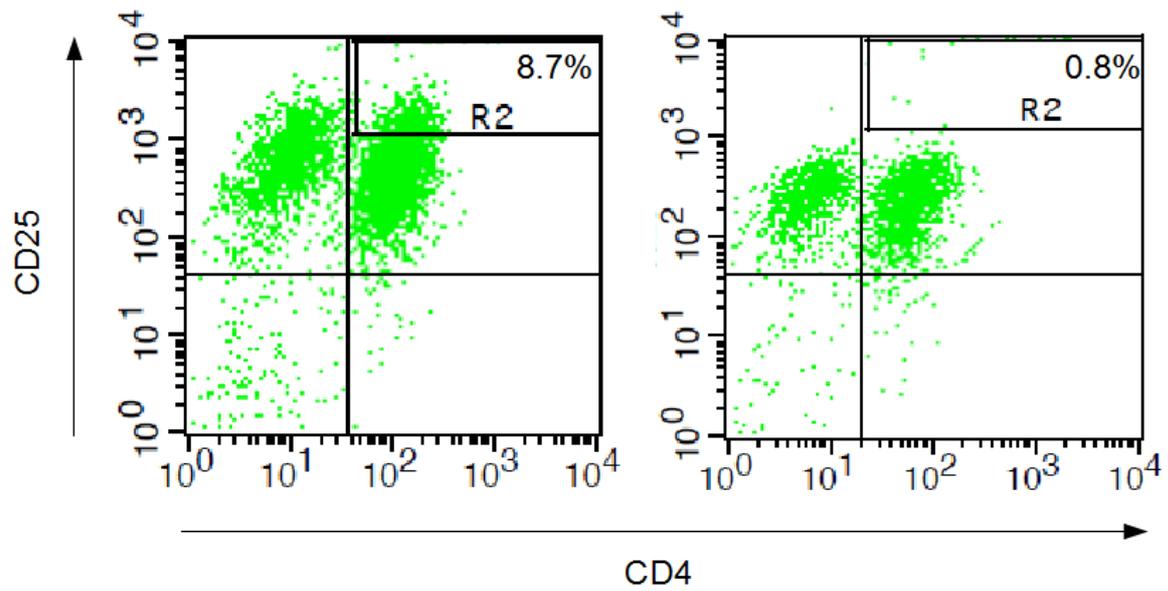
Supplementary Table 1

Characteristics of CRC patients

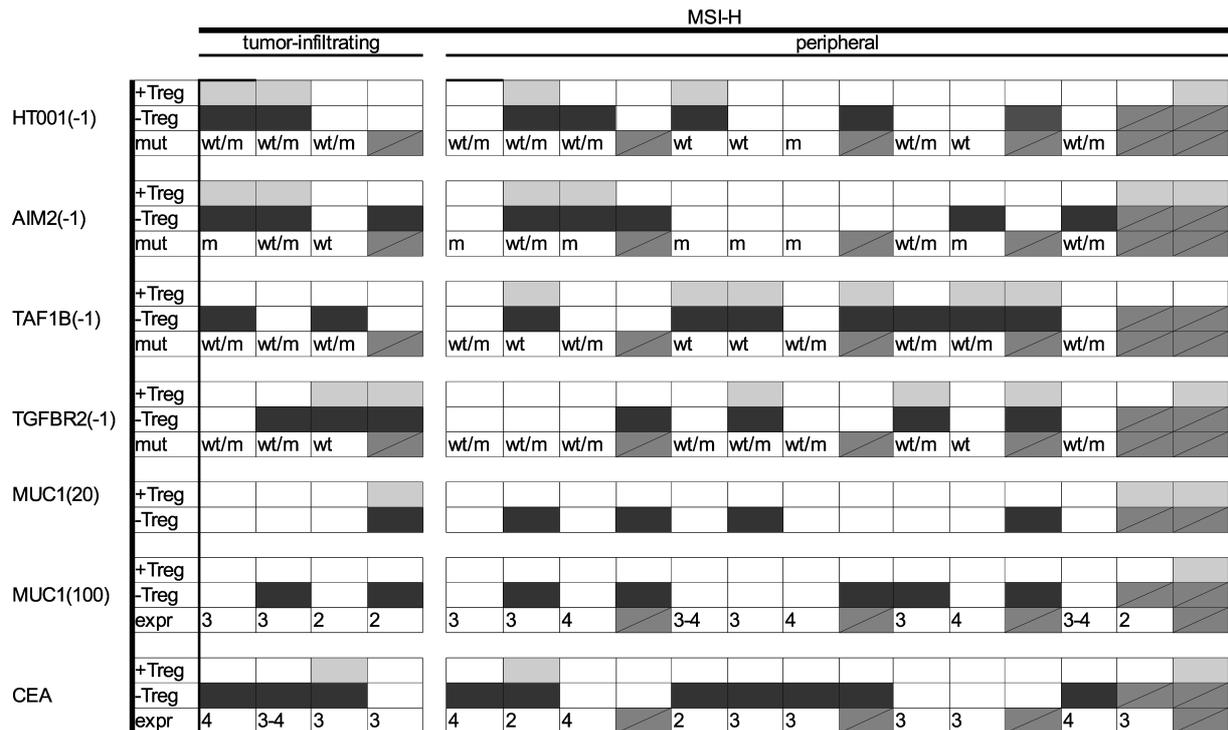| | | MSI-Status | |
	Total	MSI-H	MSS
Number of patients	n=48	n=16	n=32
Tumor-infiltrating T cell samples	n=30	n=4	n=26
Peripheral T cell samples	n=31	n=14	n=17
Age (years, median;range)	60 (30;85)	46 (33;77)	65 (30;85)
Gender			
male	31 (64.6)[a]	13 (81.3)	18 (56.3)
female	17 (35.4)	3 (18.8)	14 (43.8)
Stage of disease (UICC)			
I	5 (10.4)	4 (25.0)	1 (3.1)
II	6 (12.5)	2 (12.5)	4 (12.5)
III	13 (27.1)	2 (12.5)	11 (34.4)
IV	10 (20.8)	1 (6.3)	10 (31.3)
NA	14 (29.2)	7 (43.8)	6 (18.8)

NA not available

[a] Numbers in parentheses indicate percentage values if not otherwise indicated

Supplementary Figure 1

