

Creation and implementation of an institutional workflow for systematic collection of fresh renal cell carcinoma tumor biospecimens for investigative studies.

Authors: Katherine E. Sadak¹, Nicholas R. Schindler¹, Yalai Bai², Dongqing Liu², Dinesh Singh³, Michael S. Leapman³, Harriet Kluger^{4,5}, Mario Sznol^{4,5}, Daniel P. Petrylak^{4,5}, Peter A. Humphrey², Adebowale Adeniran^{*2}, Michael Hurwitz^{*4,5}, Patrick A. Kenney^{*3}, David A. Braun^{*1,2,3,4} *Co-Senior authors

¹Center of Molecular and Cellular Oncology, Yale Cancer Center, Yale School of Medicine, New Haven, CT, USA. ²Department of Pathology, Yale School of Medicine, New Haven, CT USA. ³Department of Urology, Yale School of Medicine, New Haven, CT USA. ⁴Section of Medical Oncology, Department of Internal Medicine, Yale School of Medicine, New Haven, CT USA. ⁵Yale Cancer Center, Yale School of Medicine, New Haven, CT USA.

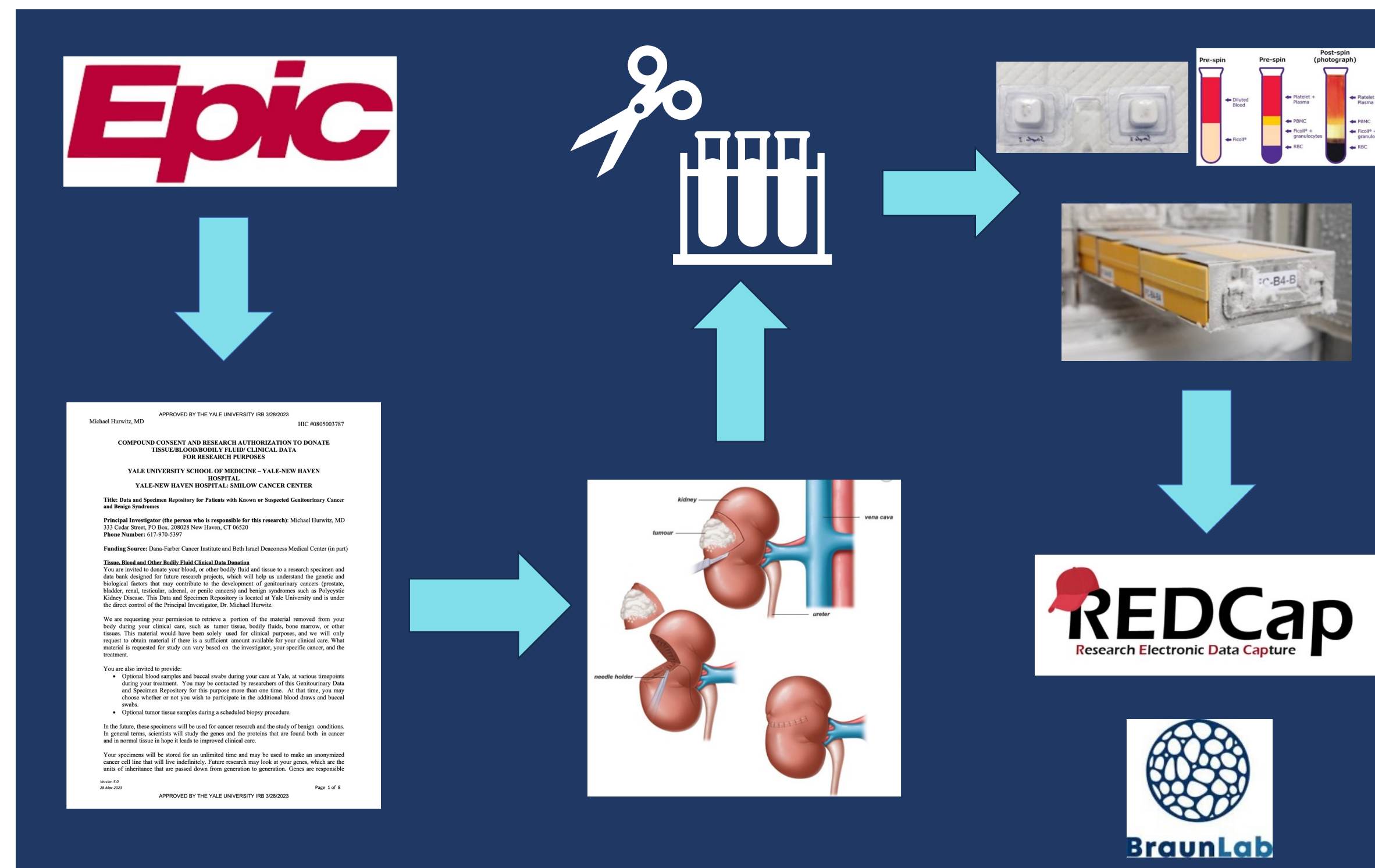
Introduction

- Growing need for **fresh** biospecimen collection in molecular oncology research
- Emerging experimental assays require fresh tumor tissue, including:
 - Single-cell RNA-seq analysis
 - Patient-derived model systems
- Due to their larger size, renal cell carcinoma (RCC) tumor specimens provide a unique opportunity to perform functional analyses on primary human tumors.
- Concomitant blood collection allows for comparison of systemic immune function to that of the tumor microenvironment.
- For effective fresh collection, biospecimens must be received by researchers in a timely and efficient manner
