Exhibit 379

Dr. Irving C. Allen, M.S., M.B.A., PH.D.

Response to "Expert Report on the Case of Jacqueline Y. Tukes for the Department of Justice" prepared by Gail H. Vance, M.D., dated April 8, 2025

May 13, 2025

GENERAL RESPONSE

I have reviewed the expert report of Dr. Gail H. Vance, MD, dated April 8, 2025 provided to me by the attorneys for the plaintiff Jacqueline Y. Tukes. Based on the information in the introduction section of the report, Dr. Vance is a medical geneticist at Indiana University Health Physicians' Group and Eskenazi Health. The first approximately half of Dr. Vance's report provides an overview of cancer genetics. Overall, I generally agree with the information presented in her background materials on pages 3-14 of the report. She then presents the background information and history of pertinent illness, which I also generally agree with on pages 15-16 of the report. Genetic testing was initially conducted to assess the *VHL* gene in 2013. The next genetic assessment was conducted in 2018 by Invitae Laboratories (LabCorp) and utilized the Renal/Urinary Tract Cancers Panel of 30 genes. I agree with Dr. Vance's statement on page 18 of her report, referring to the panel of genes evaluated by Invitae Laboratories, that states "**These genes are inclusive for all the known hereditary renal cell disorders outlined in the NCCN 2025 guidelines (Figures 11-12)** [14,15]." We both appear to agree that the results of these genetic tests are "negative" for the genes evaluated and that two "Variants of Unknown Significance" (VUS) were identified in the *PMS2* and *SMARCA4* genes. Areas where we disagree in our opinions can be generally grouped into the four categories discussed in more detail below.

1) GENE-CANCER RELATIONSHIPS

Dr. Vance does not fully account for the role of environmental exposures to carcinogens and their role in causing cancer in her opinions. In her report, Dr. Vance provides an accurate gene-cancer relationship overview. As she notes there are 3 categories: Sporadic Cancer; Hereditary Cancer; and Familial Cancer. As pointed out in her report, most cancers (~70-80%) are sporadic in nature with no clear familial or inherited association. While there is no clear genetic cause, environmental exposure to carcinogens is well known to significantly increase the risk of developing sporadic cancer. This can occur through a variety of biological mechanisms as outlined in my report, and has been detailed in works defining the key characteristics of carcinogens[1]. The key characteristics of carcinogens that can cause the transformation of cells includes agents that are electrophilic or can be metabolically active to

electrophiles, are genotoxic, can alter DNA repair or cause genomic instability, induce epigenetic alterations, induce oxidative stress, alter inflammation, induce immunosuppression, modulate receptor mediated effects, cause immortality, and/or alter cell proliferation, cell death, or nutrient supply[1].

Dr. Vance then notes that approximately 15-20% of cancer is familial cancer, where there is an increased number of individuals in a family with cancer, without a clearly identified genetic or heritable pattern. She then further notes on page 11 that familial cancer is "potentially due to a combination of genetic and environmental factors." It is well accepted in the field that "environmental factors" include exposure to carcinogens[2].

The last category is Hereditary Cancer, which is noted to accounts for approximately 5-10% of cancers and is associated with an identified, genetic or heritable pattern in Dr. Vance's report. She discusses features that suggest an inherited predisposition to cancer that include the following: (1) two or more close relatives affected; (2) early age of onset; (3) cancers of a specific type occurring together; (4) multiple or bilateral cancers; and (5) rare cancers. She then discusses Knudson's two hit theory of cancer on page 11. This theory and the Figure shown on Page 11 basically states that a patient could have a mutation that pre-disposes to cancer (hit one), and is then exposed to another "hit" later in life that essentially triggers the biological processes that result in cancer. The "second-hit" in the two-hit model is identified in Figure 9 in Dr. Vance's report as the "rare event" for both non-hereditary and hereditary cancers. It is well accepted in the field that exposure to carcinogens are examples of the "rare event" in Knudson's two-hit theory.

In the field of cancer biology, as outlined here, regardless of the source of the genetic mutation, genetic mutations alone are considered "enabling characteristics"[3-5]. Patients can often have gene mutations, but will not necessarily develop cancer because of the mutation alone. Additional biological changes are necessary for the cell to acquire the "hallmarks of cancer" required for the development of disease[3-5], which is often associated with external or extrinsic events that can include exposure to carcinogens. In the examples provided by Dr. Vance, exposure to carcinogens can be the "environmental factor", "second hit", or "rare event" necessary to ultimately cause cancer.

