Exhibit 444

EXPERT REPORT ON THE CASE OF JACQUELINE Y. TUKES FOR THE DEPARTMENT OF JUSTICE

GAIL H. VANCE, M.D.

Gail H. Vance, M.D. Digitally signed by Gail H. Vance, M.D. Date: 2025.04.07 15:01:07 -04'00'

Gail H. Vance, M.D.

April 8, 2025

Contents

I. Introduction

I have been retained by attorneys at the U. S. Department of Justice (DOJ) for the purpose of review of the medical records of Jacqueline Y. Tukes and to render an opinion as to the etiology of her extensive disease of bilateral and multifocal renal cell carcinoma. This report also provides background information for the understanding of genetics and cancer genetic disease as well as my assessment. It is my opinion that Ms. Tukes meets criteria for Hereditary Renal Cell Carcinoma. Despite the absence of a gene mutation in genes tested, Ms. Tukes has features consistent with hereditary renal cancer, including her age at diagnosis, her family history, and her bilateral and multifocal cysts and tumors. My opinions are based on the information currently available to me and I reserve the right to supplement these opinions based on new information.

I am qualified to review the documentation and offer an opinion regarding Ms. Tukes' genetic testing and her Hereditary Renal Cell Carcinoma. I have over 30 years of experience as a practicing medical geneticist with a specialty in cancer genetics. My training includes two residencies: Clinical Pathology and Pediatrics. I also completed two fellowships: Clinical Genetics and Clinical Cytogenetics. I am a board-certified clinical pathologist by the American Board of Pathology (active); board certified clinical geneticist (active) and board-certified clinical cytogeneticist (active) by the American Board of Genetics and Genomics; and board-certified pediatrician by the American Board of Pediatrics (inactive). I am a practicing cancer geneticist and a member of the medical staffs of Indiana University Health (IUH) Physicians' Group and Eskenazi Health. I attend weekly cancer genetic clinics at IUH University Hospital/IU Simon Comprehensive Cancer Center and IUH Schwartz Cancer Center. I am emeritus professor of the departments of Medical and Molecular Genetics and Pathology and Laboratory Medicine and the Sutphin Chair of Cancer Genetics at Indiana University School of Medicine in Indianapolis, Indiana. My CV is attached to this report.

I have not testified at deposition or trial in the past four years.

I am being compensated at a rate of $350 per hour for my work on this case.

A. What is genetics?

Genetics is the science of genes, gene products, and their effect on conception, intrauterine formation and development, physical phenotype, and overall health.

Genes are the basic units of heredity, passed down from parents to their children. Genes are located on chromosomes and found in the nucleus of the cell (Figure1).

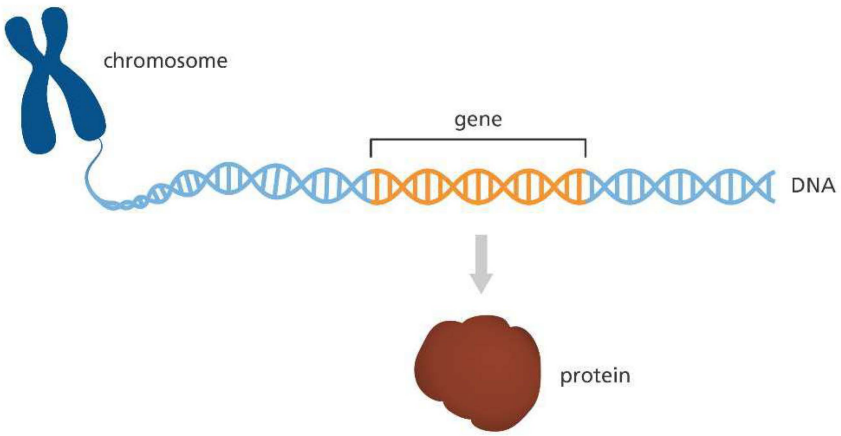


Figure 1. (Yourgenome.org)

Gene products are primarily RNAs and proteins. There may be multiple proteins (isoforms) from one gene (Figure 2).

Genotype is the genetic constitution of an organism, as distinguished from its physical appearance.

Missense refers to a mutation that changes a codon specific for one amino acid to specify another amino acid. This is in contrast to a deletion mutation, which results in a loss of nucleotides and possibly a loss-of-function mutation, depending on the deletion. If there is a loss-of-function mutation, the protein would not work in its normal manner.

Codon: The sequence of nucleotides, coded in triplets (codons) along the mRNA, that determines the sequence of an amino acid in protein synthesis.

Transcription is the process of making an RNA copy of a gene's DNA sequence. The copy is called messenger RNA (mRNA).

Translation is the process through which information encoded in messenger RNA directs the addition of amino acids during protein synthesis. Translation takes place on ribosomes in the cell cytoplasm, where mRNA is read and translated into the string of amino acid chains[1].

Phenotype is the physical characteristics of an organism or the presence of a disease that may or may not be genetic.

Proteins are large, complex molecules that play many important roles in the body. They are required for the structure, function, and regulation of the body's tissues and organs. A protein is made up of one or more long, folded chains of amino acids (each called a polypeptide), whose sequences are determined by the DNA sequence of the protein-encoding gene [1].

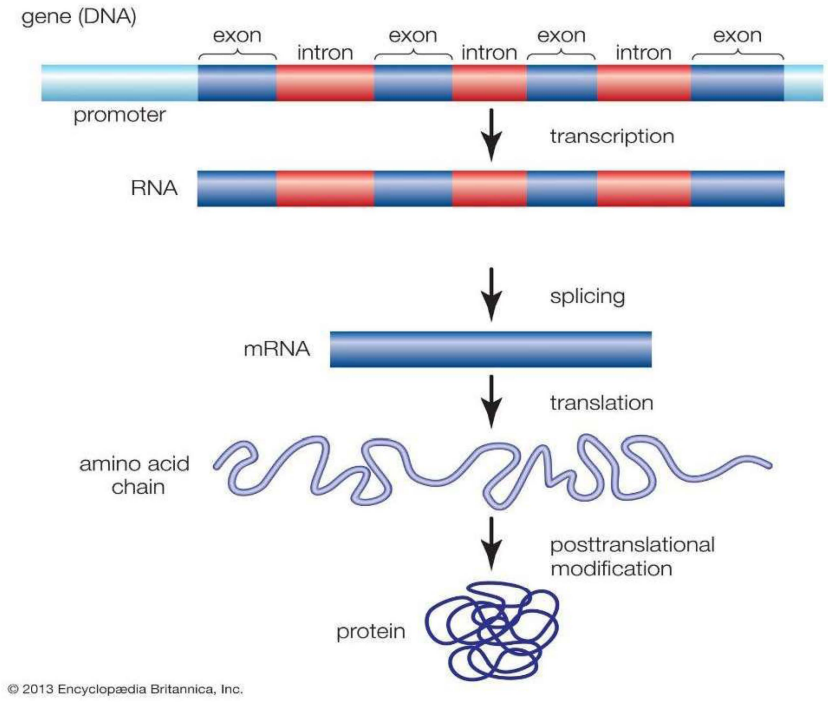


Figure 2.

B. Cancer Genetics

What is carcinogenesis? Carcinogenesis (oncogenesis or tumorigenesis) is the process of transformation of normal cells to cancer cells due to disruption of normal cell cycle controls leading to uncontrolled proliferation (Figure 3). All cancer is genetic in that cancer results from dysregulation/disruption of genes that control cell growth and differentiation. Cancer typically develops with an accumulation of genetic mutations in targeted genes such as tumor suppressor genes, DNA repair genes, and oncogenes.

