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TITLE: Functionally Characterizing the Enhancer Cistrome in Advanced Human Kidney Cancer

PRINCIPAL INVESTIGATOR: Mark Pomerantz, M.D.

CONTRACTING ORGANIZATION: Dana-Farber Cancer Institute, Boston, MA

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14. ABSTRACT
The mechanism by which genes are turned "on" is driven by proteins called transcription factors (TFs) binding to certain locations in the human genome called **regulatory elements**. TFs recognize and bind to specific regulatory elements (i.e., stretches of specific DNA sequences) and this interaction can result in a gene being turned on. These regulatory elements are proving to be important in cancer biology. Regulatory elements are located throughout the 98% of the human genome that *does not code for genes*. Due to the lack of tools available for studying the noncoding region, until recently it was difficult **to identify**, **to functionally characterize**, and **to therapeutically target** these 'non-genic' genomic regions. Advances are enabling the systematic identification and testing of relevant regulatory elements as well as the ability to target their associated TFs, which were once considered 'undruggable'.

The ultimate goals of our proposal are to identify and characterize critical regulatory elements that contribute to advanced kidney cancer. Aim 1 will utilize a method called chromatin immunoprecipitation followed by sequencing (ChIP-seq) in clinically relevant patient samples to characterize the regulatory landscape during progression to metastatic disease. We will also use a novel technique called HiChIP to create a 3D model of the kidney cancer genome to determine which regions communicate with each other. Gene regulation often occurs when distal enhancers come into contact with target genes in 3D space. Finally, using an innovative technology termed genome editing, Aim 2 will identify candidate regulatory elements that are functionally relevant for mediating resistance to therapy. By focusing on epigenetics and the non-coding portions of the genome, our proposal takes a decidedly innovative approach to identifying functionally important gene regulatory elements. These regions have been understudied compared with the protein coding genome. We have designed our proposal to benefit patients with advanced kidney cancer. The study will open new areas for drug target discovery. We believe that our approach paves the way for systematically studying cancer and identifying targets through an epigenetic perspective.

15. SUBJECT TERMS
Renal cell carcinoma, massively parallel sequencing, ChIP-seq, regulatory elements, transcription factors, ATAC-seq, clear cell renal cell carcinoma.

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REPORT OUTLINE

1. **INTRODUCTION:**

Epigenetic aberrations direct much of clear cell renal cell carcinoma (RCC) pathogenesis. Indeed, genes fundamental to epigenetic programming are recurrently mutated in the disease. Despite the clear dependency on epigenetic transcriptional regulation, large-scale investigation into the epigenetic mechanisms underlying RCC have lagged behind genetic studies. Our ability to now robustly perform chromatin immunoprecipitation (ChIP) in primary kidney tumor specimens is enabling the first generation of comprehensive epigenomic studies. These tools are enabling the production of large-scale epigenomic datasets in clinically relevant samples, just as next generation sequencing facilitated tumor sequencing over the past decade. There is an increasing appreciation of the role of the non-coding genome in cancer biology and we believe that one of the next frontiers in treating cancer will be in the realm of epigenetics.

2. **KEYWORDS:**

Renal cell carcinoma, massively parallel sequencing, ChIP-seq, regulatory elements, transcription factors, ATAC-seq, chromophobe renal cell carcinoma, papillary renal cell carcinoma, clear cell renal cell carcinoma.

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**
  - The ultimate goals of this proposal are to comprehensively characterize the epigenetic landscape in advanced RCC in order to gain insights into key mechanisms driving lethal disease, which can then be rationally targeted. The anticipated outcomes are that we will identify **areas of vulnerability (Aims 1)** that are **functionally relevant (Aim 2)** and ultimately can be rationally targeted. The aims of the proposal require specific domains of expertise and to address this point, we have assembled an outstanding team with the appropriate scientific depth to go from target identification to analysis of function.
  - Aim 1 will generate the most comprehensive epigenomic ChIP-seq datasets to date in advanced RCC. Aim 1 will also use HiChIP, combining chromosome conformation analysis with ChIP to link the regulatory elements with their target genes. Aim 2 will utilize the powerful tools of genome editing to identify regulatory elements that are functionally relevant in the development of metastatic RCC. The outcome of this study will be a compendium of candidate regulatory elements that influence RCC progression.
- **What was accomplished under these goals?**
  - 1) Major Activities:

Initially, we profiled the regulatory element landscape associated with enhancers and promoters by H3K27ac and H3K4me2 ChIP-seq in 30 patient-derived fresh-frozen RCC tumor samples of different histologies: 12 clear cell RCC (ccRCC), 12 chromophobe RCC (chRCC), and 6 papillary RCC (pRCC). We used publicly available ATAC-seq data from 16 ccRCCs and 34 pRCCs and generated in-house ATAC-seq data for seven chRCC samples[1]. We generated RNA seq, DNA-seq through targeted panel sequencing, and SNP profiling through SNP arrays on 28, 28, and 21 patient-derived samples, respectively.

We identified another cohort (cohort 2) of patients with metastatic ccRCC who had fresh-frozen tumor tissue banked at our institution (total of 32 patients); 13 of which also had

paired normal and local tumor banked in addition to tissue from the metastatic site. We performed H3K27ac ChIP-Seq, HIF-2a ChIPSeq, ATAC-Seq, and RNA-Seq on each available tissue site. Two patients did not have adequate tissue amounts from the metastatic site for profiling. Cohort 2 included at least one sample from 15 different metastatic sites, most commonly lung (18%), adrenals (15%), and soft tissue (12%).

The resulting data set represents, to our knowledge, the most extensive interrogation of RCC epigenomes in primary specimens.

- 2) Specific Objectives:

- Profile the regulatory element landscape, including enhancers and promoters, across the different RCC histologies
- Characterize epigenetic difference between different RCC histologies
- Identify histotype specific transcription factors governing cellular identity and transcriptional programs
- Characterize reprogramming of regulatory elements during oncogenesis and distant metastasis

- 3) Significant Results:

Using H3K27ac data from cohort 1, we identified a total of 154,075 active regulatory elements across all the samples, most of which (n=134,468) were common to at least two histologies. A relatively small fraction of regulatory regions (n=16,852) were histology-specific (defined as having ≥2-fold increase in H3K27ac peaks in one histology versus the others). Of the histology-specific sites, most (n= 8,939) were chRCC-specific, 3,653 were pRCC-specific, and only 316 were ccRCC-specific. Using transcriptional start site (TSS)-distal H3K27ac peaks, we identified 137,044 putative active enhancers spanning the renal cell carcinoma epigenome. Unsupervised hierarchical clustering and principal component analysis (PCA) clearly segregated chRCC from pRCC and ccRCC, indicating a distinctive enhancer landscape defining RCC histologies. Unsupervised hierarchical clustering and PCA analysis of H3K4me2 peak calls from 24 samples showed analogous results to H3K27ac with distinctive separation of chRCC from the other two RCC subtypes. The same correlation was observed across the different histotypes, r=0.76, r=0.77 and r=0.66 for clear cell, papillary and chromophobe RCC respectively, providing further compelling evidence for their active enhancer classification **(Figure 1)**.

