Rini et al. Active Surveillance in Metastatic Renal Cell Carcinoma: a Prospective Phase 2 Trial: Supplementary Appendix

Table of Contents

Methods

Study Design

The HADS questionnaire includes seven items reflecting anxiety and seven items reflecting depression. Each item is answered by the patient on a four point (0–3) response category, and the possible scores range from 0 to 21 for anxiety and 0 to 21 for depression. A score of 0 to 7 for either subscale is regarded as being in the normal range, a score of 11 or higher indicating probable presence of the mood disorder and a score of 8 to 10 being suggestive of the respective state.

Immune Cell Analysis

For phenotypic and functional studies all samples from a single patient were thawed together and used in the same experiment. For MDSC analysis, surface staining of PBMCs for HLADR, CD15, CD14, and CD33 (BD Bioscience) was performed as described.[1, 2] Total MDSC were defined as $CD33^{+}$ HLA-DR $^{low/-}$; PMN-MDSC were defined as $CD33^{+}$ HLA-DR $^{low/-}$ $CD14^{-}CD15^{+}$; M-MDSC were defined as $CD33^{+}$ HLA-DR $^{low/-}$ $CD14^{+}$ $CD15^{-}$, and I-MDSC were defined as $CD33^{+}$ HLA-DR $^{low/-}$ $CD14^{-}$ $CD15^{-}$. Phenotypic analysis of regulatory T cells (Treg) cells in patient PBMCs was done by surface marker staining of CD3, CD4, (BD Bioscience) and CD25 (StemCell Technologies), followed by permeabilization using a FoxP3 buffer kit (eBioscience) and staining for FoxP3 (236A/E7 clone, eBioscience). Results are expressed as percentage of $CD25^{+hi}$/$Foxp3^{+}$ cells out of total $CD3^{+}$/$CD4^{+}$ viable cells.[3] For the analysis of T cell function, we accessed the ability of the T cells to produce IFNγ after stimulation with anti-CD3 and anti-CD28 coated Dynalbeads (25 μl/ml) and 20U/mL of IL-2 as previously described.[3] All flow data were acquired using Cellquest on a BD FACS Calibur and were analyzed using Cellquest software.

Table S1. Univariable Analysis of Surveillance Duration and Time to RECIST Progression

	Surveillance Duration			Time to RECIST Progression		
Factor	**Median/Hazard Ratio[1]**	**95% C.I.**	**p[2]**	**Median/Hazard Ratio[1]**	**95% C.I.**	**p[2]**
Age	1.02	0.98-1.05	.40	1.03	0.99-1.06	.11
Gender						
Male	14.9	10.6-28.5		9.4	7.4-13.5	
Female	14.8	3.2-74.7	.64	9.7	3.2-22.2	.93
KPS						
100	13.9	9.0-25.0		9.1	4.1-13.1	
90	31.6	11.4-69.7		13.5	8.6-17.2	
80	5.2	3.0-9.2	.86	3.0	2.2-9.2	.86
Time from diagnosis to metastatic disease	1.00	1.00-1.00	.96	1.00	1.00-1.00	.82
No. IMDC risk factors						
0	13.8	5.5-74.7		11.4	2.8-17.2	
1	19.3	13.4-30.1		13.4	9.2-19.3	
2-3[3]	8.2	3.0-15.6	.0213[4]	4.8	3.0-9.5	.0011[4]
IMDC prognostic group						
Favorable (0 risk factors)	13.8	5.5-74.7		11.4	2.8-17.2	
Intermediate/Poor (1-3 risk factors) [3]	15.6	10.6-25.0	.28	9.2	6.2-13.4	.99
No. MSKCC risk factors						
0	13.8	5.5-74.7		11.4	2.8-17.2	
1	20.7	12.3-33.3		12.3	8.6-19.3	
2	7.4	3.0-15.6	.0184[4]	6.2	2.3-9.5	.0024[4]
MSKCC prognostic group						
Favorable (0 risk factors)	13.8	5.5-74.7		11.4	2.8-17.2	
Intermediate (1-2 risk factors)	15.6	9.2-25.0	.30	9.2	7.4-13.4	.94

Metastatic Sites[5]						
Lung only	19.3	9.1-74.7		11.4	3.0-17.2	
Other organ(s) only	16.9	11.2-69.7		13.2	4.1-69.7	
Both lung and other organs	9.0	3.0.-25.0	.0280	9.0	3.0-9.5	.13
Number of organ sites with metastases						
1	18.1	13.4-34.5		13.4	6.2-19.3	
2	15.6	3.0-31.6		9.2	3.0-12.2	
>2[6]	7.9	2.8-8.4	.0239	7.9	2.7-N/A	.11
Tumor burden at baseline						
(sum in cm of RECIST measurements)	1.01	1.00-1.01	.15	1.00	0.99-1.01	.90