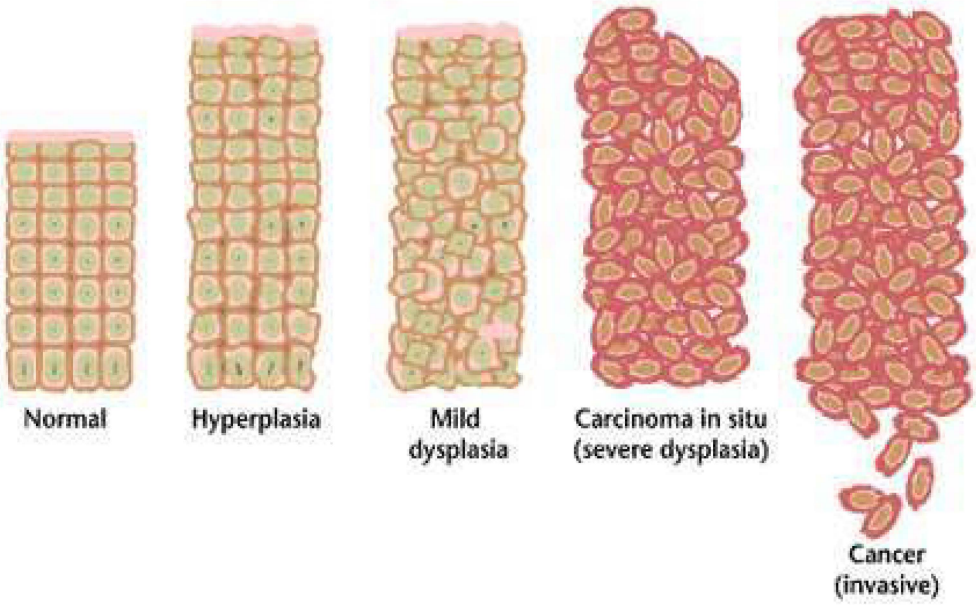


Figure 3. Spectrum from normal tissue to cancer- to metastasis -Natl Cancer Institute

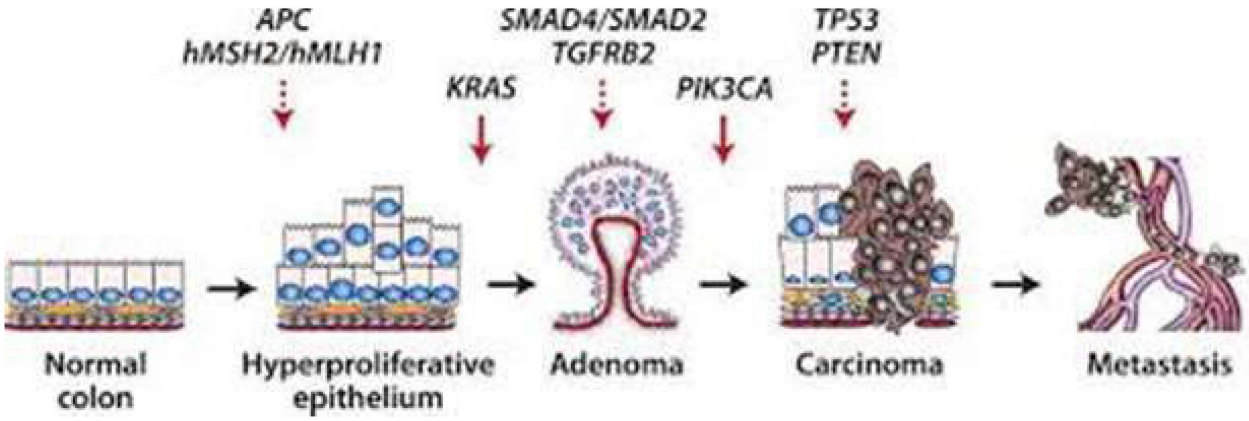


Figure 4. Spectrum from normal colonic cells showing gene mutations at the different stages of carcinogenesis. Adapted from Vogelstein and Ferron by Scott A. Waldman

In the image above (Figure 4), a mutation in the *APC* gene is an early event in the development of colorectal cancer. The early *APC* mutation leads to cellular instability and the acquisition of additional mutations including DNA repair genes such as *MSH2* and *MLH1*.

The *APC* gene is class of cell regulatory genes known as **tumor suppressor genes**. These genes are essentially the "brakes" of the cell producing a negative stimulus for cell proliferation and whose function must be inactivated (lost) for cell transformation to occur. When a cellular error or mutation occurs to a cell, these genes may arrest further cell cycle progression until repair is completed. If repair is not possible, then there are cell signals to eliminate the cell causing cell death or apoptosis.

An error in a tumor suppressor gene leads to cellular instability. Loss of the second tumor suppressor gene contributes to cancer.

Another class of genes associated with cancer is the **oncogene**. Proto-oncogenes are normal genes which produce a positive, controlled stimulus for cell proliferation. However, when mutated, **oncogenes** lead to a gain of function or "transformed" cellular phenotype, meaning the cell continues to proliferate uncontrollably. *KRAS* is an example of an oncogene (Figure 5).

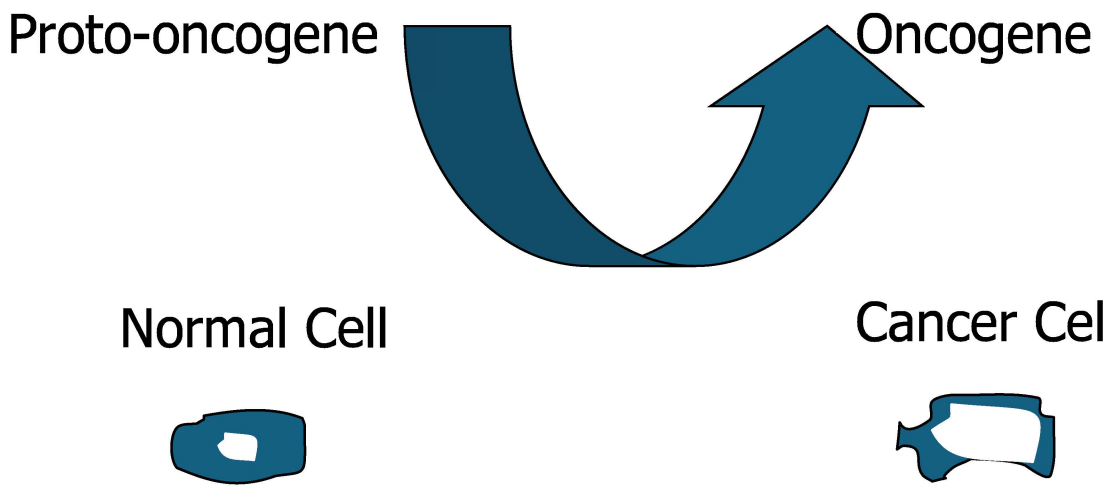


Figure 5. A proto-oncogene when mutated leads to a gain of function, promoting cellular proliferation.

Another category of cancer genes includes the DNA repair genes which support and maintain genetic stability and are specifically involved in the repair of damaged DNA. They exert an indirect effect on cell proliferation or survival by influencing the ability of the cell to repair damage to other genes including tumor suppressor genes and proto-oncogenes. In Figure 4, the image shows progressive acquisition of mutations and cellular proliferation in the colon, with DNA repair genes identified as *MSH2* and *MLH1*.

C. *Examples of known genetic-cancer relationships*

As illustrated above (Figure 4), a mutation in the *APC* gene (Adenomatous Polyposis Coli) is an early event in the development of colon cancer in the general population. Typically, an *APC* mutation occurs sporadically, i.e. it is acquired over the lifetime of the individual with colon cancer. This is referred to as sporadic disease and it is for those common and sporadic cancers that the American Cancer Society has established population screening guidelines such as getting a colonoscopy starting at age 45 years[2]. Most cancer, ~70-80% of the total burden of cancer, is sporadic in nature and acquired during the lifetime of an individual. So, for colon cancer as an example, exposures would include exposures to chemicals and other toxins, UV radiation, infection, and aging. Colon cancer because of toxic exposure would be considered sporadic disease.

However, there is an inherited predisposition to colon cancer associated with a germline (inherited) mutation of the *APC* gene, called Familial Adenomatous Polyposis. Germline means that the mutation

was inherited from a parent and not because of a toxic exposure, for example. A germline mutation is inherited from a parent and therefore generally occurs in every cell of the body. Another mutation of the *APC* gene in a colon cell with a germline mutation could lead to the development of a tubular adenoma and further proliferation to colon cancer. An inherited genetic syndrome called Familial Adenomatous Polyposis (FAP) is a well-known and characterized colon cancer syndrome resulting from inheritance of a mutated *APC* gene leading to a "predisposition" to colon cancer. Features of FAP include the development of hundreds of polyps in the colon beginning, on average, at age 16 years. Individuals with a diagnosis of classic FAP have a 70%-100% estimated lifetime risk of colon cancer if left untreated [3].

Other inherited cancer predisposition syndromes include:

- **Hereditary Breast and Ovarian Cancer (HBOC)** with elevated lifetime risks for breast, ovarian, prostate, melanoma, and pancreatic cancers. Female breast cancer is common with an approximate lifetime risk of 12.5% for the development of breast cancer. However, individuals with a germline mutation of *BRCA1* have an elevated lifetime risk of ~60% for female breast cancer and a 39-58% risk of epithelial ovarian cancer. Men carry a 7-26% risk of prostate cancer and men and women carry ~5% risk for pancreatic cancer. Other genes associated with a high risk of breast cancer due to an inherited gene mutation include germline mutations of *BRCA2*, *PALB2*, *TP53* and *STK11*[4]. Disease onset, particularly for breast cancer, is often earlier than that of the general population, i.e. < 50 years.

