**SUPPLEMENTARY MATERIALS AND METHODS**

**Tumor specimens**

Patients with RCC were classified as responders (R) or non-responders (NR) to anti-PD-1 therapy based on radiographic staging according to Response Evaluation Criteria in Solid Tumors (RECIST) ([1](#_ENREF_18)). Non-responders included patients whose disease progressed as well as those with stable disease (SD). Responding (R) patients included those with complete or partial responses (CR, PR). From among 35 potential pretreatment tumor specimens derived from 26 patients on four clinical trials of nivolumab, 21 formalin-fixed paraffin-embedded (FFPE) tumor specimens were available for study. They were characterized for PD-L1 expression by immunohistochemistry (IHC) as previously described (2). In brief, a tumor specimen was defined as PD-L1+if ≥5% of tumor cells showed cell surface staining with the murine anti-human PD-L1 mAb 5H1 (from Lieping Chen, Yale University). Among the 21 tumors examined, 14 specimens (67%) from 13 unique patients demonstrated PD-L1 expression. One specimen from each of 13 patients was selected for further analysis. Only 12 specimens yielded sufficient RNA for gene expression analyses (**Supplementary Table S1**).

**Immunohistochemical (IHC) analysis**

The molecules CD3, CD4, CD8, CD68 and FoxP3 were detected with standard automated IHC methods. IHC for PD-1, PD-L2, and LAG-3 was performed as previously described ([2](#_ENREF_7)). TIM-3 was detected with a primary murine anti-human TIM-3 mAb (clone F38-2E2; BioLegend, San Diego, CA) at 1.5 ug/ml, after antigen retrieval for 10 min in citrate buffer, pH 6.0 at 120oC; a secondary anti-mouse IgG1 was used at 1.0 ug/ml, amplification was performed with the CSA kit (DAKO #1500, Carpinteria, CA), and visualization was accomplished with DAB (Sigma, St. Louis, MO). UGT1A6 expression was detected using the same antigen retrieval conditions**,** with application of a primary rabbit anti-human UGT1A6 mAb (clone EPR11068, Abcam, Cambridge, MA) at 1.25 ug/ml (1:250), followed by application of the Novolink anti-rabbit polymer detection system (RE7112, Leica, Buffalo Grove, IL) and visualization with DAB.

The intensity of immune cell infiltrates was scored as mild, moderate or severe, as previously described ([2](#_ENREF_7)). CD3 and CD68 immunostains were performed on each specimen and were used to guide assignment of an intensity score for immune infiltrates and to determine which cell types were expressing PD-1 ligands. Intratumoral CD4:CD8 ratios were estimated at 1:1, 1:2, 1:4, or 2:1. The proportion of TILs expressing PD-1, LAG-3, TIM-3 or FoxP3 was scored as “none”, “focal” (isolated, <5% of lymphocytes), “moderate” (5-50% of TILs), or “severe” (>50% of TILs). PD-L2 expression on infiltrating immune cells (TILs or histiocytes) was scored on the same semi-quantitative scale of “none”, “focal”, “moderate” or “severe”. Extent of UGT1A6 expression was assessed as the percentage of tumor cells with positive cytoplasmic staining and was scored at 5% intervals. Comparisons of IHC results from PD-L1+ tumors derived from responding vs. non-responding patients were performed using The R Project for Statistical Computing (https://www.r-project.org/).

**Multiplex qRT-PCR assays and statistical analysis**

PD-L1+ RCC tumor areas were isolated and subjected to total RNA isolation, performed with the High Pure RNA Paraffin Kit (Roche, Indianapolis, IN) according to manufacturer’s instructions. RNA quality and quantity was estimated using a NanoDrop spectrophotometer (Thermo Scientific), resulting in a 260/280 ratio ≥ 1.7. Fifty ng of total RNA was reverse-transcribed in a 10 ul reaction volume using qScript cDNA SuperMix (Quanta Biosciences, Gaithersburg MD) per protocol. From each RT reaction, 7.5 ul was pre-amplified in a total volume of 30 ul using a 14-cycle PCR reaction per PreAmp protocol (Applied Biosystems, Foster City CA). Fourteen ul of each pre-amplification reaction was expanded into a 440 ul total volume reaction mix and added to TaqMan Array Micro Fluidic Cards per protocol (Applied Biosystems). These cards were custom designed with 64 gene-specific primers/probes in triplicate wells, including 4 internal controls (*18S,* 18S ribosomal RNA; *ACTB,* beta-actin; *GUSB,* beta-glucuronidase; and *PTPRC,* CD45 pan-immune cell marker). qRT-PCR was run using a QuantStudio 12K Flex Real-Time PCR System, and expression analysis was performed with the manufacturer’s software (Applied Biosystems). Results were calculated with the ΔΔCt method and analyzed according to clinical response to anti-PD-1 therapy, using the Student t-test.