2) GENETIC TESTING RESULTS

The panel of genes tested are inclusive for all the known hereditary renal cell disorders, which includes renal cell carcinoma. Dr. Vance states that "Negative testing on a 30 gene panel does not rule out a hereditary renal cancer syndrome." (Page 18, Section D). However, the conclusion of the report provided by UNC Chapel Hill (for example on page 10 of 286) in the letter

dated 11/26/2018 from Mary K. Garbarini (Genetic Counselor) states the following: “Testing did not reveal a known pathogenic mutation in any of these genes. Since the current test is not perfect, it is possible there may be a mutation that current testing cannot detect, but that chance is small.”. It further states the following: “This normal result is reassuring and indicates that you do not likely have well understood hereditary predisposition to renal cancer.” There are approximately 22,000 genes in the human genome. Of these, the panel of 30 genes utilized by Invitae are inclusive of all the genes currently identified and validated as causative for a hereditary predisposition to renal cancer. This was noted by Dr. Vance, who stated the following in her report: “These genes are inclusive for all the known hereditary renal cell disorders outlined in the NCCN 2025 guidelines (Figures 11-12)[14,15].” **Thus, based on where science stands today, the contribution of mutations in any other genes in Ms. Tukes Renal Cell Carcinoma are speculative and unconfirmed**.

It was additionally noted in Dr. Vance’s report that other genetic panels are available that are much larger in size. Specifically, Dr. Vance discusses the Renasight Panel, which includes an assessment of 397 genes based on the most current information for the panel by the manufacturer (Natera). However, in reviewing this panel, it is not specific for renal cancer and polycystic kidney disease. It is currently marketed as a comprehensive kidney gene panel. Indeed, based on the Natera website, most of the genes in this panel have no relationship to cancer. Conversely, some genes well known to be directly associated with renal cell carcinoma, for example MET (discussed on page 22 of my report), do not appear to be included on this panel. Likewise, PMS2 and SMARCA4, also do not appear to be on this panel. Thus, while this panel is much larger, it is less relevant to Ms. Tukes disease. In sum, while there may be other genetic panels that are larger, we are left with the data from the panel that we have, which is inclusive for the genes currently known to science as the most relevant to Ms. Tukes disease.

In summary, the genetic testing did not reveal any pathogenic variants (genetic mutations) currently known to be associated with a genetic risk of renal cell carcinoma. This includes both inherited mutations and congenital mutations that are associated with the disease. Thus, it is more likely than not that the patient’s RCC is not directly associated with an inherited or congenital genetic mutation.

3) CLINICAL PHENOTYPE

In the absence of a positive genetic test result, Dr. Vance relies on the NCCN Guidelines for Hereditary Renal Cell Carcinoma for her opinion. This opinion is based on (1) the clinical phenotype (disease presentation) and (2) family history, rather than genetic findings. In the clinical

findings, Dr. Vance seems to focus on two findings related to the multiple, bi-lateral presentation of the disease (multiple tumors in both kidneys) and the relatively young age of disease on-set. We both agree that these presentations would typically support a heritable genetic causation for the patient's disease, and in my opinion, this presentation was almost certainly a significant consideration for ordering Ms. Tukes genetic testing. However, to my knowledge the NCCN Guidelines referred to in Dr. Vance's report do not account for carcinogen exposure. Indeed, a review of the NCCN Guidelines Version 3.2025 for Kidney Cancer does not have any mention of carcinogen exposure. Bilateral renal cell carcinoma can occur without a genetic association and it can occur in people with no known history of kidney cancer. This is especially true in the context of carcinogen exposure, where carcinogens can accumulate in the kidney cortex of both kidneys eventually leading to renal cancer in both organs (bilateral disease). Carcinogen exposure can also be associated with early age of onset. It was also noted by both Dr. Vance and I that there was some question as to whether Ms. Tukes mother's cancer was specifically renal cancer (noted in Dr. Vance's report on page 16; noted in the UNC Chapel Hill report on page 48 of 286). From the UNC Chapel Hill report: "Mother (Christine, d. 66) - diagnosed with an unknown cancer which was metastatic at diagnosis. Ms. Tukes remembers that her mother had a renal mass, but it is unclear if it truly was a renal primary cancer." Thus, (1) the negative genetic testing results on the panel of genes currently known to science and validated as causative for hereditary renal cell carcinoma; (2) the bi-lateral disease and early age of on-set presentation being consistent with carcinogen exposure; and (3) the ambiguous maternal family history, would all exclude a heritable cause for Ms. Tuke's kidney cancer.

3) PMS2 VARIANT

Dr. Vance and I both agree that the PMS2 mutation is a VUS and is classified as "likely benign". The VUS designation refers to there being insufficient data to determine if a mutation directly causes a disease. The further classification of the mutation as being "likely benign" generally refers to findings from human population studies and is in reference to gene mutations that directly cause disease. These designations do not account for exposure to carcinogens or environmental exposures. Rather, they are limited to the assessment of sequencing genetic mutations in affected patient populations. Specifically, the "likely benign" classification does not account for the role of PMS2 in contributing to cancer development following carcinogen exposure. Ms. Tukes is heterozygous for the mutation in the *PMS2* gene. Dr. Vance and I both rely, in part, on the ClinVar Database. The ClinVar Database, accessed 04/13/2025 clearly states that there is "sufficient evidence for dosage pathogenicity" in the haploinsufficiency (HI) score.