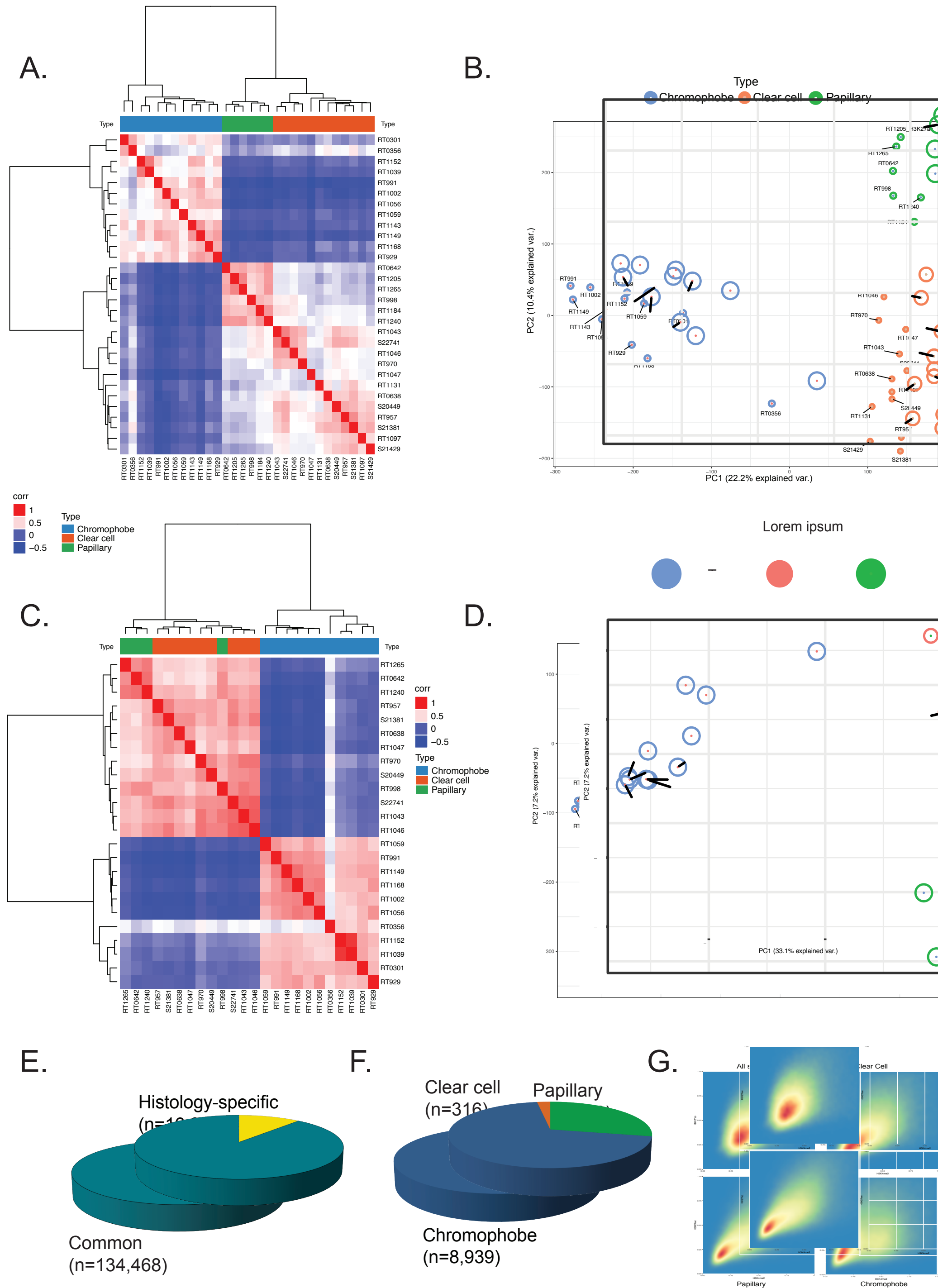
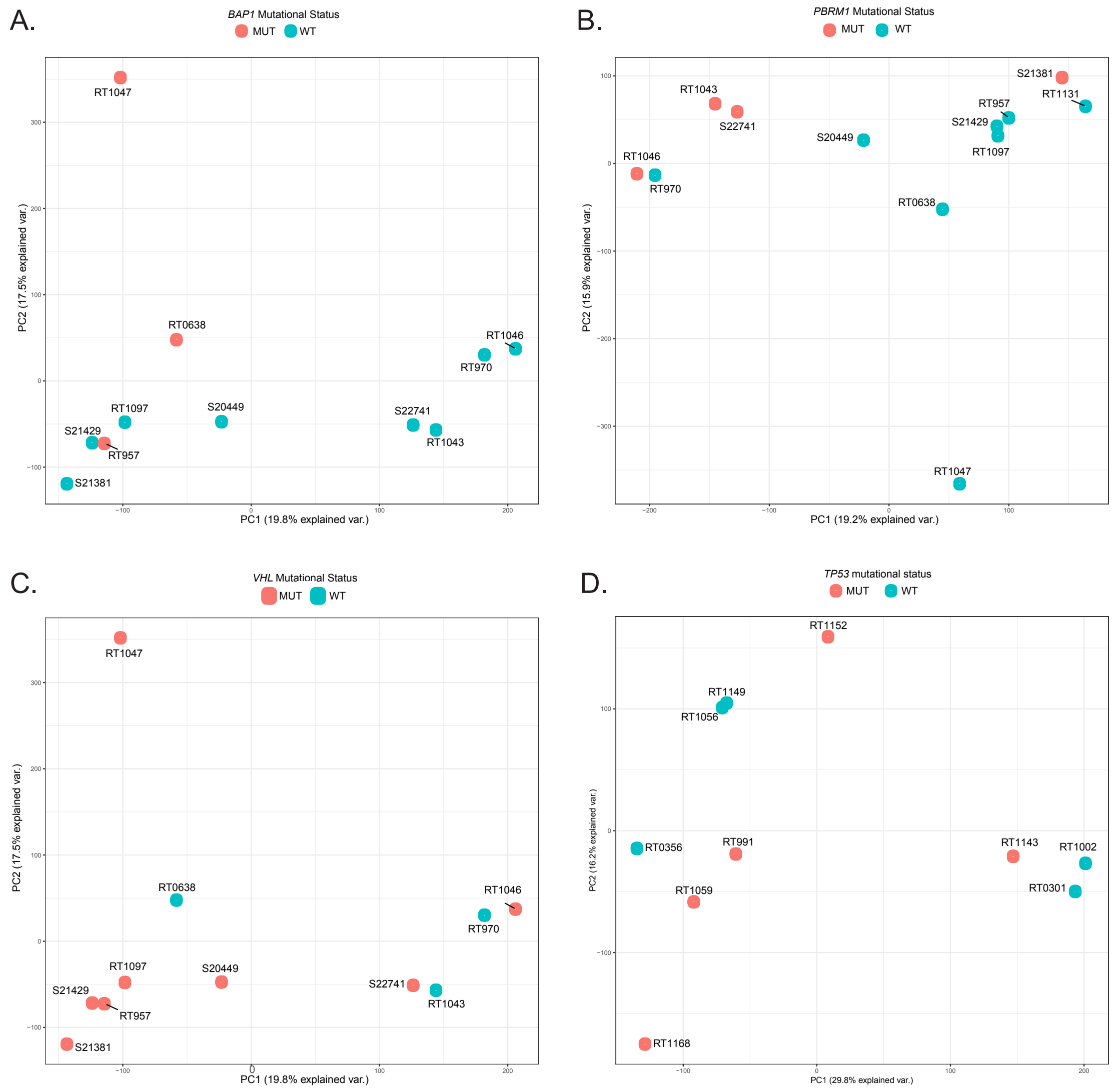