- Implementation of an effective workflow requires careful coordination between the clinical setting and research laboratory.

Methods

- Potential subjects are identified using the electronic medical record system, through manual review of clinic and surgical schedules with pre-specified criteria.
- Informed consent for an IRB-approved protocol is obtained.
- Pathology receives the tissue and allocates a portion (not affecting clinical diagnosis) in chilled RPMI media to a research team member who immediately transports the specimen to the research laboratory.
- Any remaining tissue is stored using 4 different methods: formalin-fixed paraffin-embedded, frozen using optimal cutting temperature media, fresh frozen, and cryopreservation of viable tissue fragments.
- Blood is also collected at the time of surgery. Samples undergo density centrifugation for isolation and storage of plasma and peripheral blood mononuclear cells (PBMCs).
- Patients are tracked post-operatively for future blood collections.
- De-identified clinical data for each subject is encoded into a clinical RedCap database.

Fresh RCC Tumor Collection Workflow

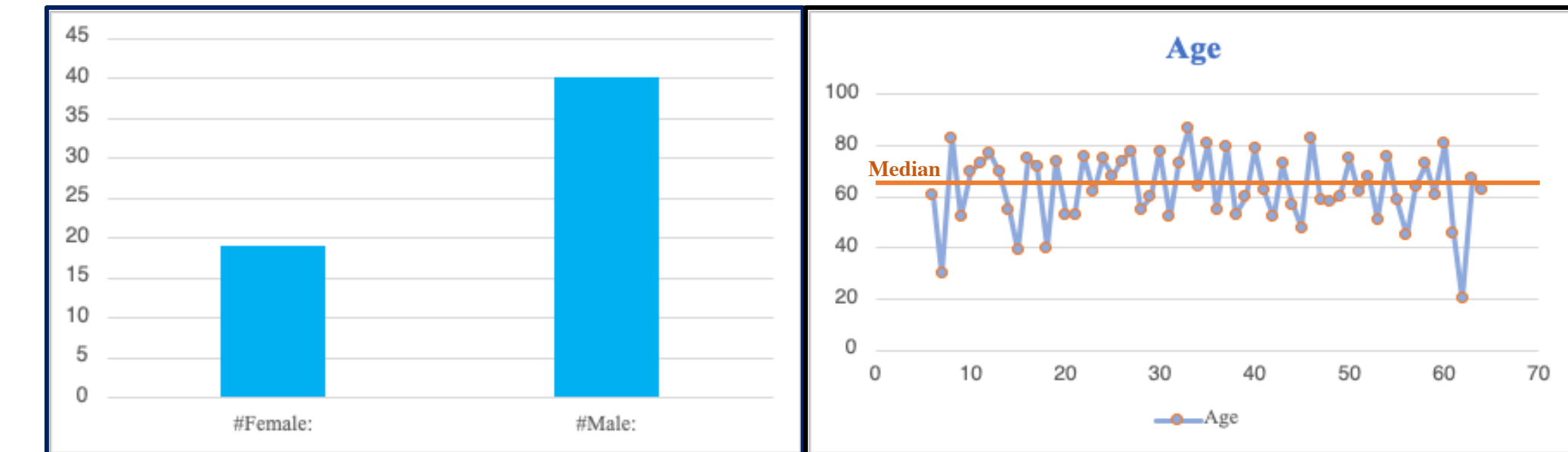


Results

- In the time period of **September 2022 through May 2023**, clinical data from 59 patients were added to the RedCap Database.
- From these patients, we have collected tumor tissue from 43, adjacent normal tissue from 40, and blood from 49 and registered 63 patients to our protocol.

