SUPPLEMENTARY MATERIALS - ONLINE ONLY

To the research article:

Impact of a preceding radiotherapy on the outcome of immune checkpoint inhibition in advanced metastatic melanoma: a multicenter retrospective cohort study of the DeCOG

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Supplementary Table 1. Covariate distribution after re-weighting with IPTW

	Anti-CTLA-4 (n=596)		Anti-PD-1 (n=239)		All patients with preceding RT (n=235)	
	No preceding RT %	Preceding RT %	No preceding RT %	Preceding RT %	RT last before ICI %	RT not last before ICI %
Primary site						
Skin	76.6	75.5	69.1	66.5	74.7	77.5
Occult (MUP)	12.9	10.2	19.3	20.0	16.5	16.7
Mucosa	7.1	8.6	10.3	12.2	6.5	4.9
Uvea	3.4	5.7	1.3	1.4	2.3	0.9
BRAF V600 mutational status						
Wildtype	65.2	63.1	70.6	67.0	61.4	59.4
Mutation	34.8	36.9	29.4	33.0	38.6	40.6
M stage						
M1a (skin, LN)	9.5	6.0	6.9	12.5	4.2	3.2
M1b (lung)	24.6	25.5	22.5	22.3	9.1	10.1
M1c (other organ)	37.8	39.2	37.6	33.1	29.2	29.5
M1d (brain)	28.2	29.3	32.6	26.9	57.4	57.1
Bone metastases						
No	77.8	75.9	73.3	79.9	67.7	64.6
Yes	22.2	24.1	26.7	20.1	32.3	35.4
Systemic pre-treatment in stage IV						
No	27.9	29.9	12.9	10.7	16.8	14.4
Yes	72.1	70.1	87.1	89.3	83.2	85.6
Duration of stage IV disease at start of ICI (months) Median (p10; p90)	6.7 (0.9; 28.7)	7.4 (0.8; 31.7)	12.6 (2.3; 34.2)	15.5 (2.7; 41.9)	11.0 (2.3; 32.7)	10.5 (2.5; 43.8)

This table shows the distribution of covariates after re-weighting according to inverse probability treatment weighting (IPTW). M stage categories refer to the AJCC-v8 classification system; systemic pre-treatment describes systemic therapies received by the patient for inoperable stage IV disease (not adjuvant) prior to the investigated immune checkpoint inhibition (ICI) therapy. RT, radiotherapy; MUP, melanoma of unknown primary; p10, 10th percentile; p90, 90th percentile.

Supplementary Table 2. Covariate distribution after re-weighting with IPTW in patients with brain metastasis

	Patients with brain metastasis (n=223)	
	No preceding RT (n=86) %	**Preceding RT (n=137)** %
Primary site		
Skin	77.0	76.9
Occult (MUP)	18.0	17.4
Mucosa	2.9	2.7
Uvea	2.1	3.1
BRAF V600 mutational status		
Wildtype	50.6	52.1
Mutation	49.4	47.9
Systemic pre-treatment in stage IV		
No	23.1	22.4
Yes	76.9	77.6
Duration of stage IV disease at start of ICI (months) Median (p10; p90)	8.7 (2.3; 24.7)	8.4 (1.9; 32.5)

This table shows the distribution of covariates after re-weighting according to inverse probability treatment weighting (IPTW) in all patients with brain metastasis treated with anti-CTLA-4 or anti-PD-1. M stage categories refer to the AJCC v8 classification system; systemic pre-treatment describes systemic therapies received by the patient for inoperable stage IV disease (not adjuvant) prior to the investigated immune checkpoint inhibition (ICI) therapy. RT, radiotherapy; MUP, melanoma of unknown primary; p10, 10th percentile; p90, 90th percentile.