Figure 1. A. Hierarchical clustering of chRCC, ccRCC, and pRCC based on sample-to-sample pairwise correlation of the H3K27ac ChIP-seq signals (red – strong correlation, pink – intermediate correlation, blue – opposite correlation). The diagonal of cells with red outlines denotes the line of identity. **B.** Principal component analysis of ccRCC, chRCC, and pRCC based on H3K27ac profiles. **C.** Hierarchical clustering of chRCC, ccRCC, and pRCC based on sample-to-sample pairwise correlation of the H3K4me2 ChIP-seq signals (red – strong correlation, pink – intermediate correlation, blue – opposite correlation). The diagonal of cells with red outlines denotes the line of identity. **D.** Principal component analysis of ccRCC, chRCC, and pRCC based on H3K4me2 profiles. **E.**Genome-wide distribution of H3K27ac signals. **F.** Distribution of histology-specific active regulatory elements among RCC subtypes. **G.** H3K27ac versus H3K4me2 ChIP-seq signals at RCC regulatory elements across subtypes.

The interplay between genetics and epigenetics in RCC is evident from the significant number of tumors harboring genomic alterations in genes encoding histone and chromatin modifiers. For example, somatic mutations are found in *PBRM1* (21-41%), *SETD2* (3-12%), *KDM5C* (3-8%), *KDM6A*(1%) and *BAP1* (8-15%)[2-5]. We therefore investigated the impact of known driver mutations on the enhancer landscape in renal cell carcinoma. Consequently, we

compared the enhancer landscape in ccRCC patients between *VHL* mutants (MT, n=8) vs. *VHL* wild type (WT, n=3), *PBRM1* MT (n=4) vs. *PBRM1* WT (n=4), and *BAP1* MT (n=3) vs. *BAP1* WT (n=7) respectively. Similarly, in chRCC, we compared the enhancer landscape between *TP53* MT (n=6) vs. *TP53* WT (n=5). The number of mutations in our papillary RCC cohort did not allow for formal comparisons. No significant differences in the active regulatory element landscape were discerned. Samples clustered irrespective of underlying genomic alterations, suggesting that other underlying factors were driving epigenetic heterogeneity **(Figure 2A-D)**.



Supplementary Figure 2. ABC. Principal component analysis of H3K27ac ChIP-Seq data of ccRCC samples based on mutational status of *BAP1, PBRM1, and VHL* genes. **D.**Principal component analysis of H3K27ac ChIP-Seq data of chRCC samples based on mutational status of *TP53* gene. MUT: mutant; WT: Wild Type

Cellular identity in both tumor and normal tissues is defined by a small number of master TFs that dominate control of the transcriptional program. There is no single defining feature of a master TF, but a series of key properties commonly exhibited by master TFs can be exploited to identify candidates. Master TFs 1) bind to large cell-type-specific enhancers,

termed super-enhancers (SEs) [6,7], 2) are often regulated by SEs, and 3) regulate one another in what has been termed a transcriptional core regulatory circuitry (CRC)[8]. To identify the specific master TFs governing the transcriptional programs in each RCC histology, we and others employ a meta-analysis approach [9,10], leveraging multi-omic data profiling to prioritize candidate histotype-specific master TFs. The overall approach is shown in **Figure 3A**. Briefly, a TF must be differentially expressed in one histology *versus* the other RCC subtypes and must also exhibit evidence of connectivity (in motif-based analyses) and/or specificity of expression relative to other tumor types and/or SE-association. This meta-analysis approach aims to minimize effects of limitations inherent to each approach used individually; for example, SEs are commonly misassigned to genes, and some TF motifs are absent from the motif databases used for circuitry mapping.

Individual TF clique enrichment score (CES: the percentage of total cliques in which a TF is a member) for each TF in each sample, SE ranking, RNA-seq differential expression and Cancer Core Transcription factor Specificity (CaCTS)[11] analyses identified more than 200 TFs with histotype-specific behavior. Using this approach **Figure 3A, 3B**, **3C**), we prioritized 50 histotype-specific master TFs. We nominated the TFs *GATA2*, *TFCP2L1*, and *DMRT2* as candidate oncogenic drivers in chRCC (**Figure 3B, 3C**). For ccRCC, *ETS1* was nominated as a putative master TF. *BARX2*, *ZNF395,* and *EPAS1* were other potential ccRCC-specific master TFs identified by this approach (**Figure 3B, 3C**). For pRCC, CES and SE and differential gene expression analyses elucidated *HNF1B*, and *NR2F2* as putative pRCC-specific master TF (**Figure 3B, 3C**). Of note, several TFs were identified in more than one histology including *PAX8*, a well-known MTF in ccRCC[12]. Using CaCTS, *PAX8* was found to be an important TF in all three histologies (**Figure 3D**). CES analysis also identified *PAX8* as a master TF in both chRCC and ccRCC. SE ranking, gene expression, and CES of the 50 histotype-specific TFs selected using this meta-analysis approach was able to cluster the samples according to their respective histologies (**Figure 3E**).