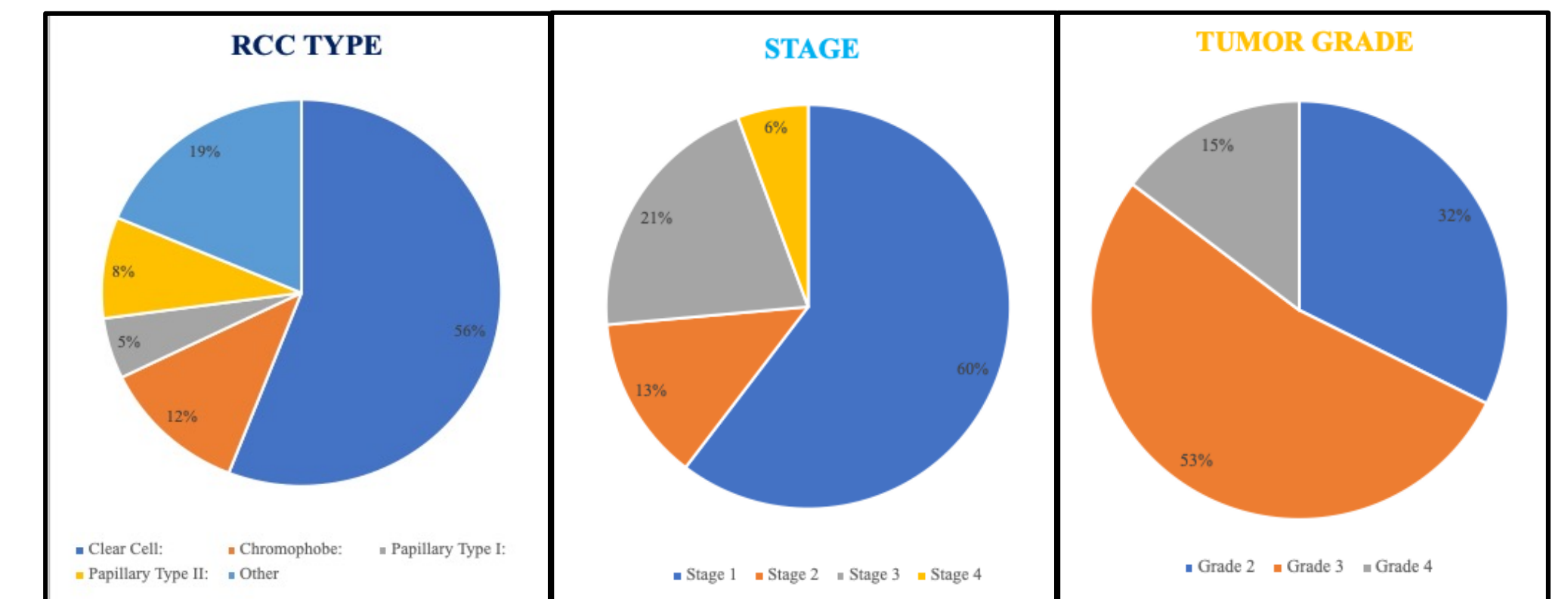
Patient Demographics

- There were 40 males (68%) and 19 females (32%) with a M:F ratio of 2.1.
- The median age was 63, with a range of 20-87.
- 52 (88%) patients identified as White or Caucasian, 5 (8%) as Black or African American, 1 (2%) as Hispanic or Latino, and 1 (2%) as Asian.



RCC Characteristics

- 33 patients had clear cell RCC (57%), 7 with chromophobe RCC (13%), 8 with papillary RCC (13%), 11 considered “Other” (17%) (includes benign oncocytomas, mucinous tubular and spindle cell RCC, and eosinophilic RCC).



Conclusions and Future Directions

- This workflow has led to the successful collection and distribution fresh specimen for research, including single-cell RNA-sequencing, the creation of *ex vivo* patient-derived tumor-models, and the isolation and functional interrogation of tumor-infiltrating immune cells.
- This work demonstrates the feasibility of implementing a multidisciplinary fresh biospecimen collection protocol for improving our understanding of RCC specifically and human tumor immunology more generally.

