

**Tissue based biomarkers in non-clear cell RCC: Correlative analysis from the ASPEN clinical trial**

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**SUPPLEMENTARY DATA**

**TABLES.**

**Supplementary Table 1.** Summary of 5 tissue biomarkers analyzed in the present study using immunohistochemistry according to antibody source, isotype, dilution, antigen retrieval method, and clinical thresholds examined.

<b>Biomarker</b>	<b>Antibody Source</b>	<b>Antibody Isotype</b>	<b>Dilution Factor</b>	<b>Antigen Retrieval Method</b>	<b>Primary Threshold</b>	<b>Secondary Threshold</b>
<b>pAkt (S473)</b>	Cell Signaling Technologies	Rabbit mAb	1:50	pH 9.0 EDTA	0 vs. 1-3	0-1 vs. 2-3
<b>pS6 (S235/236)</b>	Cell Signaling Technologies	Rabbit mAb	1:100	pH 9.0 EDTA	0 vs. 1-3	0-1 vs. 2-3
<b>HIF-1<math>\alpha</math></b>	Abcam	Rabbit mAb	1:200	pH 9.0 EDTA	0 vs. 1-3	0-1 vs. 2-3
<b>c-kit</b>	Dako	Rabbit pAb	Pre-dilute	pH 6.1 Citrate	0 vs. 1-3	0-1 vs. 2-3
<b>c-MET</b>	Novus	Mouse mAb	1:50	pH 6.1 Citrate	0 vs. 1-3	0-1 vs. 2-3

**Supplementary Table 2.** Baseline characteristics of ASPEN patients according to MSKCC prognostic risk group. NR indicates the estimate was not reached.

	<b>Good (n=23)</b>	<b>Intermediate (n=44)</b>	<b>Poor (n=11)</b>	<b>Total (N=78)</b>
<b>Treatment</b>				
Sunitinib	12 (52.2)	21 (47.7)	3 (27.3)	36 (46.2)
Everolimus	11 (47.8)	23 (52.3)	8 (72.7)	42 (53.8)
<b>Age (years)</b>	64 [46, 81]	63 [23, 89]	64 [28, 72]	63 [23, 89]
<b>Male Gender</b>	16 (69.6)	30 (68.2)	7 (63.6)	53 (67.9)
<b>Ethnicity</b>				
Hispanic/Latino	0 (0)	2 (4.5)	0 (0)	2 (2.6)
Not Hispanic/Latino	23 (100)	41 (93.2)	11 (100)	75 (96.2)
Missing	0 (0)	1 (2.3)	0 (0)	1 (1.3)
<b>Race</b>				
White	20 (87)	38 (86.4)	11 (100)	69 (88.5)
Black	3 (13)	6 (13.6)	0 (0)	9 (11.5)
Other	0 (0)	0 (0)	0 (0)	0 (0)
<b>Histologic Subtype</b>				
Papillary Type I	0 (0)	2 (4.5)	1 (9.1)	3 (3.8)
Papillary Type II	6 (26.1)	13 (29.5)	2 (18.2)	21 (26.9)
Papillary Unspecified	5 (21.7)	11 (25.0)	2 (18.2)	18 (23.1)
Chromophobe	5 (21.7)	7 (15.9)	1 (9.1)	13 (16.7)
Poorly Differentiated	1 (4.3)	2 (4.5)	3 (27.3)	6 (7.7)
Mixed Papillary/Chromophobe	0 (0)	1 (2.3)	0 (0)	1 (1.3)
Other	6 (26.1)	8 (18.2)	2 (18.2)	16 (20.5)
<b>Overall Survival (months, 95% CI)</b>	NR (37.9, NR)	22.2 (13.2, NR)	7.3 (6.0, NR)	22.2 (14.8, 39.5)
<b>Progression-free Survival (months, 95% CI)</b>	11.0 (8.3, 19.7)	5.5 (4.2, 11.0)	5.8 (2.8, NR)	6.0 (5.6, 8.4)
<b>Objective Response</b>	2 (8.7)	8 (18.2)	1 (9.1)	11 (14.1)

**Supplementary Table 3.** Number (%) of patients of IHC scoring for each biomarker by histology and MSKCC prognostic risk group.