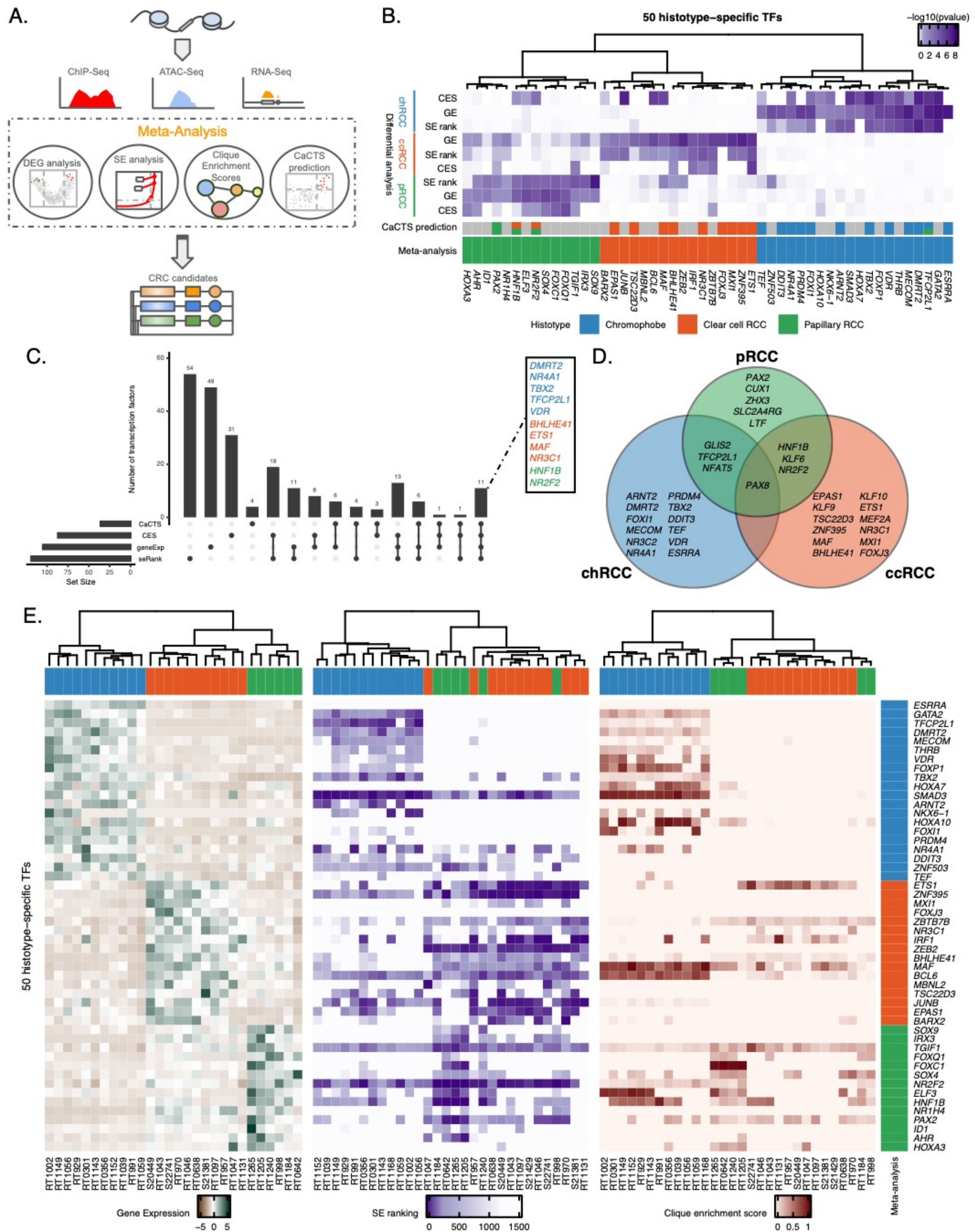


Figure 3. Multi-dimensional integrative analysis identifies histology-specific transcription factors (TFs). A. Overview of the model approach used to identify histology-specific master TFs participating in core regulatory circuitries (CRC). **B.** Heatmap integrating the 50 histology-specific TFs identified by the meta-analysis approach (CES, differential expression, SE rank analysis, and CaCTS). **C.** UpsetR plot showing the number of transcription factors identified using each individual method (differential gene expression, differential SE rank, differential CES and CaCTS). **D.** Venn diagram showing common and histology-specific master TFs using the CaCTS algorithm. **E.** Sample-to-sample clustering by the gene expression, SE rank and CES of the 50 candidate master TFs.

- **What opportunities for training and professional development has the project provided?**

  - The project has provided the opportunity to mentor three post-docs in the lab – Sylvan Baca, M.D., Sarah Alaiwi, M.D. and Amin Nassar, M.D. Dr. Baca is a medical oncology fellow at Dana-Farber Cancer Institute. He is in the midst of his post-doctoral triaing in genomics and bioinformatics. He conducted much of the bioinformatics work described above under the mentorship of the PIs on this project. Drs. Abou Alaiwi and Nassar are both internal medicine residents at Brigham and Women's Hospital. As part of this project, they mastered ChIP-seq in human tissue specimens and worked with the bioinformatics team to analyze the data. Our team – the PIs on this project plus Drs. Abou Alaiwi and Nassar – have been invited to present this work at the International Kidney Cancer Symposium 2020.

- **How were the results disseminated to communities of interest?**

  - Nothing to Report.

- **What do you plan to do during the next reporting period to accomplish the goals?**

  - Now that we have defined the universe of enhancers associated with RCC pathogenesis and identified master TFs and downstream target genes, we will functionally characterize these potential non-coding drivers of RCC progression. Our H3K27ac ChIP seq data (above) revealed the enhancer landscape in RCC tumorigenesis, with both loss and gain of enhancers. We will use these enhancer profiles to perform an in vitro screen renal cell carcinoma cell lines. We will focus on the A498 and 798-O cell lines, which have been used as models for localized and metastatic ccRCC, respectively. To determine the functional significance of these epigenetic alterations, we will perform an unbiased pooled CRISPR/Cas9-based screen to systematically suppress each differentially activated enhancer. Recruitment of a nuclease dead version of Cas9 fused to the Kruppel-associated box repressor domain (dCas9-KRAB) results in loss of H3K27ac and silencing of the enhancer. We will clone a pool of gRNAs targeting each enhancer into a lentiviral expression system. We will use five gRNAs per enhancer targeting the center of each H3K27Ac ChIP-seq peak. We anticipate approximately 4000 enhancers to be differentially activated in tumors versus normal tissue based on our preliminary results, which is well within the range typically done with genome-scale screens involving 20,000 genes and libraries consisting of 100,000 gRNAs. As a result, we will have generated the first comprehensive functional enhancer landscape required for proliferation of primary and metastatic RCC.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**

  - To date, the majority of RCC epigenomic studies have focused on promoter DNA methylation in the context of tumor suppressor gene silencing[13]. On the other hand, little is currently known about distal regulatory elements in the various subtypes of RCC. While some studies have begun interrogating the epigenetic landscape of RCC, the majority of epigenetic analyses have been conducted in immortalized or malignant cancer cell lines[10,14]. **In this project, we have directly addressed this void in our understanding of RCC by generating a comprehensive compendium of ATAC-Seq, RNA-Seq, and histone modification data across different RCC histologies with the**

goal of identifying histology-specific epigenetic mechanisms driving RCC pathogenesis and potentially explaining its diverse clinical behavior.