**Whole genome expression profiling and analysis**

Global gene expression in tumor specimens from anti-PD-1 responders (R, n=4) and non-responders (NR, n=7) was measured by DASL (cDNA-mediated Annealing, Selection, extension, and Ligation) assays arrayed on the Illumina Human HT-12 WG-DASL V4.0 R2 expression BeadChip, per the manufacturer's specifications (Illumina, San Diego, CA). This platform detects 29,377 annotated transcripts and is designed to detect partially degraded mRNAs such as typically found in FFPE tissue specimens. Briefly, total RNA was reverse transcribed into 1st-strand cDNA and then annealed with an assay-specific oligo pool for 2nd -strand cDNA synthesis. The cDNA was further amplified by PCR using universal primers. PCR products were then purified and denatured to obtain labeled single-strand DNA for DASL array hybridization, after which the BeadChip was washed and scanned to acquire the intensity data. A single intensity (expression) value for each Illumina probe on the DASL array was obtained using Illumina GenomeStudio software with standard settings and no background correction.  For each sample, the expression values for all the probes were scaled to have median 256 (28) and were then log (base 2) transformed before performing statistical analysis. Transcriptional data were further analyzed using BRBArrayTools developed by the Biometric Research Branch, NCI (<http://linus.nci.nih.gov/BRB-ArrayTools.html>) (3), and were visualized by the Stanford Cluster 3.0 Program and custom programs written in the Interactive Data Language (IDL) (product of Excelis/Harris, Melbourne, FL). For each Illumina probe on the DASL array, expression levels in R and NR patients were compared. A heat map based on genes derived from R vs. NR class comparison using a Student’s t test p-value ≤ 0.01 and fold change ≥1.5 (calculated using the geometric mean of the expression levels in each group) was visualized by Stanford Cluster 3.0 and TreeView software. For functional analysis, two lists of genes (i.e., Illumina probe IDs) for which the p-value was ≤ 0.05 and the fold change was ≥1.5 (either positive or negative fold change) were examined for significant enrichment in gene annotation categories and in functional categories including KEGG pathways, using the DAVID web tool (<http://david.abcc.ncifcrf.gov/>) ([4](#_ENREF_20), [5](#_ENREF_21)). Principal component analysis (PCA) was performed using a custom program written in IDL which output the first several principal axis coordinate values for each patient sample, formatted for 3D display using the Mage software (kinemage.biochem.duke.edu/software/mage.php).

**RCC cell lines**

Eight commercially available RCC cell lines (ACHN, UO-31,TK-10, A498, RXF-393, SN12C, 786-O and Caki-1) were cultured in RPMI 1640 + 10% heat-inactivated FBS supplemented with 10 mM HEPES buffer and 1% antibiotic/antimycotic solution (Life Technologies, Grand Island, NY). Cell cultures were maintained at 37°C, 5% CO2 and were confirmed to be mycoplasma-free in our laboratory with the Venor®GeM Mycoplasma Detection kit (Sigma Aldrich). Characterization by morphology, cytogenetics, and other attributes can be found at https://dtp.cancer.gov/discovery\_development/nci-60/characterization.htm.