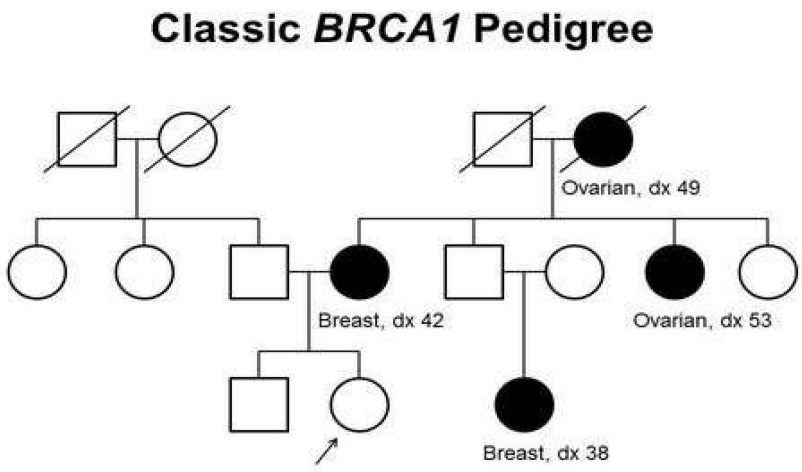


Figure 6. https://visualsonline.cancer.gov/details.cfm?imageid=10436 Circles represent females and squares represent males. A filled in circle indicates a cancer. A line through the circle indicates the individual is deceased. Note breast and/or ovarian cancer in each generation. Dx=diagnosis.

- **Lynch Syndrome** is a hereditary colon cancer syndrome associated with inherited germline mutations of *EPCAM*, *MLH1*, *MSH2*, *PMS2* and *MSH6*. Individuals with germline mutations in

these genes have increased lifetime risks of colon, endometrial, ovarian, upper gastrointestinal, and urinary tract cancers. For example, individuals with germline mutations of *MLH1* have a 46-61% lifetime risk of colon cancer compared to a general population risk of ~4%. Other risk estimates include a 34-54% lifetime risk of endometrial cancer, 4-20% risk of ovarian cancer and 0.2-5% risk of renal pelvis/ureter cancer[5].

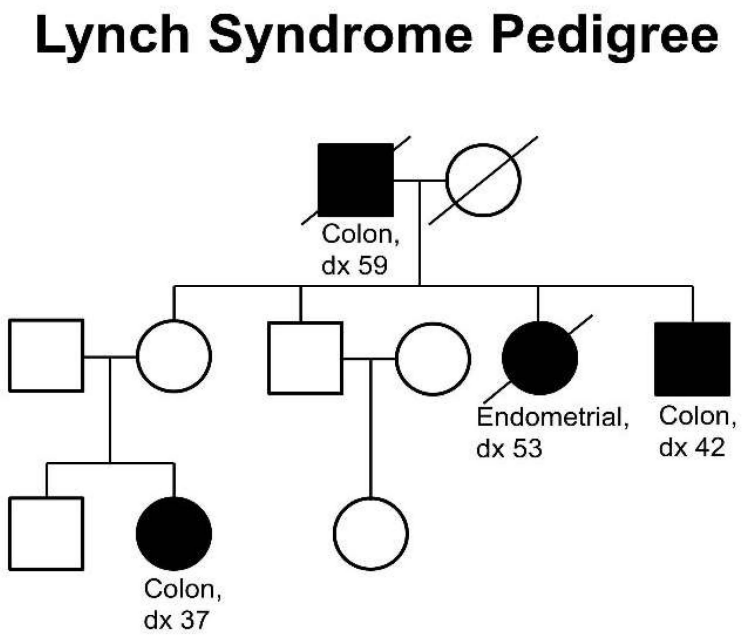


Figure 7. https://visualsonline.cancer.gov/details.cfm?imageid=9843 Circles represent females and squares represent males. A filled-in circle indicates a cancer. A line through the circle indicates the individual is deceased. Note colon cancer in each generation. Dx=diagnosis.

- **Multiple Endocrine Neoplasia syndromes** have features of neuroendocrine, parathyroid, thyroid, and renal cancers. Multiple Endocrine Neoplasia Type 1 is due to a gene mutation of *MEN1*. Features include hyperparathyroidism, pituitary adenomas and neuroendocrine tumors of the gastro-entero-pancreatic organ systems. *MEN2* is associated with a gene mutation of *RET*. Characteristic features include medullary thyroid carcinoma, pheochromocytoma, primary hyperparathyroidism and mucosal and gastrointestinal ganglioneuromas[6,7].

- **Von Hippel Lindau** is a hereditary kidney cancer syndrome associated with hemangioblastomas of the brain, retina, and spine; clear cell renal cancer; and neuroendocrine cancer. Other features include pheochromocytoma and pancreatic, renal, epididymal and broad ligament cysts. Von Hippel Lindau syndrome is caused by mutations in the *VHL* gene[8,9].

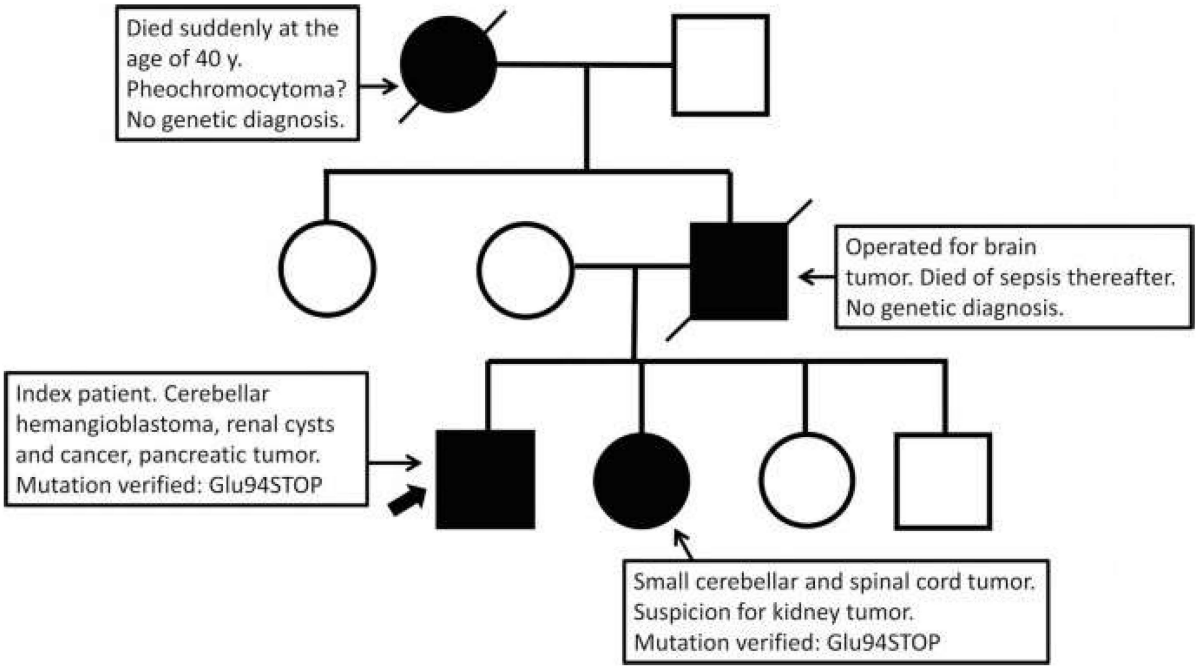


Figure 8. Von Hippel–Lindau Syndrome | SpringerLink https://link.springer.com/chapter/10.1007/978-3-030-62011-0_52 Circles represent females and squares represent males. A filled in circle indicates a cancer. A line through the circle indicates the individual is deceased.

Individuals genetically predisposed to cancer account for approximately ~5-10% of the cancer population. Features suggesting an inherited predisposition to cancer include:

1. **Two or more close relatives affected,** e.g., first-degree relatives such as mother/father or sister/brother and second-degree relatives such as aunt/uncle or grandmother/grandfather.
2. **Early age of onset**- earlier than the general population e.g., breast cancer <50 years or colon cancer less than 45 years.
3. **Cancers of a specific type occurring together** (breast and ovary; colon and endometrial cancer associated with hereditary cancer syndromes as indicated above for HBOC and Lynch syndromes).
4. **Multiple or bilateral cancers** occurring in one person; multifocal (more than one solitary tumor) or bilateral disease e.g., cancer in both eyes, both breasts, both kidneys as observed in retinoblastoma, hereditary breast and ovarian cancer, and hereditary kidney cancer, respectively. In contrast to unilateral cancer (one kidney) and a single focus of cancer in that kidney.
5. **Rare cancers** such as ovarian cancer with a general population lifetime risk of 1.5%.