  - In line with histological, expression and mutational data, the regulatory element landscapes of ccRCC, pRCC and chRCC are distinct. While the majority of gene regulatory elements were common across all three histologies, large sets of epigentic marks were unique to each histology. Interestingly, within individual histologies, **mutations in commonly altered chromatin modifying genes did not have a significant impact on the regulatory element landscape**. This suggests that the highly prevalent genomic alterations discovered in large-scale genomic sequencing projects such as TCGA may not be the key players in driving the regulatory element landscape heterogeneity and, hence, the transcriptional diversity in RCC.

  - Prior work in medulloblastoma and ependymoma demonstrated how molecularly-defined cancer subgroups exhibit specific core regulatory circuitries. These studies showed that subgroup-specific TFs are faithful tracers of cell-of-origin and important regulators of tumor dependencies[15-18]. In attempt to first understand the epigenetic orchestrators of cellular identity and second to highlight potential drivers of carcinogenesis in RCC, we mapped putative master TFs in each RCC histology. **Using an integrative approach, we identified 50 histotype-specific TFs**.

  - **This project characterized a novel master TF, ETS-1, in ccRCC pathogenesis**. ETS proto-oncogene 1 (ETS-1) is a transcription factor that is involved in differentiation of hematopoetic cells and angiogenesis[19-22]. ETS-1 also plays a crucial role in carcinogenesis. As a proto-oncogenic factor, ETS-1 is capable of activating genes associated with angiogenesis, metastasis and invasive behavior in multiple tumor types[23-25]. Additionally, ETS-1 expression correlates with microvessel density in some non-glial tumors and is an independent negative prognostic marker in different tumor entities such as breast, ovarian, pancreatic and colorectal cancer[23,26,27]. Nonetheless, the role of ETS-1 in RCC has mainly focused on its interaction with HIF2-alpha[28]. Here, we show that ETS-1 may play a role as a master TF specific to ccRCC and not chRCC or pRCC. Prospective studies should focus on understanding the global epigenomic effect of ETS1 in driving ccRCC pathogenesis. Furthermore, HIF2-alpha, a known driver in ccRCC, was found to be a ccRCC-specific SE.

- **What was the impact on other disciplines?**

  - This is among the first studies in which that histone modifications ChIP-Seq, ATAC-seq and RNA-seq data have been combined into a unified analysis to capture the deep complexity of the epigenomic landscape across tumor histologies within a single organ. Our work highlights the histology-specific nature of these regulatory elements and the increasing need for cataloging the epigenetic landscapes across various specific tumor sub-types.

- **What was the impact on technology transfer?**

  - Nothing to Report.

- **What was the impact on society beyond science and technology?**

  - Nothing to Report

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**

- Nothing to Report

- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - The COVID-19 pandemic had a significant impact on our work this year. By the winter/spring 2019-20 we had generated our epigenomics and genomics in human specimens. As we were preparing to perform the functional work outlined in Aim 2 of the project, our laboratories at Dana-Farber Cancer Institute and the National Cancer Institute were closed. Both institutions placed strict restrictions on access to the laboratory for activities unrelated to direct patient care. Our team of investigators gained full access to the laboratory and tools necessary to continue our work by early September. We have resumed the work described above and have begun processing data generated pre-quarantine that have not yet been analyzed.

- **Changes that had a significant impact on expenditures**
  - Nothing to Report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  - Nothing to Report

- **Significant changes in use or care of human subjects**
  - Nothing to Report

- **Significant changes in use or care of vertebrate animals.**
  - Nothing to Report

- **Significant changes in use of biohazards and/or select agents**
  - Nothing to Report

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**
  - **Journal publications.** "Mapping the epigenetic landscape of renal cell carcinoma" is under review at *Nature Cancer* and we have provided acknowledgement of federal support and specifically cited this grant.
  - **Books or other non-periodical, one-time publications.** Nothing to Report
  - **Other publications, conference papers, and presentations.** "Mapping the epigenetic landscape of renal cell carcinoma"* to be presented in October 2020 at the International Kidney Cancer Symposium 2020

- **Website(s) or other Internet site(s)**
  Nothing to Report

- **Technologies or techniques**
  Nothing to Report. Techniques developed in our lab for performing epigenetic analysis in human specimens has been reported in previous publications.

- **Inventions, patent applications, and/or licenses**
  Nothing to Report

- **Other Products**
  The epigenetics data generated by the project thus far will be made publicly available via the NCBI Gene Expression Omnibus upon publication of our manuscript.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

Name:	Mark Pomerantz, M.D.
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0003-4914-1157
Nearest person month worked:	0.91 CM
Contribution to Project:	Dr. Pomerantz has led overall study design, data generation and data analysis. He directs the database and biobank that provides the materials for the project.
Funding Support:	

Name:	Matthew Freedman, M.D.
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0002-0151-1238
Nearest person month worked:	0.48 CM
Contribution to Project:	Dr. Freedman has been involved in overall study design, data generation and data analysis
Funding Support:	

Name:	David Takeda, M.D.
Project Role:	Co-PI

Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0002-5986-1169
Nearest person month worked:	0.46 CM
Contribution to Project:	Dr. Takeda is leading the functional molecular biology experiments
Funding Support:	

Name:	Toni Choueiri, M.D.
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0002-9201-3217
Nearest person month worked:	0.46 CM
Contribution to Project:	Dr. Choueiri has been involved in overall study design, data generation and data analysis
Funding Support:	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

POMERANTZ, MARK

NEW AWARDS

W81XWH-19-1-0565 09/30/19 – 09/29/22 0.36 CM
DoD (Freedman)
Defining and Functionally Characterizing the Epigenome in Lethal Prostate Cancer
Goals/Aims: 1) To characterize the transcriptome and genome-wide landscape of open chromatin (ATAC-seq) and active enhancers (H3K27ac) in 150 patients from metastatic hormone-sensitive PCa (N=50) to mCRPC (N=50) to enzalutamide-resistance (N=50); 2) To perform epigenome-wide CRISPR-based screens to identify regulatory elements associated with enzalutamide resistance; 3) To use novel technology (GloPro) to identify the key proteins binding to clinically relevant enhancers.
Role: Co-Investigator
Funding Agency Contact:
Michelle Cromwell, Grants Specialist
Department of Defense
Phone:
Email: michelle.l.cromwell.civ@mail.mil

W81XWH-19-1-0554 09/15/19 – 09/14/21 0.90 CM
DoD
Functionally Characterizing the Enhancer Cistrome in Advanced Human Kidney Cancer
Goals/Aims: 1) To characterize the genome-wide landscape of open chromatin and active regulatory elements and their connections to target genes in the progression from localized to metastatic ccRCC; 2) To perform epigenome-wide CRISPR-based screens across the epigenome to identify the functional relevance of regulatory elements associated with ccRCC in model systems.
Role: Principal Investigator
Funding Agency Contact:
Michelle Cromwell, Grants Specialist
Department of Defense
Phone:
Email: michelle.l.cromwell.civ@mail.mil