Haploinsufficiency describes the condition where a single functional copy of a gene is not sufficient to ensure normal cellular function. The most common form of haploinsufficiency arises from a loss-of-function mutation in one copy of a gene. Dr. Vance's report notes that the variant in the *PMS2* gene is a missense variant and is not a variant with obvious loss of function or a deletion variant. However, in the Invitae report, it is noted that the missense change identified in Ms. Tukes genetic testing is likely to result in the deletion/inactivation of the protein. As stated on page 26 of the Invitae report: "An algorithm developed specifically for the PMS2 gene suggests that this missense change is likely to be deleterious"[6].

Animal studies are the foundation of biomedical research and are relied upon in the field of genetics and molecular biology to provide functional data for genetic mutations. In cancer research, this has been true for well over a century. This is also the case for toxicology and carcinogen analysis, where human studies are typically limited to epidemiology studies, with the animal studies used to define functional effects, dosage and exposure limits. In the case of PMS2, the animal studies conducted with deletions of the *Pms2* gene noted in my report show that animals that are heterozygous for the gene (with 1 functional copy and 1 deleted copy) do not have an increase in spontaneous tumors. However, when exposed to a carcinogen, the heterozygous animals develop cancer at higher levels compared to the control animals with normally functioning PMS2. This is clear evidence of dosage pathogenicity.

As noted in my report, based on the function of PMS2 as a tumor suppressor, the predicted loss of function mutation observed in the patient, the "sufficient evidence for dosage pathogenicity" haploinsufficiency score in the ClinVar database, and the data related to carcinogen exposure in heterozygous animal models, it is as likely as not that this mutation results in insufficient DNA mismatch repair in the patient. As observed in the 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.

4) SMARCA4 VARIANT

Dr. Vance and I both agree that the *SMARCA4* mutation is a VUS and is classified as "likely benign". The VUS designation has all the caveats noted above for *PMS2*. Ms. Tukes is heterozygous for the mutation in the *SMARCA4* gene. The defense report suggestion that there is no evidence for dosage pathogenicity is not supported by the information listed in the ClinVar record for the variation in SMARCA4 present in the patient. The curation status for this mutation is complete and finalized. The ClinVar Database, accessed 04/13/2025 states that there is "sufficient evidence for dosage pathogenicity". Dr. Vance's report notes that this variant is a

missense variant and is not a variant with obvious loss of function or a deletion variant. In the Clinvar database, it is true that the functional consequence of this mutation is currently listed as undefined. The defense report seems to infer that because this is a missense variation, then there is likely no effect on the functionality of the protein. However, the algorithms employed by Invitae appear to have produced conflicting results regarding the functional effect of the missense mutation on the protein that is encoded by the *SMARCA4* gene, suggesting that this mutation could be either deleterious or benign. As stated on page 26 of the Invitae report, "Algorithms developed to predict the effect of missense changes on protein's structure and function do not agree on the potential impact of this missense change (SIFT: Deleterious; PolyPhen2: Benign; Align-GVGD: Class C0)."

Similar to PMS2, animal studies and cell studies provide the functional support for the role of SMARCA4 in cancer. Here, studies reveal that heterozygous loss of *Smarca4* results in more tumors following carcinogen exposure compared to the control animals where both pairs of the gene are functional. Likewise, there was no effect following complete loss of *Smarca4*. These data show that the increased cancer risk following carcinogen exposure is associated with heterozygosity. The mechanism of action is associated with the dysregulation of cell death. It was also noted that inactivation of the protein encoded by *Smarca4* could not, by itself, initiate tumor development in untransformed cells; thus, requiring carcinogen exposure to initiate the cancer development.

As noted in my report, based on the biology of SMARCA4 and the experimental data from the functional studies, <u>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.</u>

CONCLUDING REMARKS

In my opinion, with a reasonable amount of scientific certainty, evidence exists to satisfy the burden of proof that Ms. Tukes renal cell carcinoma is more likely than not caused by factors other than an inherited genetic mutation. Furthermore, in my opinion the mutations identified in the genetic testing, while not directly causative for renal cell carcinoma, are at least as likely as not to increase the susceptibility of Ms. Tukes to cancer development following exposure to carcinogens.

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DISCLOSURES

All the opinions expressed in this report are my own, are based on my training and expertise in molecular biology and cancer biology and were provided in my capacity as President of Allen Consulting and Management, LLC. While I am also employed by Virginia Polytechnic Institute and State University (Virginia Tech), this work was conducted independently from my position at this University. The content of this report is the responsibility of the author, and does not necessarily represent the official views of Virginia Tech or any funding agencies that support my academic research program. My opinions are based on my evaluation of the medical records of Jacqueline Tukes, the peer-reviewed scientific literature, and the other sources noted in reference section of this report. I reserve the right to supplement these opinions if new information, data, and/or evidence becomes available and to address the opinions and/or testimony of other parties that reference the opinions expressed in this report. I have provided expert testimony in other court proceedings related to environmental exposures to chemicals and cancer. I am being compensated for my time generating this report at a rate of $400 per hour.

Dr. Irving C. Allen
President and Managing Partner
Allen Consulting and Management, LLC