Representative T cell sample prior (left) and upon Treg cell depletion (right). Treg cell

depletion resulted in a decrease of CD4-positive CD25-high T cells from 8.7 % to 0.8%

	MSI-H															
	tumor-infiltrating				peripheral											
HT001(-1) +Treg / -Treg / mut	wt/m	wt/m	wt/m	⊠	wt/m	wt/m	wt/m	⊠	wt	wt	m	⊠	wt/m	wt	⊠	wt/m
AIM2(-1) +Treg / -Treg / mut	m	wt/m	wt	⊠	m	wt/m	m	⊠	m	m	m	⊠	wt/m	m	⊠	wt/m
TAF1B(-1) +Treg / -Treg / mut	wt/m	wt/m	wt/m	⊠	wt/m	wt	wt/m	⊠	wt	wt	wt/m	⊠	wt/m	wt/m	⊠	wt/m
TGFBR2(-1) +Treg / -Treg / mut	wt/m	wt/m	wt	⊠	wt/m	wt/m	wt/m	⊠	wt/m	wt/m	wt/m	⊠	wt/m	wt	⊠	wt/m
MUC1(20) +Treg / -Treg																
MUC1(100) +Treg / -Treg / expr	3	3	2	2	3	3	4	⊠	3-4	3	4	⊠	3	4	⊠	3-4
CEA +Treg / -Treg / expr	4	3-4	3	3	4	2	4	⊠	2	3	3	⊠	3	3	⊠	4