N (%)	Histology			MSKCC Risk Group			Overall
	Papillary (n=43)	Chromophobe (n=13)	Undifferentiated (n=22)	Good (n=23)	Intermediate (n=44)	Poor (n=11)	(N=78)
<b>pAkt</b>							
0	19 (44.2)	9 (69.2)	13 (59.1)	12 (52.2)	23 (52.3)	6 (54.5)	41 (52.6)
1+	23 (53.5)	4 (30.8)	8 (36.4)	9 (39.1)	21 (47.7)	5 (45.5)	35 (44.9)
2+	1 (2.3)	0 (0)	0 (0)	1 (4.3)	0 (0)	0 (0)	1 (1.3)
Missing	0 (0)	0 (0)	1 (4.5)	1 (4.3)	0 (0)	0 (0)	1 (1.3)
<b>pS6</b>							
0	4 (9.3)	6 (46.2)	0 (0)	6 (26.1)	4 (9.1)	0 (0)	10 (12.8)
1+	19 (44.2)	4 (30.8)	8 (36.4)	9 (36.1)	20 (45.5)	2 (18.2)	31 (39.7)
2+	14 (32.6)	1 (7.7)	6 (27.3)	4 (17.4)	14 (31.8)	3 (27.3)	21 (26.9)
3+	6 (14)	1 (7.7)	7 (31.8)	3 (13)	5 (11.4)	6 (54.5)	14 (17.9)
Missing	0 (0)	1 (7.7)	1 (4.5)	1 (4.3)	1 (2.3)	0 (0)	2 (2.6)
<b>HIF-1<math>\alpha</math></b>							
0	10 (23.3)	3 (23.1)	8 (36.4)	5 (21.7)	11 (25)	5 (45.5)	21 (26.9)
1+	27 (62.8)	6 (46.2)	7 (31.8)	14 (60.9)	23 (52.3)	3 (27.3)	40 (51.3)
2+	2 (4.7)	1 (7.7)	2 (9.1)	0 (0)	3 (6.8)	2 (18.2)	5 (6.4)
3+	3 (7)	2 (15.4)	0 (0)	2 (8.7)	3 (6.8)	0 (0)	5 (6.4)
Missing	1 (2.3)	1 (7.7)	5 (22.7)	2 (8.7)	4 (9.1)	1 (9.1)	7 (9)
<b>c-kit</b>							
0	39 (90.7)	4 (30.8)	20 (90.9)	19 (82.6)	36 (81.8)	8 (72.7)	63 (80.8)
1+	0 (0)	6 (46.2)	0 (0)	3 (13)	3 (6.8)	0 (0)	6 (7.7)
2+	2 (4.7)	2 (15.4)	0 (0)	1 (4.3)	1 (2.3)	2 (18.2)	4 (5.1)
3+	1 (2.3)	0 (0)	0 (0)	0 (0)	1 (2.3)	0 (0)	1 (1.3)
Missing	1 (2.3)	1 (7.7)	2 (9.1)	0 (0)	3 (6.8)	1 (9.1)	4 (5.1)
<b>c-MET</b>							
0	4 (9.3)	3 (23.1)	0 (0)	3 (13)	3 (6.8)	1 (9.1)	7 (9.0)
1+	14 (32.6)	6 (46.2)	9 (40.9)	8 (34.8)	19 (43.2)	2 (18.2)	29 (37.2)
2+	15 (34.9)	3 (23.1)	6 (27.3)	6 (26.1)	13 (29.5)	5 (45.5)	24 (30.8)
3+	6 (14)	0 (0)	4 (18.2)	4 (17.4)	4 (9.1)	2 (18.2)	10 (12.8)
Missing	4 (9.3)	1 (7.7)	3 (13.6)	2 (8.7)	5 (11.4)	1 (9.1)	8 (10.3)