The clinical phenotype or features of an individual with a germline gene mutation associated with a hereditary cancer syndrome was first outlined by Dr. Alfred Knudson in his analysis of individuals with retinoblastoma, a rare retinal cancer identified in children. He established the “two hit theory” to explain that children with bilateral eye cancer must have been predisposed to cancer and born with

an inherited gene mutation in *RB*, the tumor suppressor gene associated with retinoblastoma, and later acquired a second, tumor-activating hit (Figure 9). The second hit occurs somatically (sporadically) in the other allele of the same gene. These children, born with a first hit, not only had bilateral disease but also multifocal cancer (multiple tumors) and an overall younger age of onset than those children with unilateral disease and wildtype (non-mutated) *RB* genes [10,11].

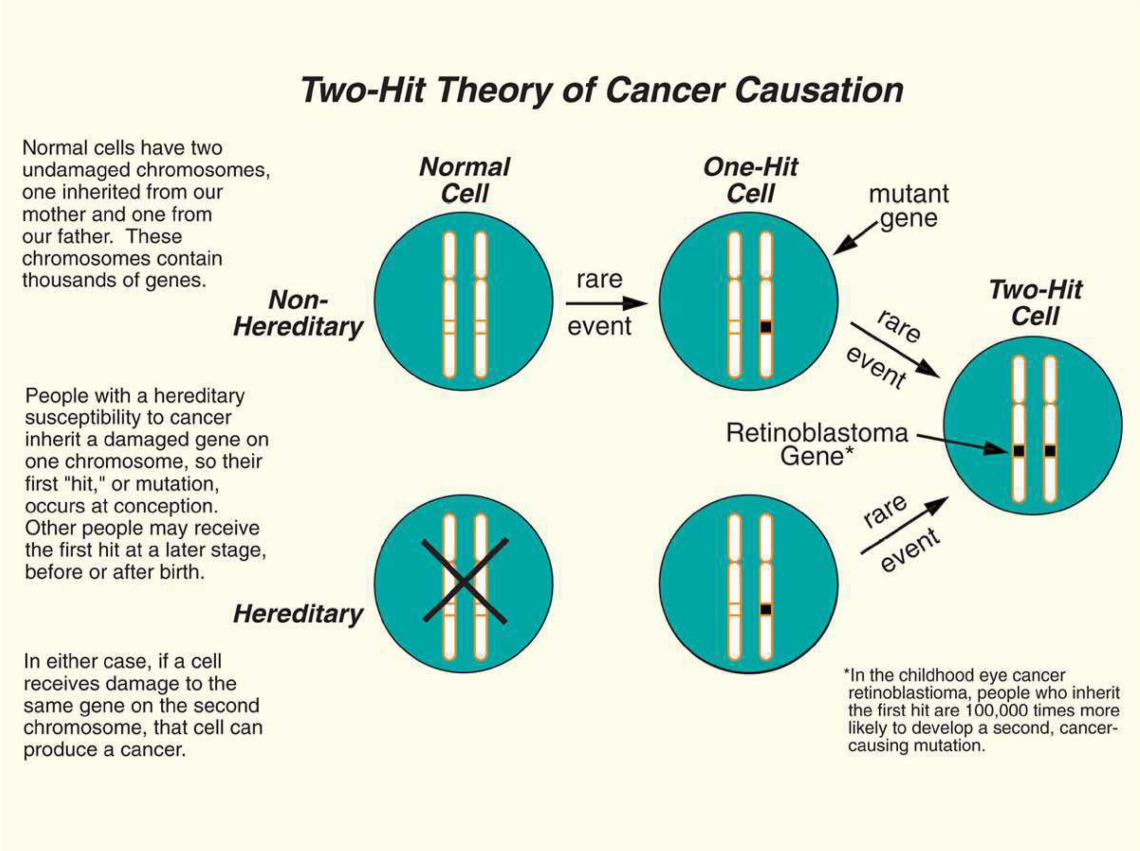


Figure 9. Knudson's two hit theory of cancer- https://www.foxchase.org/about-us/history/discoveries-fox-chase-research/knudsons-two-hit-theory-cancer-causation

In addition to hereditary cancer and sporadic cancer there is a third classification called "familial cancer". Familial cancer is recognized as an increased number of individuals in a family with cancer, without a clearly identified heritable pattern, i.e. not autosomal dominant or autosomal recessive transmission, potentially due to a combination of genetic and environmental factors. Familial cancer represents ~15-20% of cancers and is usually associated with more common cancers such as breast, colon, and prostate cancer[12] (Figure 10).

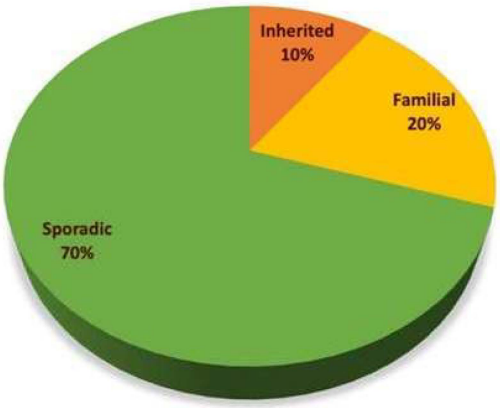


Figure 10. Figure representing approximate proportions of cancer in inherited, familial, and sporadic cancer classifications.

D. Cancer Genetic Services

Cancer genetic services include genetics evaluation, risk assessment, genetic counseling, genetic testing, and medical management. Typically, a person will meet with a genetic counselor and medical geneticist for an appointment in which the following activities occur.

- Reviews referral and contacts patients in advance of their appointment as needed.
- Collects family history information and medical records in advance of the appointment when possible.
- Conducts a genetics evaluation including assessment of personal and or family cancer history as well as review of laboratory studies, past medical, social and surgical history.
- Obtains and evaluates, at minimum, a three-generation family history primarily focused on history of cancer, tumors, and polyps or other abnormal cellular growth.
- Performs a physical examination when needed to evaluate for clinical features associated with various cancer predisposition syndromes.
- Constructs a differential diagnoses list as potential etiology for the personal and//or family cancer histories.
- Educates the patient and his/her family regarding cancer risks, medical management, benefits and limitations of genetic testing and inheritance patterns. Identifies at-risk family members who may also benefit from medical-management options and/or genetic testing.
- Obtains informed consent for genetic testing for individuals in which testing is considered appropriate and who pursue this option.
- Obtains and submits samples for genetic testing to genetic testing laboratories located throughout the United States. Testing is typically performed on peripheral blood or saliva. Skin biopsy samples are collected for individuals who have undergone non-autologous stem cell transplant.
- Interprets the results of the genetic testing with consideration of the personal and family cancer history. Informs patient of the clinical significance of the genetic testing results.

- Recommends medical management options including cancer surveillance, cancer prevention as well as medical and surgical interventions. Coordinates medical management as needed and facilitates specialty referrals.
- Facilitates DNA storage for patients with cancer for whom genetic testing was non-informative, for individuals at the end of their life, or for individuals who choose not to pursue genetic testing but who wish to make a DNA sample available to their children for genetic testing in the future.

E. Genetic Testing

In the context of Cancer Genetics, genetic testing refers to testing of, typically, a panel of genes associated with a particular cancer syndrome or cancer. This is germline testing, i.e. looking for inherited gene mutations and not genetic testing of tumor tissue or circulating tumor cells in the blood. As stated earlier, if a gene mutation was inherited, it will reside in every cell of the body including the blood cells. Typically, germline genetic testing is performed on peripheral blood although saliva and cheek swabs may also be collected. The DNA is extracted from the white cells in the sample and subjected to molecular testing, such as high-throughput sequencing. There are several commercial and academic laboratories performing this testing. Laboratories will report variants, i.e. changes in the patient's DNA, in tested genes when compared to a reference genome, using a paradigm of five classifications: pathogenic, likely pathogenic, uncertain significance, likely benign and benign. Pathogenic and likely pathogenic results are considered significant/positive and associated with disease whereas benign or likely benign are thought to be polymorphisms/random changes and not associated with disease [13].

