WARNING: G-401 and SK-NEP-1 cell lines are not Wilms tumor cell lines.

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word count: 1030

number of Tables, Figures, Supporting Information files: /

running title: G-401 and SK-NEP-1 are not Wilms tumor cell lines.

keywords: G-401, SK-NEP-1, Wilms tumor, Rhabdoid tumor of the kidney, Ewing sarcoma

abbreviation key:

ICLAC	International Cell Line Authentication Committee
NIH	National Institutes of Health
ATCC	American Type Culture Collection

Scientific knowledge and progress, as well as human knowledge, relies on history and starts from history. We study literature on the subject of interest, and we start to imagine and plan our work from available, published data. Thus, a necessary prerequisite for our studies to add new knowledge is to have a solid, trusted base of prior knowledge to build on. Cell lines are easily accessible resources, and important models for life science research. Cancer research relies on cell lines as effective models for the cell type and for the pathology being studied. However, the use of cell lines requires a lot of caution. First of all, correct assignment of diagnosis to the cell line used as model is necessary. If the patient from whom the line was initially derived was given a diagnosis different from that which would be made nowadays, cell line origin should be corrected accordingly. Furthermore, an emerging problem is that of cell line misidentification, which refers to a cell line no longer corresponding to the donor or species from which it was originally established. This is the object of cell line authentication, promoted by the International Cell Line Authentication Committee (ICLAC, https://iclac.org/).[1] It is necessary to picture the consequences that studies performed on "wrong" cell lines can have in terms of adding misinformation to the literature, potentially generating further studies that lead to other misleading data, not to mention the waste of economical and intellectual resources. An accurate estimate has been performed and its findings are disturbing.[2] Nevertheless, not all published data obtained by the use of "wrong" lines should be considered useless. For example, if the mistake lies in a different diagnosis, the correct attribution of the findings to the right pathology can retain the value of the previous efforts made by researchers. In pediatric oncology, which represents only 0.5% of all cancers, the rarity of patients, together with the small number of researchers involved in the field worldwide, and of the corresponding research grants, make any published paper important. This is what prompted us to write this warning.

With this communication, we intend to clarify once and for all that G-401 and SK-NEP-1 are not Wilms tumor cell lines and thus, studies performed with them cannot be claimed as Wilms tumors studies. The need for this statement comes from the fact that even now, 26 & 13 years after their identification as being derived from non-Wilms tumours, respectively, some papers using these cell

lines as Wilms tumor models are still being published and reported in PubMed. This means that some Researchers, Reviewers, and Editors, are not aware of the fact that, although both cell lines were initially classified as Wilms tumor cell lines, evidence demonstrating they are not has been subsequently presented.[3,4]

We will briefly recall the facts that led to classify G-401 as a rhabdoid tumor of the kidney,[3] and SK-NEP-1 as a Ewing sarcoma cell line.[4]

The G-401 cell line was established in 1975 by a NIH fellow, Dr. P.T. Peebles, from a kidney tumor, classified at that time as Wilms tumor, surgically resected from a three-month-old male who eventually died.[3] It has to be remembered that 1975 was before the completion of the first National Wilms tumor study,[5] and that other renal neoplasias, such as the entities clear cell sarcoma of kidney and malignant rhabdoid tumor of the kidney, were classified as Wilms tumor variants. The establishment of the G-401 cell line is reported in abstract form only,[6] and when Dr. A.J. Garvin and colleagues tried to locate the original tumor tissue blocks and hospital of origin, they failed.[3] Then, they used the G-401 cell line obtained from the American Type Culture Collection (ATCC) to inject nude mice and obtain tumor tissue to investigate. The evidence they collected are those obtainable in the nineties, thus they are based on histology, ultrastructure, immunohistochemical and RNA analyses which compared the heterotransplants derived from the G-401 cell line with those derived from Wilms tumor and rhabdoid tumor of the kidney, and led them to conclude that the G-401 cell line derives from a rhabdoid tumor of the kidney.[3] Very importantly, examination of the G-401-obtained tumor tissue by local pathologists and by Dr. Bruce Beckwith, director of the National Wilms tumor Study, "yielded complete agreement that the tumor did not resemble a Wilms, but resembled a rhabdoid tumor of the kidney".[3] Also the age of presentation, aggressive clinical behavior and fatal outcome (if death was due to disease) of the tumor from which these cells are derived, are consistent with it being a rhabdoid tumor of the kidney.[3] Furthermore, this cell line has subsequently been shown

to have a homozygous *SMARCB1* deletion, the molecular characteristic of rhabdoid tumours at all body sites.[7]

The SK-NEP-1 cell line was established in 1971 by Dr. J. Fogh and Dr. G. Trempe from a malignant pleural effusion in a 25-year-old female patient diagnosed with a tumor classified as anaplastic Wilms tumor.[8] This cell line was re-classified when the scientists of the Pediatric Preclinical Testing Program, which is a comprehensive program to systematically evaluate new agents against childhood solid tumors and leukemia models, molecularly investigated the lines used for these studies.[9] Due to discordance between the reported diagnoses and the gene expression profiles of several of these lines, further studies were performed. SK-NEP-1 has a mutant *TP53* and its expression profile indicates a closer relationship with Ewing sarcoma heterotransplant and cell lines than with Wilms tumor lines.[4] Furthermore, two SK-NEP-1 cells batches, one that had been passaged for years at St. Jude Children's Research Hospital, and one that had been newly acquired from ATCC, expressed the *EWS-FLI1* gene fusion transcript, that once sequenced demonstrated the joining of exon 7 of *EWS* with exon 5 of *FLI1*.[4] Also in this case, the clinical presentation is uncommon for Wilms tumor, whereas it is more consistent with Ewing tumors family.[4]

We encourage the authors of the more recent publications claiming G-401 and SK-NEP-1 as Wilms tumor cell lines to provide corrections to the journals in which their articles were published.

We trust that from now on, any studies performed with G-401 and SK-NEP-1 cells will be correctly assigned to the right pathology.

Conflict of interest statement: the authors declare to have no conflict of interest

Acknowledgements

The Authors wish to thank Dr. Amanda Capes-Davis, chair, and Dr. Christopher Korch and Dr. Barbara Parodi, members of ICLAC for helpful discussion.

Thanks to Associazione Bianca Garavaglia ONLUS, Busto Arsizio, Varese, Italy.

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