Supplementary Figure 2

Response pattern of tumor-infiltrating and peripheral T cell samples from MSI-H CRC patients without (light grey) and with (dark grey) Treg-cell depletion, FSP mutation status (wt = wildtype allele, wt/m = heterozygous mutated, m = mutations in both alleles) and expression level of hypoglycosylated MUC1 and CEA (2 = 20-50% of tumor cells express the antigen; 3 = 50-80% of tumor cells express the antigen; 4 = 80-100% of tumor cells express the antigen). Crossed boxes = data have not been obtained
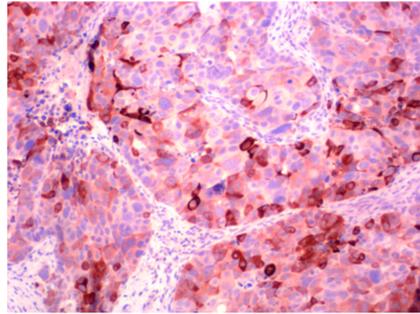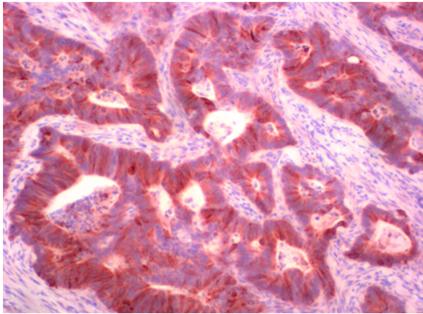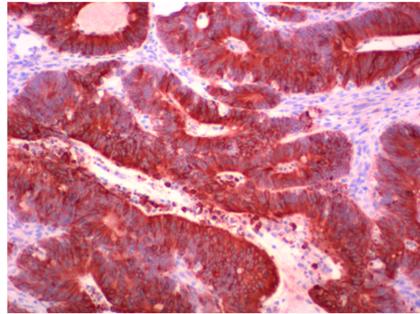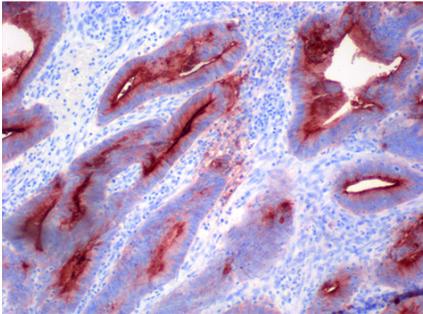
a

Score 1:
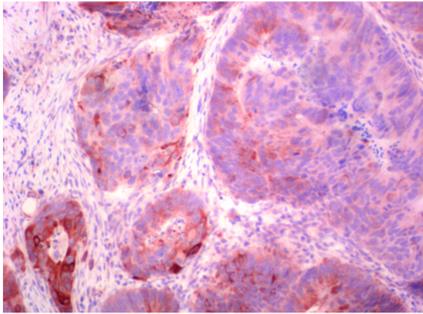
100 μm

Score 2:

100 μm

Score 3:

100 μm

Score 4:

100 μm
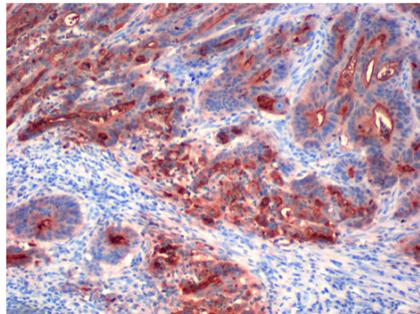
b

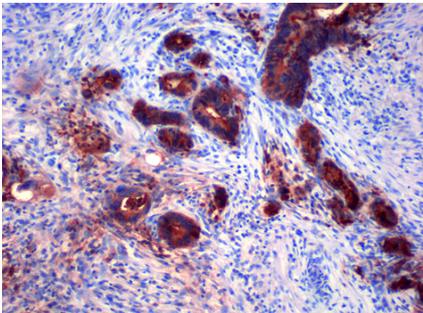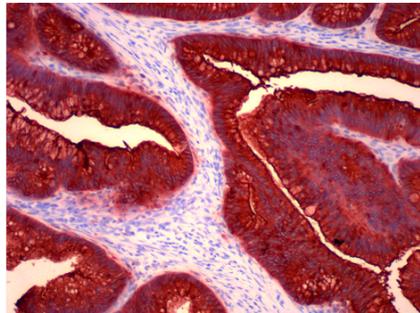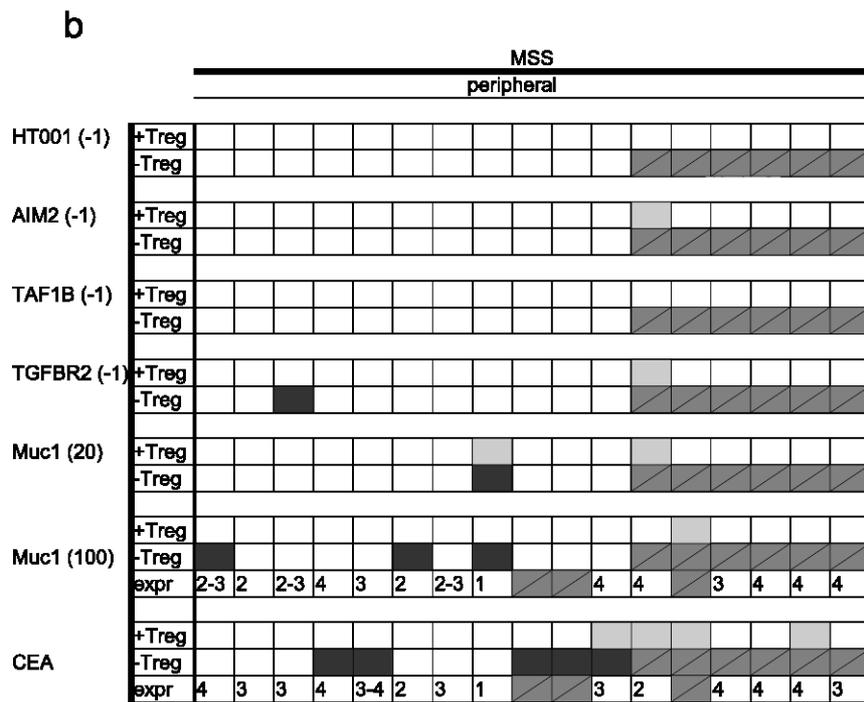Score 1:

100 μm

Score 2:

100 μm

Score 3:

100 μm

Score 4:

100 μm

Supplementary Figure 3

Representative immunohistochemical stainings illustrating the scoring for hypoglycosylated MUC1 (a) and CEA (b) expression. Score 1: antigens are expressed in up to 20% of the tumor cells; score 2: expression is detected in 20-50% of the tumor cells; score 3: antigen expression in 50-80% of the tumor cells; score 4: 80-100% of the tumor cells express the respective antigen. Pictures were taken with a Leica DMRBE microscope and a digital camera DFC480 (Leica Microsystems, Solms, Germany)

a

MSS
tumor-infiltrating

HT001 (-1) | +Treg / -Treg
AIM2 (-1) | +Treg / -Treg
TAF1B (-1) | +Treg / -Treg
TGFBR2 (-1) | +Treg / -Treg
Muc1 (20) | +Treg / -Treg

Muc1 (100) | +Treg / -Treg / expr
expr: 2-3 | 2 | 2-3 | 4 | 2 | 3 | 3 | 3 | | 3 | 4 | 4 | 2-3 | 3 | 4 | 1 | 3 | 4 | 4 | 3 | 3 | | 2 | 1 | 4 | 1-2

CEA | +Treg / -Treg / expr
expr: 4 | 3 | 3 | 4 | 2 | | 4 | 4 | | 3-4 | 2 | 4 | 3 | 3 | 4 | 3 | 4 | 3-4 | 2 | 2-3 | 4 | | 2 | 1 | 4 | 3

b

MSS
peripheral

HT001 (-1) | +Treg / -Treg
AIM2 (-1) | +Treg / -Treg
TAF1B (-1) | +Treg / -Treg
TGFBR2 (-1) | +Treg / -Treg
Muc1 (20) | +Treg / -Treg

Muc1 (100) | +Treg / -Treg / expr
expr: 2-3 | 2 | 2-3 | 4 | 3 | 2 | 2-3 | 1 | | | 4 | 4 | | 3 | 4 | 4 | 4

CEA | +Treg / -Treg / expr
expr: 4 | 3 | 3 | 4 | 3-4 | 2 | 3 | 1 | | | 3 | 2 | | 4 | 4 | 4 | 3

Supplementary Figure 4

Response pattern of tumor-infiltrating (a) and peripheral (b) T cell samples from MSS CRC patients without (light grey) and with (dark grey) Treg-cell depletion and expression level of hypoglycosylated MUC1 and CEA (1 = 0-20% of tumor cells express the antigen; 2 = 20-50%

of tumor cells express the antigen; 3 = 50-80% of tumor cells express the antigen; 4 = 80-100% of tumor cells express the antigen). Crossed boxes = data have not been obtained