***UGT1A6* transfectants**

The established melanoma cell lines 397-mel and 888-mel were generated from metastatic melanoma lesions in our laboratory and maintained as previously described (6, 7); they were characterized by morphology, expression of melanoma antigens, and HLA typing to confirm identity with the donor of origin. They were confirmed to be mycoplasma-free. They were transfected with the empty vector pCMV6-Entry or with pCMV6-UGT1A6 (RC215957; Origene Technologies, Rockville, MD) using Lipofectamine 2000 (Life Technologies, Grand Island, NY), and were selected in geneticin (G418). Melanoma cell expression of *UGT1A6* mRNA and the endogenous control genes *18S* rRNA and *GUSB* was detected with qRT-PCR using TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA) and the QuantStudio 12K Flex Real-Time PCR System (Applied Biosystems). To assess UGT1A6 protein expression, whole cell proteins were extracted by M-PER mammalian protein extraction reagent (ThermoFisher Scientific, Grand Island, NY) and analyzed by Western blotting with an anti-UGT1A6 mAb (Abcam, Cambridge, UK). Blots were counterstained with peroxidase-conjugated rabbit anti-human IgG (Sigma, St. Louis, MO, USA).

**Cytotoxicity assays**

A modified flow-cytometry-based assay detecting the disappearance of CFSE-labeled tumor cells upon incubation with effector cells was used to measure tumor lysis (8). Tumor infiltrating lymphocyte cultures (TILs) from two melanoma patients demonstrated specific lysis of autologous cultured melanoma cells, 397-mel, and 888-mel, as described (6, 7). Non-specific lymphokine activated killer (LAK) cells were generated in 3-4 day cultures from peripheral blood mononuclear cells in the presence of 6000 IU/ml recombinant IL-2 (6). Melanoma target cells that were stably transfected with *UGT1A6* or an empty control vector were labeled with 15 uM carboxyfluorescein diacetate succinimidyl ester (CFSE; ThermoFisher Scientific, Grand Island, NY) in PBS + 5% heat-inactivated human AB serum for 15 minutes at 37°C, then further incubated for 30 minutes in RPMI 1640 medium + 10% human AB serum to allow CFSE acetate hydrolysis. After 3 washes, labeled target cells were co-cultured with effector cells (TILs or LAK cells) in a total volume of 1.5 ml in 5 ml round-bottom polypropylene tubes at various effector:target ratios for 12-15 hours. Following incubation, cell mixtures were stained with an anti-human CD45-APC mAb (BD Biosciences) to detect effector cells, followed by viability staining with Live/Dead Fixable Aqua (Life Technologies). Samples fixed with 1% paraformaldeyde were acquired with the BD FACSAria II (Becton Dickinson, Mountain View, CA). Flow cytometry data were analyzed with Flow Jo Version 10.1 (Treestar, Ashland, OR). Briefly, the number of CFSE+CD45(-)-gated events, or target cells, were determined and analyzed for viability. Cells that were completely lysed by effector cells were gauged by calculating the difference between the actual starting number of target cells and the acquired number of CFSE+CD45(-)-gated events. Then, for each E:T condition, the total % dead whole tumor cells was determined according to the following equation: [(Aqua-positive CFSE+CD45(-)-gated events + “missing” events)/ starting number of target cells] x 100.

**In silico correlation of gene expression with overall survival in RCC**

To investigate potential associations between differentially expressed genes identified in this study with RCC clinical stage and the overall survival of patients with RCC, RNA sequencing data from The Cancer Genome Atlas project (TCGA), including 444 clear cell RCC samples and 72 matched normal kidney samples (9), were used for in silico analysis. Level 3 RSEM normalized data were downloaded from the TCGA Data Portal (<https://tcga-data.nci.nih.gov/tcga/>). Analysis was performed using R/Bioconductor software with the survival package and custom routines for data analysis (10). Association of gene expression level with tumor stage was tested by fitting a linear model using continuous expression level of a gene and the tumor stage as a numeric value (11).The p-value was adjusted using the Benjamini-Hochberg procedure for estimating the false discovery rate (FDR) (12). For survival analysis, the estimated logarithm of the hazard ratio and p-value were computed from the Cox regression model (13) using continuous expression values and the tumor stage for all tumor samples, or just using continuous expression values for stage IV samples. All p-values were adjusted using the Benjamini-Hochberg procedure. Kaplan-Meier curves were made with the help of the survfit function from the survival package using the median expression level to split samples into two groups: high or low expression of the gene of interest.

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