**Supplementary Table 4.** Summary of IHC scoring for each biomarker by treatment group assignment.

<b>N (%)</b>	<b>Everolimus (n=42)</b>	<b>Sunitinib (n=36)</b>
<b>pAkt</b>		
0	25 (59.5)	16 (44.4)
1+	15 (35.7)	20 (55.6)
2+	1 (2.4)	0 (0)
Missing	1 (2.4)	0 (0)
<b>pS6</b>		
0	6 (14.3)	4 (11.1)
1+	16 (38.1)	15 (41.7)
2+	10 (23.8)	11 (30.6)
3+	8 (19.0)	6 (16.7)
Missing	2 (4.8)	0 (0)
<b>HIF-1<math>\alpha</math></b>		
0	13 (31)	8 (22.2)
1+	20 (47.6)	20 (55.6)
2+	3 (7.1)	2 (5.6)
3+	2 (4.8)	3 (8.3)
Missing	4 (9.5)	3 (8.3)
<b>c-kit</b>		
0	34 (81)	29 (80.6)
1+	1 (2.4)	5 (13.9)
2+	3 (7.1)	1 (2.8)
3+	0 (0)	1 (2.8)
Missing	4 (9.5)	0 (0)
<b>c-MET</b>		
0	3 (7.1)	4 (11.1)
1+	14 (33.3)	15 (41.7)
2+	14 (33.3)	10 (27.8)
3+	5 (11.9)	5 (13.9)
Missing	6 (14.3)	2 (5.6)

**Supplementary Table 5A.** Median overall survival (OS) by tissue IHC biomarkers for all evaluable patients, univariate and multivariable hazard ratios of OS for biomarkers. Cut-point of 2+ scoring. NR and NE indicate the median or hazard ratio was not reached/estimated.

Biomarker	Median OS		N	Univariate		Multivariable	
	(months, 95% CI)			HR (95% CI)	P Value	HR* (95% CI)	FDR
	0-1	2+					
<b>pAkt</b>	19.0 (14.7-39.5)	NR (NR-NR)	77	NE	0.997	NE	1.000
<b>pS6</b>	29.3 (17.7-NR)	16.0 (12.5-NR)	76	1.8 (1.0-3.2)	0.067	1.5 (0.7-3.0)	0.811
<b>HIF-1<math>\alpha</math></b>	27.2 (17.0-NR)	16.8 (11.0-NR)	71	1.5 (0.6-3.7)	0.351	0.8 (0.3-2.3)	0.819
<b>c-kit</b>	26.9 (16.8-NR)	NR (12.5-NR)	74	0.6 (0.1-2.3)	0.426	0.3 (0.0-2.2)	0.811
<b>c-MET</b>	27.2 (14.7-NR)	22.2 (16.0-NR)	70	1.3 (0.7-2.5)	0.412	1.2 (0.6-2.6)	0.819

\*Adjusting for treatment arm and stratification variables (histology and MSKCC risk groups)

**Supplementary Table 5B.** Median PFS by tissue IHC biomarkers for all evaluable patients; univariate and multivariable hazard ratios of PFS for biomarkers. Cut-point of 2+ scoring. NR and NE indicate the median or hazard ratio was not reached/estimated.

Biomarker	Median PFS		N	Univariate		Multivariable	
	(months, 95% CI)			HR (95% CI)	P Value	HR* (95% CI)	FDR
	0-1	2+					
<b>pAkt</b>	5.9 (5.5-8.4)	NR (NR-NR)	77	NE	0.996	NE	0.997
<b>pS6</b>	5.7 (5.5-11.3)	6.1 (5.5-11.0)	76	1.0 (0.6-1.6)	0.976	0.8 (0.4-1.4)	0.555
<b>HIF-1<math>\alpha</math></b>	6.0 (5.6-8.4)	6.5 (4.2-NR)	71	0.8 (0.4-1.8)	0.638	0.7 (0.3-1.7)	0.555
<b>c-kit</b>	6.0 (5.6-8.5)	6.1 (5.7-NR)	74	0.7 (0.3-2.0)	0.516	0.4 (0.1-1.2)	0.522
<b>c-MET</b>	7.6 (5.6-11.5)	5.7 (5.5- 8.5)	70	1.3 (0.8-2.2)	0.313	1.3 (0.7-2.2)	0.555

\*Adjusting for treatment arm and stratification variables (histology and MSKCC risk groups)

**Supplementary Table 6A** . Median OS by treatment group and by tissue IHC biomarkers using a cut-point of 2+, including biomarker-treatment interaction p-values. NR indicates the estimate was not reached.