P01CA228696 09/01/19 – 08/31/24 0.48 CM
NIH (Kantoff, Pomerantz)
The Impact of DNA Damage Repair Abnormalities in Prostate Cancer
Administrative Core
Goals/Aims: 1) To provide seamless budgetary oversight for the projects and cores; 2) To schedule and support the program meetings, as well as facilitate evaluation and communications among the projects; 3) To distribute and archive the publications, reports, and grant elements from the components of the P01; 4) To schedule, facilitate, and manage the meetings of the Principal Investigators and the Scientific Advisory Board.
Role: Core Co-Director
Funding Agency Contact:
Rogers Gross, Grants Management Specialist
National Cancer Institute
Phone:
Email: rogers.gross@nih.gov

P01CA228696 09/01/19 – 08/31/24 0.48 CM
NIH (Kantoff, Pomerantz)
The Impact of DNA Damage Repair Abnormalities in Prostate Cancer
Project 1: The Clinical and Epidemiological Characterization of Pathogenic DNA Damage Repair Pathway Variation in Prostate Cancer
Goals/Aims: 1) To determine the association between inherited pathogenic variants in DDR genes in the germline genome and long-term risk of lethal PC in men with high-risk, localized PC; 2) To interrogate the somatic genome in high-risk, localized PC patients for pathogenic variants in DDR genes and examine associations with risk of lethal PC; 3) To determine the prevalence of germline DDR mutations among African American PC patients, and compare and contrast them with those of European American men.
Role: Project Leader
Funding Agency Contact:
Rogers Gross, Grants Management Specialist
National Cancer Institute
Phone:
Email: rogers.gross@nih.gov

P01CA228696 09/01/19 – 08/31/24 0.20 CM
NIH (Kantoff, Pomerantz)
The Impact of DNA Damage Repair Abnormalities in Prostate Cancer
Project 3: Functional Evaluation and Interpretation of DNA Damage Response Variants in Prostate Cancer
Goals/Aims: 1) To use CRISPR technology to engineer specific tumor-derived mutations into the endogenous DDR gene locus of PC cell lines and to analyze the cellular phenotype.
Role: Co-Investigator
Funding Agency Contact:
Rogers Gross, Grants Management Specialist
National Cancer Institute
Phone:
Email: rogers.gross@nih.gov

FREEDMAN, MATTHEW

NEW AWARDS

R01CA244569 (Gayther) 06/01/20-05/31/25 0.84 CM
NIH
Characterizing noncoding somatic and germline variant interactions in ovarian cancer
Goals/Aims: Dr. Freedman will lead Aim 2, which will focus on genome editing. His training in human genetics and molecular biology provides the necessary expertise to lead Aim 2.
Role: Co-Investigator
Funding Agency Contact: TBD

W81XWH-19-1-0554 (Pomerantz) 09/15/19 – 09/14/21 0.48 CM
DoD
Functionally Characterizing the Enhancer Cistrome in Advanced Human Kidney Cancer
Goals/Aims: 1) To characterize the genome-wide landscape of open chromatin and active regulatory elements and their connections to target genes in the progression from localized to metastatic ccRCC; 2) To perform epigenome-wide CRISPR-based screens across the epigenome to identify the functional relevance of regulatory elements associated with ccRCC in model systems.
Role: Co-Investigator

Funding Agency Contact:
Michelle Cromwell, Grants Specialist
Department of Defense
Phone:
Email: michelle.l.cromwell.civ@mail.mil

W81XWH-19-0553 (Choueiri) 09/15/19-09/14/22 0.24 CM
DOD / KCRP TDA
Development and testing of circulating-free methylation DNA as a prognostic biomarker for recurrent kidney cancer
Goals/Aims: 1) To evaluate the relationship between cfmeDNA and recurrence in patients with early-stage RCC treated with placebo; 2) To evaluate the relationship between cfmeDNA and recurrence in patients with early-stage RCC treated with pazopanib.
Role: Co-Investigator
Funding Agency Contact
Michelle Cromwell, Grant Specialist
Department of Defense
Phone:
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P01CA228696 (Kantoff / Pomerantz multi-PI) 09/01/19–08/31/24 2.40 CM
NIH/NCI
The Impact of DNA Damage Repair Abnormalities in Prostate Cancer
Project 3: Functional Evaluation and Interpretation of DNA Damage Response Variants in Prostate Cancer
Goals/Aims: 1) To use CRISPR technology to engineer specific tumor-derived mutations into the endogenous DDR gene locus of PC cell lines and to analyze the cellular phenotype.
Role: Co-Investigator
Funding Agency Contact:
Rogers Gross, Grants Management Specialist
National Cancer Institute
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CHOUEIRI, TONI
NEW AWARDS

KC180129 (Choueiri) 09/30/19-09/29/21 1.20 CM
DOD / KCRP IDA
Host Immune Signatures as Therapy Response Biomarkers in Metastatic Renal Cell Carcinoma
Goals/Aims: 1) Identify immune response factors by characterizing the tumor immune microenvironment from pre-treatment mRCC tumor RNA-seq data; 2) Evaluate whether observing an expansion of tumor-infiltrating immune repertoire in post treatment blood is associated with patient response to VEGF-TT, ICB and ICB+VEGF-TT; 3) Validate the results using other mRCC cohorts with VEGF-TT, ICB and ICB+VEGF-TT treatment.
Role: Principal Investigator
Funding Agency Contact:
Michelle Cromwell, Grants Specialist,
U.S. Army Medical Research Acquisition Activity
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KC180206 (Choueiri) 09/15/19-09/14/22 0.84 CM
DOD / KCRP TDA

Development and testing of circulating-free methylation DNA as a prognostic biomarker for recurrent kidney cancer
Goals/Aims: 1) To evaluate the relationship between cfmeDNA and recurrence in patients with early-stage RCC treated with placebo; 2) To evaluate the relationship between cfmeDNA and recurrence in patients with early-stage RCC treated with pazopanib.
Role: Principal Investigator
Funding Agency Contact:
Joshua D. McKean, Grants Officer,
Department Of The Army
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KC190130 (Choueiri, Wu, MPI) 09/01/20-08/31/23 0.48 CM
DOD/ KCRP-TRPA
Combining Locally Administered Ipilimumab with a Personalized Neoantigen Cancer Vaccine to Improve T Cell Priming and Anti-Tumor Immunity in High-Risk Renal Cell Carcinoma
Goals/Aims: 1) Identify and characterize antigen presenting cells (APCs) that stimulate neoantigen-specific T cells following personal neoantigen vaccine with local IPI; 2) Determine the magnitude and characterize the phenotype, state, and functional avidity of neoantigen-specific T cells following personal neoantigen vaccine with local IPI.
Role: Principal Investigator
Funding Agency Contact:
Joshua D. McKean, Grants Officer,
Department Of The Army
Phone: joshua.d.mckean3.civ@mail.mil
Email:

KC190128 (Braun, D.) 10/01/20 – 09/30/24 0.60 CM
DOD
Understanding CD8+ T-Cell Specificity and Function in Renal Cell Carcinoma
Goals/Aims: Systematically identify the classes of tumor antigens in ccRCC, Link the T cell clonality of TILs in ccRCC with antigen specificity, and connect specific ccRCC target antigens and TIL clonotypes with response or resistance to anti-PD-1-based treatments.
Role: Mentor
Funding Agency Contact:
Joshua D. McKean, Grants Officer,
Department Of The Army
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KC180276 DOD (Pomerantz) 09/30/19-09/29/22 0.48 CM
W81XWH-18-KCRP-IDA
Functionally Characterizing the Enhancer Cistrome in Advanced Human Kidney Cancer
Goals/Aims: 1) To characterize the genome-wide landscape of open chromatin and active regulatory elements and their connections to target genes in the progression from localized to metastatic ccRCC; 2) To perform epigenome-wide CRISPR-based screens across the epigenome to identify the functional relevance of regulatory elements associated with ccRCC in model systems.
Role: Co-Investigator
Funding Agency Contact:
Joshua D. McKean, Grants Officer,
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R21CA238053 (Choueiri, Signoretti, MPI) 12/01/19-11/30/21 0.72 CM
NIH/NCI/BWH (DFCI only)
Tissue-based predictive biomarkers for Cabozantinib therapy in metastatic renal cell carcinoma
Goals/Aims: In this application, we propose to assess candidate predictive biomarkers for response to cabozantinib by utilizing pre-treatment tumor specimens from patients with metastatic ccRCC (mccRCC) treated in the randomized phase III METEOR clinical trial that compared cabozantinib to the mTOR inhibitor everolimus. 1) We will test the hypothesis that expression of angiogenesis-associated genes is predictive of clinical response to cabozantinib; 2) We will assess tumor genetic alterations as predictive biomarker of clinical response to cabozantinib. Aim 3: We will explore gene signatures of immune pathways activation that might be associated with response or resistance to cabozantinib.
Role: Partnering PI
Funding Agency Contact:
Mishell Alberto, Sr. Grant Administrator,
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(RENEWED)
P50CA101942 (McDermott/Kaelin) 09/01/20-08/31/25
NIH/ DF/HCC Kidney Cancer SPORE
Beth Israel Deaconess Medical Center

DF/HCC Kidney Cancer SPORE Project 1 0.60 CM
Goals/Aims: Given the critical dependency of clear cell RCC on HIF2α and our expertise in this area, we remain focused on this target in a completely revised project. The current proposal is based on a drug-like small molecule that directly binds to HIF2α and inhibits its function.
Role: Clinical Co-Leader (Basic Co-Leader: Kaelin)

DF/HCC Kidney Cancer SPORE Project 2 (PDL-1) *no salary taken
Goals/Aims: In his role as Co-Investigator, Dr. Choueiri will work closely with the co-PIs to analyze the results of the proposed trial and its correlatives.
Role: Clinical Co-investigator

DF/HCC Kidney Cancer SPORE Core 2 (TAPCD) (Choueiri, Signoretti) 0.90 CM
Goals/Aims: Dr. Choueiri is the Co-Director of Core 2 (TAPCD) on the submitted grant. Dr. Choueiri will oversee the administration of the Tissue Acquisition, Pathology and Clinical Data Core at DFCI and will supervise the Data Coordinator. He will ensure regulatory compliance, guide trouble-shooting, and contribute to planning of analyses and their interpretation.
Role: Co-Director (Director: Signoretti)

Funding Agency Contact:
Igor A. Kuzmin, PhD, Program Director,
National Cancer Institute
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ENDED AWARDS

A10383 SRA (Choueiri) 03/23/17-09/22/20 0.12 CM
AstraZeneca
Epi Study
Research activities are conducted as a contribution to AstraZeneca's retrospective observational study of outcomes and MET status in PRCC.
Role: Principal Investigator
Funding Agency Contact:

Heather Snyder, PhD, Clinical Scientist
AstraZeneca Pharmaceuticals LP
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Email: heather.snyder@astrazeneca.com

A11730 SRA (Choueiri) 01/08/18-07/08/20 0.24 CM
Exelixis, Inc
Differential expression of c-Met and PD-L1 expression/other immune biomarkers on the CABOSUN trial.
The investigation of the prognostic and/or predictive value of c-MET concurrently with other biomarkers of immune response on the CABOSUN trial (such as PD-L1) will constitute 1) preliminary data for correlative studies of the planned PDIGREE study that is planned by the Alliance. 2) It will also provide a benchmark for cabozantinib activity in PD-L1 (+) vs. PD-L1 (-) populations.
Role: Principal Investigator
Funding Agency Contact:
Lauren Kohlhoff, JD, Corporate Counsel, Contracts
Exelixis, Inc.
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A10674 SRA (Choueiri) 10/26/17-09/30/19 0.60 CM
Novartis
Correlation of mutation findings in TSC1, TSC2 and MTOR with response to rapalog therapy in patients with metastatic renal cell carcinoma (mRCC)
In this study, we will complete the mutation analysis of a full cohort of trial patients treated with rapalog therapy, to empower the statistical analysis in order to determine the positive predictive value of a mutation in TSC1,TSC2 and MTOR for response to rapalog therapy.
Role: Principal Investigator
Funding Agency Contact:
Maritza Crawford, Clinical Research Scientist
Novartis Pharmaceuticals Corp
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R21CA220253 (Brastianos, Carter, Choueiri, MPI) 09/01/2018-08/31/2020 0.60 CM
NIH/NCI/MGH
Identification of genomic drivers of brain metastases in renal cell carcinoma
Goals/Aims: 1) To test the hypothesis that brain metastases have unique molecular drivers, we will perform whole exome sequencing and epigenetic profiling of matched intracranial and extracranial sites. Using genomic characterization of matched primary renal cell tumors, brain metastases, and extracranial distant metastatic sites, and comparing to TCGA data, we aim to identify genetic alterations that are significantly enriched in brain metastases; 2) To test the hypothesis that recurrent brain metastases harbor specific genomic alterations driving their clinical behavior including resistance to therapy, we propose to analyze CSF samples, plasma samples and brain metastases before, during therapy and after progression on targeted therapy and PD-1 inhibitors.
Role: Co-PI
Funding Agency Contact:
Mario DeSantis, Senior Grant Administrator
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2018 Medical Oncology Award (Choueiri) 07/01/17-12/31/19 *No salary support
Dana-Farber Cancer Institute