The purpose of genetic testing is to determine the risk for developing a cancer or cancers; the etiology of a cancer; direct clinical management; identify carriers; and possible prognoses in individuals, families, or populations. Genetic testing is voluntary and informed consent about the risks and benefits of genetic testing and potential genetic test results is required.

There are three possible results for each gene tested. A positive result indicates a mutation or pathogenic/likely pathogenic variant associated with disease was identified. A negative result is an indeterminate result meaning no additional information was gained. It is not known if the person was truly negative for a gene mutation; or the current technology couldn't find the mutation; or another gene is involved but medical science has not yet identified the association of the gene with disease. Test reports do not usually report benign or likely benign results unless a variant has been reclassified as such. The test report will report negative results. The third possible result is a variant of unknown/uncertain significance meaning there is a change when compared to a reference, but the significance of the change is not yet known. The interpretation of genetic test results is delivered in the context of the laboratory report and the medical and family history of the patient.

When a **positive result (pathogenic/likely pathogenic variant)** is identified, medical screening is recommended. Often the medical screening for the cancer syndrome is outlined in the guidelines produced by the National Comprehensive Cancer Network (NCCN). NCCN is a non-profit alliance of U.S. cancer centers. Expert panels are established with experts from various fields of oncology and cancer therapy. The panel members review current literature and provide clinical practice guidelines

as evidence-based recommendations for the diagnosis, treatment, and follow-up of various types of cancer including hereditary cancers. These guidelines are continuously updated as new knowledge is gained. Using FAP as an example again, a patient with a pathogenic variant in the *APC* gene would be counseled to undergo colonoscopy every 12 months beginning at age 10-15 years until a colectomy is recommended. Other screening measures include upper gastrointestinal endoscopy; ultrasound of the thyroid; and if five years or younger, screening for hepatoblastoma[3].

The first individual in a family diagnosed with a cancer-associated gene mutation syndrome is often labeled the proband. First degree (mother/father; sister/brother) and possibly second-degree relatives (grandparent; aunt/uncle) of the proband are recommended to have genetic testing for the cancer gene mutation. The testing of family members is referred to as "cascade" testing. Typically, testing is ordered for the familial variant identified in the proband and not a panel of genes. In the scenario of a known familial gene mutation or variant, the interpretation of the genetic test result is either positive (the familial gene mutation was identified) or negative (the familial gene mutation was not identified).

A **negative result (includes benign or likely benign variant)** in a person with cancer indicates the person does not have an identifiable pathogenic variant in any of the genes analyzed. The person would still have a clinical diagnosis of the cancer and should undergo recommended surveillance for the cancer. Again, a negative gene test result in an individual with a suspected hereditary cancer syndrome does not exclude a heritable cause for the cancer. Advances in genetic testing technology and genetic knowledge may identify gene mutations not currently recognized. Generally, it is suggested that updated testing be performed in 5-10 years from the date of the negative test in a person suspected of have hereditary cancer.

A **variant of uncertain significance (VUS)** on the genetic testing report indicates that a change was identified in one of the genes tested, but there is currently insufficient date available to indicate whether it is a benign or pathogenetic variant. We typically do not test other family members or change medical management based on an uncertain test result.

II. Opinions regarding Jacqueline Tukes's renal cancer

A. Background information:

The following medical information was obtained from medical records provided to me by the DOJ attorneys, including the medical records relevant to Ms. Tukes's cancer and genetic testing. These medical records provide the basis of my conclusion that Ms. Tukes displayed features of hereditary renal cancer. I also reviewed relevant deposition transcripts made available to me by the DOJ attorneys. A complete list of the facts and data considered will be provided separately.

Re: Jacqueline Y. Tukes - DOB: [redacted]/1965

B. History of pertinent illness:

Ms. Tukes was diagnosed with a renal mass in June 2010 at age 45 years. She underwent an open right partial nephrectomy on 8/20/2010 at the University of North Carolina (UNC). The pathology was classified as clear cell renal cell carcinoma (RCC). Furman Gr 2/4. On May 23, 2011, at UNC, she had an MRI/MRA of brain due to malignant hypertension and suspected transient ischemic attack (TIA). No lesions were identified on MRI.

She was seen by a geneticist/internist, Dr. James Evans, at UNC on 1/21/2013. He reported a 47-yr old woman with a medical history significant for renal cell carcinoma, rhabdomyolysis, transient ischemic attacks (TIAs), and hypertension. She stated she had never smoked cigarettes. Her surgical history was recorded as :1990-Tubal ligation; 1991- Cholecystectomy; 2010- Partial right nephrectomy with pathology as clear cell renal cancer; 2011-Hysterectomy.

Her family history was recorded as having a mother with metastatic renal cancer diagnosed at age 67 and died at age 67 years. Two of her mother's siblings were recorded as having a history with rhabdomyolysis. Two of the patient's siblings also had a history of rhabdomyolysis. The patient has three children by two fathers. All three reported to have rhabdomyolysis. Her ancestry was recorded as African American, Caucasian, American Indian. Dr. Evans assessed the patient as having renal cancer, rhabdomyolysis, hypertension and family history of rhabdomyolysis and renal cancer. He ordered genetic testing for Von Hippel Lindau disease, a genetic syndrome with autosomal dominant inheritance, clear cell renal cancer, and hypertension due to mutations in the *VHL* gene. A specimen was sent to Mayo Medical Labs, the *VHL* gene was sequenced, and duplication/deletion analysis was performed. Testing was negative for a disease-causing mutation.

She was followed by urologist, Dr. Roc McCarthy, Atlantic Urology, Wilmington and noted to have a complex left renal cyst on MRI, June 2016. An office note from 3/21/2018 indicates that Ms. Tukes had bilateral renal cysts and a 0.2 cm solid enhancing renal mass of left kidney. She subsequently underwent partial left nephrectomy 4/26/2018, removing three tumors. All three tumors were classified as low-grade clear cell papillary renal cell carcinoma.

She was again referred to the UNC genetics service and on August 8, 2018, and was seen by Dr. Evans and Mary K. Garbarini, a genetic counselor. Dr. Evans noted that since the visit in 2013, Ms. Tukes had been diagnosed with left renal cancer undergoing a partial left nephrectomy including a lower pole mass and two superficial tumors excised 4/26/2018, now indicating **bilateral and multifocal renal cancer**.

The family history was updated at this visit and there was some question as to whether Ms. Tukes mother's cancer was specifically renal cancer as previously noted in 2013. A four-generation pedigree was taken. Maternal relatives with cancer included her mother; maternal aunt diagnosed with "stomach cancer" at age ~65 (d. 71); and daughter to this aunt (maternal cousin) with breast cancer in her 30s.

Paternal relatives included a paternal uncle with lung cancer at 74 (d. ~80) non-smoker; paternal uncle diagnosed with lung cancer ~65 years, (d. 68); paternal uncle diagnosed with throat cancer ~58 years (d. 59); son of this uncle diagnosed with lung cancer, non-smoker; two paternal aunts, one with liver cancer ~55 years and paternal aunt with stomach cancer (d. 72 years); a child of this aunt died at age 20 of kidney cancer; and a paternal grandmother with "stomach" cancer d. 69.

Updated germline genetic testing was performed through Invitae Laboratory (now LabCorp) for the Renal/Urinary Tract Cancer Panel. Thirty genes were tested: *BAP1, BUB1B, CDC73, CDKN1C, CEP57, DICER1, D153L2, EPCAM, FH, FLCN, GPC3, MET, MITF, MLH1, MSH2, MSH6, PMS2, PALB2, PTEN, SDHA, SDHB, SDHC, SDHD, SMARCA4, SMARCB1, TP53, TSC1, TSC2, VHL, WTI*.

Results: Two Variant of Uncertain Significance (VUS) calls.