[1] Median outcomes are reported for categorical factors, hazard ratios from a proportional hazards model are reported for measured factors

[2] Logrank test for unordered categorical factors, logrank trend test for ordered categorical factors, Wald test for measured factors

[3] One patient had 3 risk factors

[4] 0 or 1 risk factor versus >1

[5] 25 patients had lung metastasis only; 14 had involved organs other than lung (10 patients had a single site, primarily lymph nodes, bone, or adrenal; 4 had 2 (n=2) or 3 (n=2) involved organs, primarily kidney with lymph nodes and/or bone); 19 had both

[6] 6 patients had 3 and 2 had 4

Table S2. Multivariable Analysis

Surveillance Duration

Factor	Hazard Ratio (95% C.I.)[1]	p[2]
Number of organ sites with metastases		
1 versus 2 versus >2	1.61 (1.02-2.55)	.0414
No. IMDC risk factors		
0 or 1 versus >1	2.12 (1.03-4.34)	.0403

Time to Progression

Factor	Hazard Ratio (95% C.I.)	p[2]
No. Heng Poor Prognostic Factors		
>1 versus 0 or 1	3.48 (1.68-7.22)	.0004

[1] The first group listed is the reference group. Hazard ratios being >1 indicate a decreased surveillance period for the other group(s)

[2] Wald test

Table S3: Peripheral Blood Immune Cell Populations at Baseline

Median (IQR) percent of live cells	Healthy Controls (n=20-22)	Active Surveillance cohort (n=40)	Immediate systemic therapy cohort (n=30-34)[3]	p*	p**
MDSC	1.14 (0.74-1.41)	2.92 (1.75-4.15)	3.56 (2.47-5.99)	***<.0001***	***<.0001***
Treg	1.44 (1.25-1.86)	0.59 (0.35-0.97)	2.02 (1.32-2.58)	***<.0001***	***.0284***
CD3+ IFN-gamma T cells	16.32 (12.64-21.78)	17.19 (9.42-23.64)	9.35 (5.07-15.05)	.50	***.0004***
CD4+ IFN-gamma T cells	15.84 (12.46-21.14)	16.78 (9.07-22.13)	7.67 (4.43-11.80)	.76	***.0002***

Abbreviations: MDSC; myeloid- derived suppressor cells; Treg; regulatory T cells; IFN; interferon
* Wilcoxon rank sum test for the comparison of active surveillance patients to controls
** Wilcoxon rank sum test for the comparison of active surveillance patients to immediate systemic therapy cohort

Table S4: Peripheral Blood Immune Cell Populations at Baseline, Month 6, Month 12 and at Last Assessment in Surveillance Cohort

Median (IQR) percent of live cells	Baseline	Month 6	Month12	Last Assessment	p*
MDSC	2.92 (1.75-4.15)	2.84 (1.25-6.47)	2.45 (1.19-4.40)	2.71 (1.21-5.04)	***0.90***
Treg	0.59 (0.35-0.97)	1.45 (0.32-4.69)	1.56 (0.35-2.78)	0.54 (0.26-1.93)	***0.83***
CD3+ IFN-gamma T cells	17.19 (9.42-23.64)	15.90 (8.03-27.12)	20.49 (8.80-23.32)	17.24 (2.09-41.37)	***0.58***
CD4+ IFN-gamma T cells	16.78 (9.07-22.13)	14.58 (7.32-23.32)	20.90 (7.02-23.10)	15.90 (2.04-45.23)	***0.52***

Abbreviations: MDSC; myeloid- derived suppressor cells; Treg; regulatory T cells; IFN; interferon.
* Wilcoxon signed-rank test for absolute changes from pre-treatment to last assessment

Table S5: Participating Centers

Study Site	Principal Investigator	Number of Patients Recruited
Cleveland Clinic Taussig Cancer Institute	Brian I. Rini, MD	32
Fox Chase Cancer Center	Elizabeth R. Plimack, MD	11
USC Norris Comprehensive Cancer Center	Tanya Dorff, MD	5
Vall d'Hebron University Hospital	Cristina Suarez Rodriguez, MD	3
Royal Marsden NHS Foundation Trust	James Larkin, MD	1

References

1. Ko JS, Zea AH, Rini BI, et al. Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell carcinoma patients. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2009; **15**(6): 2148-57.
2. Raychaudhuri B, Rayman P, Ireland J, et al. Myeloid-derived suppressor cell accumulation and function in patients with newly diagnosed glioblastoma. *Neuro Oncol* 2011; **13**(6): 591-9.
3. Finke JH, Rini B, Ireland J, et al. Sunitinib reverses type-1 immune suppression and decreases T-regulatory cells in renal cell carcinoma patients. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2008; **14**(20): 6674-82.