Biomarker	Median OS (months, 95% CI)				N	Univariate P Value	Multivariate FDR
	Sunitinib		Everolimus				
	0-1	2+	0-1	2+			
<b>pAkt</b>	36.2 (14.8-NR)	NR (NR-NR)	18.6 (11.0-NR)	NR (NR-NR)	77	NR	NR
<b>pS6</b>	NR (NR-NR)	16.4 (12.5-NR)	26.9 (9.7-NR)	13.2 (7.3-NR)	76	0.268	0.999
<b>HIF-1<math>\alpha</math></b>	36.2 (14.7-NR)	16.8 (12.5-NR)	26.9 (13.2-NR)	15.0 (5.7-NR)	71	0.805	0.999
<b>c-kit</b>	36.2 (14.8-NR)	12.5 (12.5-NR)	22.2 (12.7-NR)	NR (6.1-NR)	74	0.413	0.999
<b>c-MET</b>	NR (12.5-NR)	36.2 (16.8-NR)	27.2 (13.2-NR)	19.0 (9.7-NR)	70	0.619	0.999

**Supplementary Table 6B** . Median PFS by treatment group and by tissue IHC biomarkers using a cut-point of 2+, including biomarker-treatment interaction p-values. NR indicates the estimate was not reached.

Biomarker	Median PFS (months, 95% CI)				N	Univariate P Value	Multivariate FDR
	Sunitinib		Everolimus				
	0-1	2+	0-1	2+			
<b>pAkt</b>	8.4 (5.8-19.7)	NR (NR-NR)	5.5 (4.4-7.3)	NR (NR-NR)	77	NR	NR
<b>pS6</b>	11.3 (5.7-NR)	8.4 (5.8-NR)	5.5 (3.0-8.5)	5.7 (5.5-18.2)	76	0.586	0.801
<b>HIF-1<math>\alpha</math></b>	8.4 (5.8-25.9)	6.5 (5.5-NR)	5.7 (5.5-8.3)	8.6 (4.2-NR)	71	0.338	0.801
<b>c-kit</b>	8.4 (5.8-19.7)	5.5 (5.5-NR)	5.6 (5.5-8.4)	6.1 (5.7-NR)	74	0.952	0.997
<b>c-MET</b>	11.3 (5.6-NR)	8.4 (5.7-NR)	5.7 (5.5-18.2)	5.5 (2.8-8.3)	70	0.418	0.801

**Supplementary Table 7A.** Objective response rate showing N (%) of overall responders and OR (95% CI) according to IHC 0-1 vs. 2+ biomarker status.

Biomarker	0-1 N (%)	2+ N (%)	N	OR (95% CI)
pAkt	11 (14.5)	0 (0.0)	77	NE
pS6	5 (12.2)	6 (17.1)	76	1.5 (0.4 – 5.6)
HIF-1 $\alpha$	9 (14.8)	1 (10.0)	71	0.6 (0.0 – 4.1)
c-kit	10 (14.5)	0 (0.0)	74	NE
c-MET	6 (16.7)	4 (11.8)	70	0.7 (0.2 – 2.6)

**Supplementary Table 7B.** Objective response rate showing N (%) of overall responders OR (95% CI) for patients by treatment and IHC 0 vs. 1+ biomarker status.

Biomarker	Sunitinib			Everolimus		
	N	0-1 N (%)	2+ N (%)	N	0-1 N (%)	2+ N (%)
pAkt	34	7 (19.4)	0 (0.0)	41	4 (10.0)	0 (0.0)
pS6	36	2 (10.5)	5 (29.4)	40	3 (13.6)	1 (5.6)
HIF-1 $\alpha$	33	6 (21.4)	1 (20.0)	38	3 (9.1)	0 (0.0)
c-kit	36	7 (20.6)	0 (0.0)	38	3 (8.6)	0 (0.0)
c-MET	34	4 (21.1)	3 (20.0)	36	2 (11.8)	1 (5.3)