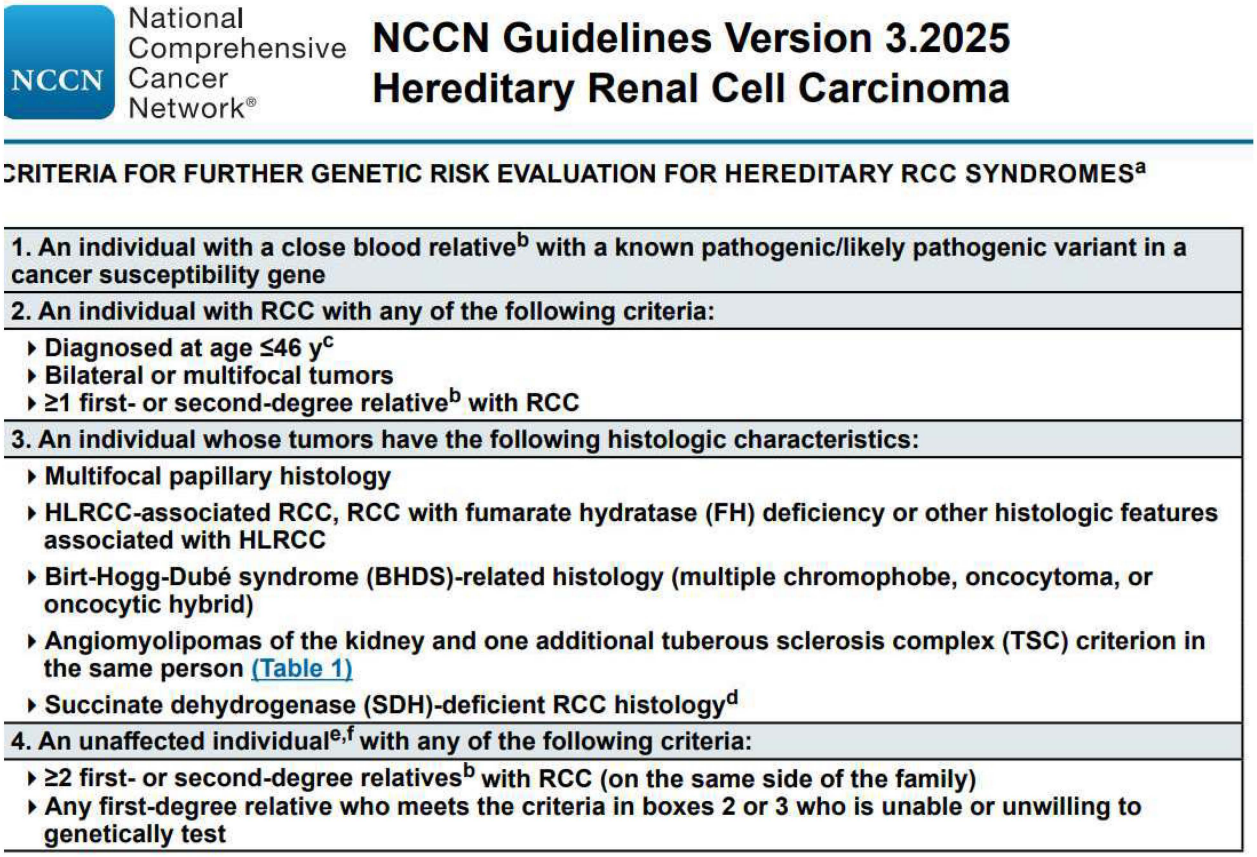
1. *SMARCA4* c.4211T>G (p.Val1404Gly) -On 4/10/2022 downgraded to likely benign (meaning additional functional data was identified to reclassify the variant from a VUS to likely benign and not associated with or a cause of renal cancer).
2. *PMS2* c.2395C>T (p.Arg 799 Trp) On 9/6/2022 downgraded to likely benign (meaning additional functional data was identified to reclassify the variant from a VUS to likely benign and not associated with or a cause of renal cancer).

Also, noted was that *RYR1* gene testing for rhabdomyolysis was performed, (Prevention Genetics) with variant of unknown significance, *RYR1* c.9242T>C (p.Met3081Thr), subsequently reclassified as likely benign.

Ms. Tukes' disease progressed with further development of cysts and tumors. On 3/14/2019 she had a **second** partial left nephrectomy. The pathology of this tumor was clear cell RCC pT1a. At this time, Ms. Tukes was diagnosed with chronic kidney disease. An MRI on 5/19/2021 demonstrated cysts of both kidneys. On 5/23/2022, age 57 years, she had a laparoscopic nephrectomy of the **right** kidney (Robotic assisted). Pathology was determined to be ccRCC pT1a. On 6/12/2023, Ms. Tukes underwent a robotic assist for a **left total nephrectomy**. Pathology indicated papillary renal cell carcinoma x 2. Hemodialysis was started following the nephrectomy, with transition to peritoneal dialysis. She underwent a kidney transplant in 2024.

C. *Methodology applied:*

As described above, the National Comprehensive Cancer Network expert panels review current literature and provide clinical practice guidelines as evidence-based recommendations for the diagnosis, treatment, screening and management of various types of cancer including hereditary cancers. Among these, NCCN has established criteria for evaluating whether a kidney cancer is a "hereditary renal cell carcinoma," which I apply here. These are the same criteria I would apply in my clinical practice.



National Comprehensive Cancer Network®

NCCN Guidelines Version 3.2025
Hereditary Renal Cell Carcinoma

CRITERIA FOR FURTHER GENETIC RISK EVALUATION FOR HEREDITARY RCC SYNDROMES[a]

1. An individual with a close blood relative[b] with a known pathogenic/likely pathogenic variant in a cancer susceptibility gene
2. An individual with RCC with any of the following criteria:
▸ Diagnosed at age ≤46 y[c] ▸ Bilateral or multifocal tumors ▸ ≥1 first- or second-degree relative[b] with RCC
3. An individual whose tumors have the following histologic characteristics:
▸ Multifocal papillary histology ▸ HLRCC-associated RCC, RCC with fumarate hydratase (FH) deficiency or other histologic features associated with HLRCC ▸ Birt-Hogg-Dubé syndrome (BHDS)-related histology (multiple chromophobe, oncocytoma, or oncocytic hybrid) ▸ Angiomyolipomas of the kidney and one additional tuberous sclerosis complex (TSC) criterion in the same person (Table 1) ▸ Succinate dehydrogenase (SDH)-deficient RCC histology[d]
4. An unaffected individual[e,f] with any of the following criteria:
▸ ≥2 first- or second-degree relatives[b] with RCC (on the same side of the family) ▸ Any first-degree relative who meets the criteria in boxes 2 or 3 who is unable or unwilling to genetically test

Figure 11: NCCN Clinical Practice Guidelines in Oncology. NCCN Guideline V.3-2025 Hereditary Renal Cell Carcinoma. https://www.nccn.org/professionals/physician_gls/pdf/kidney.pdf

The NCCN lists four different criteria when considering further genetic risk evaluation for hereditary renal cell carcinoma. These criteria, particularly #2 "An individual with RCC with any of the following criteria: including age ≤46 years; bilateral or multifocal tumors; and ≥ first or second degree relative with RCC" are often observed in individuals with a hereditary renal carcinoma syndrome and compose a phenotype that may indicate an underlying genetic predisposition to renal cancer. These criteria alert physicians to consider a genetic cause of disease. One factor doesn't outweigh another, but taken together may provide stronger evidence for hereditary-based cancer. Even in the circumstance when a germline genetic mutation is not found, a strong clinical phenotype and family history of disease in an individual with a suspected hereditary cancer

syndrome does not exclude a heritable cause for cancer and dictates that the individual should be managed as if having a hereditary cancer. Individuals with suspected hereditary cancer are at a higher risk to develop cancer and additional tumors and therefore are screened more closely than individuals without a heritable phenotype. **Thus, a negative gene test result in an individual with a suspected hereditary cancer syndrome does not exclude a heritable cause for their cancer.**

D. Genetic assessment:

Ms. Tukes meets criteria for Hereditary Renal Cell Carcinoma (Figure 11)[14]. She was **< 46 years** (45 years) at first diagnosis; had **both bilateral and multifocal cysts and renal tumors**; and a **family history of renal cancer** including a first degree (mother) and third degree (paternal cousin) relative with renal cancer. Unfortunately, genetic testing, performed during her cancer care, was unable to identify a gene mutation as a potential etiological explanation for her disease.

Her first genetic assessment for a possible underlying predisposition included germline testing for the *VHL* gene in 2013. Testing for the Von Hippel Lindau syndrome was reasonable given her history of hypertension, clear cell renal cancer, and a family history of RCC. The testing (Mayo Medical Laboratories) was negative. Further, along with the negative genetic test, she was never found to have had other features of Von Hippel Lindau syndrome such as hemangioblastomas of the head or spine or pheochromocytoma making the diagnosis of Von Hippel Lindau syndrome unlikely.

Her next genetic assessment occurred in 2018. By this time, she was noted to have bilateral kidney cancer. A panel of 30 genes was tested (sequencing and exonic dup/del) via Invitae Laboratories (report 8/22/2013). The genes tested included: *BAP1, BUB1B, CDC73, CDKN1C, CEP57, DICER1, DIS3L2, EPCAM (del/dup only), FH, FLCN, GPC3, MET, MITF (c.952G>A, p. Glu318Lys variant only), MLH1, MSH2, MSH6, PMS2, PALB2, PTEN, SDHA (sequence only), SDHB, SDHC, SDHD, SMARCA4, SMARCB1, TP53, TSC1, TSC2, VHL* and *WT1*.