T-cell receptor repertoire as a predictive biomarker of response to PD-1 inhibitors in advanced renal cell carcinoma
Goals/Aims: 1) To establish the TCR-seq experimental platform for cost-effective immune repertoire profiling; 2) To test TCR-β diversity in mRCC patients treated with nivolumab; 3) To develop bioinformatics methods to predict response nivolumab in mRCC patients.
Role: Principal Investigator
Funding Agency Contact:
Benjamin L. Ebert, MD, PhD, Chairman, Department of Medical Oncology
Dana Farber Cancer Institute
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A08749 SRA (Choueiri) 06/02/15-12/31/19 *No salary support
Bristol-Myers Squibb
Exploratory soluble factors (sPD-L1 and kynurenine and metabolites) in nivo-treated patients
Goals/Aims: Year 1: Provide proof of concept using the novel sPD-L1 and kynurenine assays; Year 2: Expand initial proof of concept from year 1 trials into testing of serum/plasma obtained as part of other nivolumab monotherapy or combination studies.
Role: Principal Investigator
Funding Agency Contact:
Donette Quamina-Edghill, Protocol Manager
Bristol-Myers Squibb Co.
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- **What other organizations were involved as partners?**
  - Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:** Nothing to Report
- **QUAD CHARTS:** Nothing to Report

9. **APPENDICES:** Nothing to Report

References:

1. Corces MR, Granja JM, Shams S, et al. The chromatin accessibility landscape of primary human cancers. *Science.* 2018;362(6413).
2. Cancer Genome Atlas Research N, Linehan WM, Spellman PT, et al. Comprehensive Molecular Characterization of Papillary Renal-Cell Carcinoma. *N Engl J Med.* 2016;374(2):135-145.
3. Chen F, Zhang Y, Senbabaoglu Y, et al. Multilevel Genomics-Based Taxonomy of Renal Cell Carcinoma. *Cell Rep.* 2016;14(10):2476-2489.
4. Dalgliesh GL, Furge K, Greenman C, et al. Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. *Nature.* 2010;463(7279):360-363.
5. Davis CF, Ricketts CJ, Wang M, et al. The somatic genomic landscape of chromophobe renal cell carcinoma. *Cancer Cell.* 2014;26(3):319-330.
6. Whyte WA, Orlando DA, Hnisz D, et al. Master transcription factors and mediator establish super-enhancers at key cell identity genes. *Cell.* 2013;153(2):307-319.
7. Hnisz D, Abraham BJ, Lee TI, et al. Super-enhancers in the control of cell identity and disease. *Cell.* 2013;155(4):934-947.
8. Boyer LA, Lee TI, Cole MF, et al. Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell.* 2005;122(6):947-956.
9. Ament SA, Blatti CA, Alaux C, et al. New meta-analysis tools reveal common transcriptional regulatory basis for multiple determinants of behavior. *Proc Natl Acad Sci U S A.* 2012;109(26):E1801-1810.
10. Roadmap Epigenomics C, Kundaje A, Meuleman W, et al. Integrative analysis of 111 reference human epigenomes. *Nature.* 2015;518(7539):317-330.
11. Reddy J, Fonseca MAS, Corona RI, et al. Predicting master transcription factors from pan-cancer expression data. *bioRxiv.* 2019:839142.
12. Bleu M, Gaulis S, Lopes R, et al. PAX8 activates metabolic genes via enhancer elements in Renal Cell Carcinoma. *Nat Commun.* 2019;10(1):3739.
13. Ricketts CJ, De Cubas AA, Fan H, et al. The Cancer Genome Atlas Comprehensive Molecular Characterization of Renal Cell Carcinoma. *Cell Rep.* 2018;23(12):3698.
14. Consortium EP. An integrated encyclopedia of DNA elements in the human genome. *Nature.* 2012;489(7414):57-74.
15. Garancher A, Lin CY, Morabito M, et al. NRL and CRX Define Photoreceptor Identity and Reveal Subgroup-Specific Dependencies in Medulloblastoma. *Cancer Cell.* 2018;33(3):435-449 e436.
16. Wang X, Prager BC, Wu Q, et al. Reciprocal Signaling between Glioblastoma Stem Cells and Differentiated Tumor Cells Promotes Malignant Progression. *Cell Stem Cell.* 2018;22(4):514-528 e515.
17. Zeid R, Lawlor MA, Poon E, et al. Enhancer invasion shapes MYCN-dependent transcriptional amplification in neuroblastoma. *Nat Genet.* 2018;50(4):515-523.
18. Mack SC, Singh I, Wang X, et al. Chromatin landscapes reveal developmentally encoded transcriptional states that define human glioblastoma. *J Exp Med.* 2019;216(5):1071-1090.
19. Anderson MK, Hernandez-Hoyos G, Diamond RA, Rothenberg EV. Precise developmental regulation of Ets family transcription factors during specification and commitment to the T cell lineage. *Development.* 1999;126(14):3131-3148.
20. Iwasaka C, Tanaka K, Abe M, Sato Y. Ets-1 regulates angiogenesis by inducing the expression of urokinase-type plasminogen activator and matrix metalloproteinase-1 and the migration of vascular endothelial cells. *J Cell Physiol.* 1996;169(3):522-531.
21. Leprince D, Gegonne A, Coll J, et al. A putative second cell-derived oncogene of the avian leukaemia retrovirus E26. *Nature.* 1983;306(5941):395-397.
22. Nunn MF, Seeburg PH, Moscovici C, Duesberg PH. Tripartite structure of the avian erythroblastosis virus E26 transforming gene. *Nature.* 1983;306(5941):391-395.
23. Dittmer J. The biology of the Ets1 proto-oncogene. *Mol Cancer.* 2003;2:29.

24. Hahne JC, Okuducu AF, Sahin A, Fafeur V, Kiriakidis S, Wernert N. The transcription factor ETS-1: its role in tumour development and strategies for its inhibition. *Mini Rev Med Chem.* 2008;8(11):1095-1105.
25. Kim E, Gunther W, Yoshizato K, et al. Tumor suppressor p53 inhibits transcriptional activation of invasion gene thromboxane synthase mediated by the proto-oncogenic factor ets-1. *Oncogene.* 2003;22(49):7716-7727.
26. Davidson B, Reich R, Goldberg I, et al. Ets-1 messenger RNA expression is a novel marker of poor survival in ovarian carcinoma. *Clin Cancer Res.* 2001;7(3):551-557.
27. Span PN, Manders P, Heuvel JJ, et al. Expression of the transcription factor Ets-1 is an independent prognostic marker for relapse-free survival in breast cancer. *Oncogene.* 2002;21(55):8506-8509.
28. Elvert G, Kappel A, Heidenreich R, et al. Cooperative interaction of hypoxia-inducible factor-2alpha (HIF-2alpha) and Ets-1 in the transcriptional activation of vascular endothelial growth factor receptor-2 (Flk-1). *J Biol Chem.* 2003;278(9):7520-7530.