Supplementary Table 3. Systemic pre-treatment in stage IV

	Anti-CTLA-4 (n=596)				Anti-PD-1 (n=239)				All (n=835)			
	No preceding RT		Preceding RT		No preceding RT		Preceding RT		No preceding RT		Preceding RT	
	n	%	n	%	n	%	n	%	n	%	n	%
Total	446	100	150	100	154	100	85	100	600	100	235	100
Systemic pre-treatment (PreT)												
No	138	30.9	27	18.0	28	18.2	11	13.0	166	27.7	38	16.2
Yes	291	65.2	123	82.0	123	79.8	72	84.7	414	69.0	195	83.0
Missing data	17	3.9	0	0.0	3	2.0	2	2.3	20	3.3	2	0.8
PreT containing chemotherapy												
No	237	53.1	55	36.7	88	57.1	49	57.6	325	54.2	104	44.3
Yes	209	46.9	95	63.3	66	42.9	36	42.4	275	45.8	131	55.7
DTIC monotherapy	129	28.9	43	28.7	34	22.1	9	10.6	163	27.2	52	22.2
Combination chemotherapy	55	12.3	34	22.7	32	20.8	26	30.6	87	14.5	60	25.5
Other monotherapy	21	4.7	16	10.7	0	0.0	1	1.2	21	3.5	17	7.2
Isolated liver chemoperfusion	4	0.9	2	1.4	0	0.0	0	0.0	4	0.7	2	0.8
PreT containing kinase inhibitors												
No	340	76.2	109	72.6	114	74.0	54	63.5	454	75.7	163	69.4
Yes	106	23.8	41	27.3	40	26.0	31	36.5	146	24.3	72	30.6
BRAF inhibitor monotherapy	53	11.9	22	14.7	27	17.5	18	21.2	80	13.3	40	17.0
MEK inhibitor monotherapy	12	2.7	2	1.3	3	2.0	3	3.5	15	2.5	5	2.1
Other monotherapy	23	5.2	7	4.7	2	1.3	2	2.4	25	4.2	9	3.8
BRAF + MEK inhibition	10	2.2	7	4.6	7	4.6	5	5.9	17	2.8	12	5.1
More than one therapy line	7	1.6	3	2.0	1	0.6	3	3.5	8	1.3	6	2.6
PreT containing immunotherapy												
No	429	96.2	141	94.0	32	20.8	15	17.6	461	76.8	156	66.4
Yes	17	3.8	9	6.0	122	79.1	70	82.4	139	23.2	79	33.6
CTLA-4 antibodies	0	0.0	0	0.0	122	79.2	70	82.4	122	20.3	70	29.8
PD-1 antibodies	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Others	17	3.8	9	6.0	0	0.0	0	0.0	17	2.8	9	3.8
PreT lines												
0	157	33.7	31	20.7	31	13.0	15	17.6	188	31.3	46	19.6
1	248	55.6	95	63.3	37	24.0	13	15.3	285	47.5	108	55.0
2	39	8.7	22	14.7	67	43.5	47	55.3	106	17.7	69	29.4
3	2	0.4	2	1.3	19	12.3	10	11.8	21	3.5	12	5.1
Number of PreT lines (mean)	0.7		1.0		1.5		1.6		0.9		1.2	

Pre-treatments (PreT) refer to systemic treatment lines given prior to the investigated immune checkpoint inhibition therapy. Percentages are given per column for each individual patient cohort. Some patients received more than one pre-treatment strategy, therefore numbers do not necessarily add up to 100%. RT, radiotherapy; DTIC, dacarbazine.

Supplementary Table 4. Characteristics of radiotherapy

	Anti-CTLA-4 (n=596)		Anti-PD-1 (n=239)		All (n=835)	
	n	%	n	%	n	%
Radiotherapy (RT)						
No	446	74.8	154	64.4	600	71.9
Yes	150	25.2	85	35.6	235	28.1
Target of RT (as % of patients with RT)						
Brain	65	43.3	35	41.2	100	42.6
Lungs	3	2.0	9	10.6	12	5.1
Bone	26	17.3	16	18.8	42	17.9
Lymph nodes	28	18.7	14	16.5	42	17.9
Skin	4	2.7	6	7.1	10	4.3
Abdominal organs	5	3.3	3	3.5	8	3.4
Soft tissue	10	6.7	5	5.9	15	6.4
unknown	22	14.7	11	12.9	33	14.0
Timing of RT (as % of patients with RT)						
Last before ICI	85	56.7	42	49.4	127	54.0
Earlier in stage IV	65	43.3	43	50.6	108	46.0
RT of the brain (as % of patients with RT of the brain)						
Total	65	100.0	35	100.0	100	100.0
Whole brain radiation	30	46.2	14	40.0	44	44.0
Stereotactic radiotherapy	22	33.8	11	31.4	33	33.0
RT of brain metastases, not further specified	13	20.0	10	28.6	23	23.0

Radiotherapies (RT) were applied in stage IV prior to the investigated immune checkpoint inhibitor therapy. Percentages are given per column for each individual patient cohort. Some patients received radiotherapies of more than one target, therefore numbers do not necessarily add up to 100%.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Directed acyclic graphs (DAGs) used to determine the confounding covariates for the survival adjustment sets of this study. The exposure of interest is marked in green (preceding radiotherapy, yes versus no); covariates are marked in light blue; confounders are marked in orange. Interactions between covariates are indicated by arrows. DAGs were build for (**A**) progression-free survival, and (**B**) overall survival in the total patient cohort, as well as (**C**) progression-free survival, and (**D**) overall survival in the subgroup of patients with brain metastases at start of ICI. The resulting confounders are (**A**) brain metastases (yes versus no), bone metastases (yes versus no), primary site of disease (categorical), systemic pre-treatment (yes versus no), (**B**) duration in stage IV (continuous), brain metastases (yes versus no), bone metastases (yes versus no), primary site of disease (categorical), BRAF V600 mutational status (yes versus no), and systemic pre-treatment (yes versus no), (**C**) primary site of disease (categorical), systemic pre-treatment (yes versus no), and (**D**) duration in stage IV (continuous), primary site of disease (categorical), BRAF V600 mutational status (yes versus no), and systemic pre-treatment (yes versus no).

Supplementary Figure 2. Kaplan-Meier curves showing the probability of progression-free (**A, B**) and overall survival (**C, D**) adjusted for confounding factors in 239 melanoma patients treated with anti-PD-1 immune checkpoint inhibitors (ICI), subdivided by preceding radiotherapy (RT). **A, C**, n=192 patients treated with anti-CTLA-4 prior to anti-PD-1 ICI; **B, D**, n=45 patients not treated with anti-CTLA-4 prior to anti-PD-1 ICI. HR, hazard ratio; CI, confidence interval.