Results: Two VUS.
SMARCA4 c.4211T>G (p.Val1404Gly) -On 4/10/2022 reclassified to likely benign
PMS2 c.2395C>T (p.Arg 799 Trp) On 9/6/2022 reclassified to likely benign.

These genes are inclusive for all the known hereditary renal cell disorders as outlined in the NCCN 2025 guidelines (Figures 11-12) [14,15].

HEREDITARY RCC SYNDROMES OVERVIEW

Syndrome/Gene	Common Histologies	Inheritance Pattern Major Clinical Manifestations	Other Specialists Involved in Screening
von Hippel-Lindau (VHL)/ *VHL* gene	Clear cell	• Autosomal dominant • Table 2	• Neurosurgery • Ophthalmology • Audiology • Endocrinology • Endocrine surgery
Hereditary papillary renal carcinoma (HPRC)/*MET* gene	Papillary	• Autosomal dominant • Multifocal, bilateral renal cell tumors	• Nephrology
Birt-Hogg-Dubé syndrome (BHDS)/*FLCN* gene[1,2]	Chromophobe, hybrid oncocytic tumors, clear cell, oncocytomas, angiomyolipomas, papillary RCC	• Autosomal dominant • Cutaneous fibrofolliculoma or trichodiscoma, pulmonary cysts, and spontaneous pneumothorax	• Pulmonology • Dermatology
Tuberous sclerosis complex (TSC)/*TSC1, TSC2* genes	Angiomyolipoma (and other PEComas), renal cysts, eosinophilic solid and cystic RCC, RCC with fibromyomatous stroma, eosinophilic vacuolated tumor, low-grade oncocytic tumor, clear cell	• Autosomal dominant • Table 1	• Neurology • Dermatology
Hereditary leiomyomatosis and renal cell cancer (HLRCC)/*FH* gene	HLRCC-associated RCC or FH-deficient RCC	• Autosomal dominant • Leiomyomas of skin and uterus, unilateral, solitary, and aggressive renal cell tumors. PET-positive adrenal adenomas	• Gynecology • Dermatology
BAP1 tumor predisposition syndrome (TPDS)/*BAP1* gene[3,4]	Clear cell	• Autosomal dominant • Melanoma (uveal and cutaneous), kidney cancer, mesothelioma	• Dermatology • Ophthalmology • Thoracic oncology
Hereditary paraganglioma/ pheochromocytoma (PGL/PCC) syndrome/*SDHA/ B/C/D* genes	SDH-deficient RCC	• Autosomal dominant • Head and neck PGL and adrenal or extra-adrenal PCCs, gastrointestinal stromal tumors (GIST)	• Endocrine • Endocrine surgery

Figure 12: NCCN Clinical Practice Guidelines in Oncology. NCCN Guideline V.3-2025 Hereditary Renal Cell Carcinoma. https://www.nccn.org/professionals/physician_gls/pdf/kidney.pdf

E. Current state of clinical germline genetic testing for kidney cancer

Invitae laboratories (now LabCorp) is a well-known and respected national clinical testing laboratory. It has updated the Renal/Urinary Cancer Panel since 2018. The changes include removal of *MITF, PALB2, SDHA, SDHD* and addition of *BLM, REST, TRIM28, TRIP13*. It is unlikely that the new panel would identify pathogenic variants in any of the genes tested for Ms. Tukes. Other options for updated testing could include looking at RNA as well as DNA which allows better assessment of potential intronic variants. The yield, though, is typically low.

Natera laboratories offers a 385 gene panel, Renasight™, for identification of genes associated with renal cancer and polycystic kidney disease. Finally, germline exome or genome testing could be performed for assessment of hereditary renal cancer as well as somatic testing of her tumors (tumor profiling) to expand the search for an underlying genetic predisposition to renal cancer. Genetic testing is continually expanding. As evidenced in Ms. Tukes case, she initially underwent testing for a single gene, the *VHL* gene in 2013. However, in 2018, five years later, a panel of genes for renal cancer was available and utilized to try to identify the underlying cause of Ms. Tukes cancer.

Despite the absence of a gene mutation in genes tested, Ms. Tukes has features consistent with hereditary renal cancer. She had significant progressive kidney cancer and complex cysts ultimately resulting in bilateral nephrectomies. From her early diagnosis, Ms. Tukes was followed closely to assess for both additional cancers in the kidneys as well as imaging for potential metastatic disease. Her disease continued to progress with extensive, bilateral, multifocal, and cystic renal disease. This clinical course is extremely unusual. Her young age, bilateral and

multifocal progressive disease support a diagnosis of a hereditary basis for her disease. Her maternal family history (first degree) is ambiguous. But even if Ms. Tukes' mother did not have RCC as initially indicated, her phenotype is still consistent with a hereditary renal cell carcinoma. Sporadic renal cell carcinoma is generally diagnosed during the **fifth to seventh decades of life**. In a review of more than 600 cases of hereditary renal cell carcinoma (RCC) from the National Cancer Institute, the median age of RCC diagnosis was 37 years, with 70% of cases being diagnosed at age 46 years or younger [16]. In contrast, the median age of renal cell carcinoma in the general population is approximately 65 years [17,18]. Further, renal cell carcinoma is more common in males than females (a ratio of approximately 3:2) [18].

Heritable RCCs are often multifocal and bilateral. In contrast to a single kidney with a single tumor, or a single kidney with multifocal tumors or bilateral renal cell cancer with a single focus in each kidney, Ms. Tukes had bilateral and multifocal disease, i.e. multiple tumors in both kidneys. A retrospective analysis of 1,235 patients with RCC who underwent genetic testing revealed that 6.1% of this population had positive genetic test results, 75.5% had negative test results, and 18.4% had a variant of unknown significance. Young age at RCC diagnosis was the only variable associated with a positive test result [19].

It is estimated that there are ~22,000 genes in the human body. Genetic knowledge is advancing rapidly, and it is certain that new genes associated with renal disease will be identified in the future[20]. Negative testing from a 30-gene panel does not outweigh these other diagnostic criteria.

As stated earlier, a negative test result (includes benign or likely benign variant) in a person with cancer indicates the person does not have an identifiable pathogenic variant in any of the genes analyzed. A negative gene test result in an individual with a **suspected hereditary cancer syndrome** does not exclude a heritable cause for the cancer. Advances in genetic testing technology and genetic knowledge may later identify gene mutations not currently recognized. Generally, it is suggested that updated testing be performed in 5-10 years from the date of the negative test in a person suspected to have hereditary cancer as occurred with Ms. Tukes.

III. Review of the Expert Report for Jacqueline Y. Tukes prepared by Dr. Irving C. Allen

I have reviewed the expert report of Irving C. Allen, PhD, as provided to me by attorneys of the U.S. Department of Justice. Dr. Allen is a professor in the Department of Biomedical Sciences and Pathobiology at Virginia Polytechnic Institute and State University. His research focuses on the role of the immune system in host-pathogen interactions, inflammatory diseases, and cancer. His group studies the interplay of the tumor microenvironment, innate immune system signaling, systemic anti-tumor responses, and therapeutic assessments in cancer. Dr. Allen's report includes references to the accepted understanding of the complexity of cancer as complex, multi-step and multifactorial, and that there are both extrinsic (environmental) and intrinsic (individual) factors that are responsible for the development of cancer.

Dr. Allen spends a significant amount of time focused on the genes and methodology in Ms. Tukes genetic testing performed by Invitae Laboratory and does not dispute the results of the testing. He notes that not all genes associated with renal cell carcinoma (Table 3 of his report) were included in the Invitae test panel and this is correct. Yet, because of the negative results on the Invitae panel, he states "it is more likely than not that the patient's RCC is **not** directly associated with an inherited or congenital genetic mutation". I disagree with this assessment. As noted above, Ms. Tukes has a significant clinical phenotype and family history that is consistent with an increased likelihood of an underlying genetic predisposition for renal cell carcinoma and as such, based on national criteria (NCCN), should be considered to have a heritable renal cancer. Negative testing on a 30-gene panel does not rule out a hereditary renal cancer syndrome. A negative result in a person with a suspected heritable cancer is an indeterminate result meaning no additional information was gained. It is not known if the person was truly negative; or the current technology couldn't find the mutation; or another gene is involved but medical science has not yet identified the association with disease.

Dr. Allen discusses the two VUS calls in the first genetic test report from 2018. Both were subsequently reclassified by Invitae laboratory (LabCorp) as **likely benign**.

> *SMARCA4* c.4211T>G (p.Val1404Gly) -On 4/10/2022 reclassified to **likely benign.**
> *PMS2* c.2395C>T (p.Arg 799 Trp) On 9/6/2022 reclassified to **likely benign**.

Dr. Allen also comments that, based on "dose pathogenicity" of *PMS2* from animal studies reported in the literature, this VUS is "as likely as not" to result in "insufficient DNA mismatch repair in the patient". Further, "as observed in animal studies, this would be expected to result in increased cancer presentation following carcinogen exposure and greater sensitivity to carcinogens, specifically those that damage DNA".

This is a leap. First, testing for inherited *PMS2* variants is hampered by a pseudogene, *PMS2CL*, which has nearly identical homology to *PMS2* in exons 12–15 of the gene. Thus, long-range sequencing is typically employed and not all articles quoting variants in exon 12-15 refer to the disambiguation technique.

When looking for the current classification of a variant identified in a genetic report, as a geneticist, I turn to the literature and data in ClinVar. ClinVar is a public database maintained by the National Center for Biotechnology Information (NCBI) that archives and shares information about genetic variations and their relationship to human health. ClinVar facilitates access to communication about the relationships asserted between human variation and observed conditions, and the history of those assertions. The database is freely accessible and frequently updated with new findings.

According to ClinVar, 11 laboratories have reported on this specific *PMS2* missense variant with six laboratories classifying the variant as "likely benign"; one as "benign", and four as "uncertain significance". There is one lab with a "flagged" submission from 2015 that does not contribute to the aggregate classification and appears to be incorrect or out of date.

Further, *PMS2* c.2395C>T (p.Arg 799 Trp) is a missense variant and is not a variant with obvious loss of function or a deletion variant. Because this is a "missense" variant—meaning a substitution of one amino acid for another—there should not be "dosage pathogenicity". There could be effects on a protein, but to date, there is no functional data to support pathogenicity, dosage or otherwise.

Similarly, for *SMARCA4*, Dr. Allen states "if the mutation carried by the patient has a deleterious effect on the protein, then it is as likely as not that this mutation results in increased cancer development following exposure to carcinogens". Again, accessing ClinVar, five laboratories have contributed to the database with two laboratories citing "uncertain significance" and three listing the variant as "likely benign". Ambry Genetics, another national genetic testing laboratory, states that the alteration is classified **as likely benign based on the combination of 1) observance in unaffected individuals; 2) population frequency; 3) intact protein function; and 4) lack of disease association in case-control studies, and/or the mechanism of disease or impacted region is inconsistent with a known cause of pathogenicity.** Again, this is a missense mutation with a substitution of the amino acid, glycine for valine. There is no evidence of "dosage pathogenicity" i.e. quantitative loss or deletion of the protein. Further, variants in the *SMARCA4* gene are mostly associated with rhabdoid tumor predisposition syndrome (RTPS), characterized by the development of rhabdoid tumors in infants and children younger than three years[21].

For both variants, Dr. Allen cites only animal studies and not human evidence. It would be important to understand if the exact same missense variants were applied to the animal studies reviewed by Dr. Allen as well. Yet, understanding that these variants are classified as likely benign, any susceptibility would most likely **not** be considered an inherited predisposition. Again, LabCorp (formerly Invitae) as well as other national testing laboratories have classified both variants as likely benign, missense variants based on the current evidence.

The majority of cancer is due to a combination of intrinsic and extrinsic factors and does not have a hereditable basis. However, in genetics we classify a syndrome as a group of symptoms that consistently occur together and compose a phenotype. For example, individuals with Down syndrome also known as trisomy 21 (three copies of chromosome 21). These individuals look more like one another than their siblings. Similarly in cancer, there are recognized syndromes in which the phenotype is similar amongst the individuals with the disorder. In hereditary renal cell

carcinoma, genes have been associated with several renal cancer syndromes, yet common amongst them is typically an earlier age of onset as well as bilateral, multifocal disease and often a family history of cancer. Such that when this phenotype is observed in the clinic, there is consideration of a hereditary contribution to the disease even without a recognized gene mutation. As stated earlier in this report, genetic knowledge is advancing and the association of genes with specific disorders continues to expand. No doubt, additional genes associated with a predisposition to renal cell carcinoma will be identified. Until that time, individuals with a clinical phenotype of hereditary cancer will be followed closely and managed according to their disease.

IV. References:

1. National Human Genome Research Institute. https://www.genome.gov/genetics-glossary/Protein accessed February 3, 2025.
2. American Cancer Society Guideline for Colorectal Cancer Screening https://www.cancer.org/cancer/types/colon-rectal-cancer/detection-diagnosis-staging/acs-recommendations.html accessed February 5 2025.
3. Yen T, Stanich PP, Axell L, Patel SG. APC-associated polyposis conditions. GeneReviews. Adam MP, Feldman j, Mirzaa GM et al. editors. Seattle WA 1993-2005 https://www.ncbi.nlm.nih.gov/books/NBK1345/ accessed February 5, 2025.
4. NCCN Clinical Practice Guidelines in Oncology. Genetic/Familial High-Risk Assessment: Breast, Ovarian, Pancreatic, and Prostate Version 2.2025-November 7, 2024 https://www.nccn.org/professionals/physician_gls/pdf/genetics_bopp.pdf
5. NCCN Clinical Practice Guidelines in Oncology. Genetic/Familial High-Risk Assessment: Colorectal, Endometrial, and Gastric Version 3.2024-October 31, 2024. https://www.nccn.org/professionals/physician_gls/pdf/genetics_ceg.pdf
6. Giusti F, Marini F, Brandi ML. Multiple Endocrine Neoplasia Type 1. GeneReviews https://www.ncbi.nlm.nih.gov/books/NBK1538/
7. Eng C, Pitt G. Multiple Endocrine Neoplasia Type 2. GeneReviews https://www.ncbi.nlm.nih.gov/books/NBK1257/
8. Van Leeuwaarde RS, Ahmad S, van Nesselrooij B et al. Von Hippel-Lindau Syndrome. GeneReviews https://www.ncbi.nlm.nih.gov/books/NBK1463/
9. Yanus GA, Kuligina ES, Imyanitov EN. Hereditary Renal Cancer Syndromes Med Sci. 2024, 12,12. https://doi.org/10.3390/medsci12010012
10. Knudson AF. Mutation and Cancer: Statistical Study of Retinoblastoma. PNAS 1971; 68(4): 820-823.
11. Knudson AF, Meadows AT, Nichols WW, Hill R. Chromosomal deletion and retinoblastoma. N Engl J Med 1976; 295:1120-1123
12. Hemminki K, Sundquist K, Sundquist J et al. Familial Risks and Proportions Describing population Landscape of Familial Cancer. Cancers (Basel) 2021; 13(17): 4385. doi: 10.3390/cancers13174385
13. Richards S, Aziz N, Bale S et al. Standards and guidelines for the interpretation of sequence variants: a join consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015. 17(5):405-24 PMID: 25741868
14. NCCN Clinical Practice Guidelines in Oncology. NCCN Guideline V.3-2025 Hereditary Renal Cell Carcinoma. https://www.nccn.org/professionals/physician_gls/pdf/kidney.pdf
15. Hass NB, Nathanson KL. Hereditary renal cancer syndromes. Adv. Chronic Kidney Dis. 2014; 21(1): 81-90.
16. Such B, Vourganti F, Ricketts CJ et al. Defining early-onset kidney cancer: implications for germline and somatic mutation testing and clinical management J. Clin Oncol 2014; 32(5): 431-437.
17. National Cancer Institute: SEER stat Fact Sheets: Kidney and Renal Pelvis cancer. Bethesda, MD. NCI. https://seer.cancer.gov/statfacts/html/kidrp.html

18. Rose Tl and Kim WY. Renal Cell Carcinoma- A review. JAMA 2024; 332(12):1001-1010.
19. Nguyen KA, Syed JS, Espenschied CR, et al. Advances in the diagnosis of hereditary kidney cancer: initial results of a multigene pane test. Cancer 2017 123(22): 4363-4371.
20. Pertea M, Salzber SL. Between a chicken and a grape: estimating the number of human genes. Genome Biology 2010; 11:206
21. Nemes K, Bens S, Bourdeaut F, et al. Rhabdoid Tumor Predisposition Syndrome. 2017. Updated 2022. In Adam MP, Feldman J, Mirzaa GM, et al editors, Gene Reviews. University of Washington, Seattle. 1993-2025.