

Peer Review File

Article information: <https://dx.doi.org/10.21037/atm-23-1208>

Reviewer A

Comment 1: The focus of this paper is very important for current research in cancer therapy. The topic is very interesting and innovative.

Reply 1: Many thanks for the time you spent in reading my manuscript.

Changes: NA

Reviewer B

Comment 1: In this editorial, Dr. Weiskirchen argues for the need of guidelines of how to avoid producing invalid research results based on using false cell lines. He exemplifies the problem by listing 21 purported liver-derived cell lines that are known to be invalid models for hepatic cancer research. The author illustrates that the use of several of these false cell lines not only continues, but is increasing despite being known to be cross-contaminated by cells of non-hepatic origin. This problem pertains to many cell lines which are used under their false identities. It has been argued since 1967 by many scientists that this is a very important problem which needs to be addressed by all concerned; namely, the research scientists, the institutions in which they work, the granting agencies that fund the research, the reviewers of grants, and finally the reviewers and editors of journal manuscripts. Journals need clear guidelines for all scientists who submit manuscripts based on research using cell lines. As the author points out, the International Journal of Cancer has one of the best set of guidelines for the handling of manuscripts based on cell line research.

Awareness of the problem of using false cell lines is not as widespread as is needed. This is reflected in the continued use of the 21 misidentified cell lines used in hepatology research that the author presents herein. Publication of this editorial would be a timely presentation for the readership of the Annals of Translational Medicine so they are made aware of the severity of the problem to not only hepatology research, but to biomedical research in general. The author describes some resources (additional ones are suggested) that can be used to address the imposter cell line problem, which if not addressed will result in the continued publication of invalid and irreproducible cell-line-based research.

Reply 1: Many thanks for the time you spent in reading my manuscript.

Changes in the text: NA

Comment 2: Suggested Additions

I suggest the following additions and edits to improve this manuscript

- Insert the Cellosaurus / RRID identification numbers of each cell line mentioned in the text; for example SMMC-7721 has the Cellosaurus Identifier CVCL_0534 which is equivalent to RRID:CVCL_0534.
- To line 90, include the reference(s) for GCCP in the reference list and add the following references:
 - Almeida and Korch, PMID 23805434
 - Geraghty et al. PMID 25117809
 - Korch CT, Hall EM, Dirks WG, Sykes GR, Capes-Davis A, Butler JM, Neve RM, Nims RW, Storts DR, Tian F, Nardone RM. 2022. Human Cell Line Authentication. Standardization of Short Tandem Repeat (STR) Profiling. ASN-0002 Revised 2022, November 2022 ed. American National Standards Institute (ANSI) - American Type Culture Collection (ATCC) Standards Development Organization, Manassas, Virginia, United States. See: <https://webstore.ansi.org/standards/atcc/ansiatccasn00022022>
 - Freshney's Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications by R. Ian Freshney and Amanda Capes-Davis. 2021. Published by Wiley / Blackwell.

Reply 2: Thanks for this comment. I have added the Cellosaurus numbers in the text and table and the additional references you suggested.

Changes in the text: Addition of Cellosaurus numbers and mentioned references.

Comment 3: Suggested Grammatical Corrections

| Line Number | Suggested Changes |
|-------------|---|
| 18 | Change "... evidence of ..." to "...evidence over..." |
| 22-23 | Delete "Exemplarily" does not mean "as an example" if that is the intended meaning here. Instead write sentence as follows: "Of these cell lines, 21 were initially thought to be of hepatic origin, but ..." |
| 30-31 | I suggest changing it to read as follows: "...1977 [17], but 38 years later it was suggested to be contaminated and taken over either by HeLa cells..." |
| 32 | Delete "already" |
| 33 | Change "... that has not ..." to read "... that was not..." |

| | |
|---------|--|
| 39-40 | Change "... cell line derived xenograft (CDX)..." by inserting hyphens so it reads as follows: "... cell-line-derived xenograft (CDX)..." |
| 41 | For the authors information, reference 18 to "Creative Bioarray" refers to a company that has most likely "pirated" cell lines from other companies and is selling the cell lines without a license to do so. |
| 45 | Spell out "22" to read "Twenty-two.." |
| 46 | Delete "also" |
| 53 | Use lower case "a" in the word "albumin" instead of "Albumin" |
| 55-56 | Change "that" to "which" and enclose the clause in parenthesis so it reads: "...that this cell line (which is also called "Liver-02", "L-02", "LO2", "human liver-7702", or "HL-7702") is a derivative of HeLa [2]." |
| 67 | Change to "...well-reputable cancer journal...." to read as: "... a highly reputable cancer journal ..." |
| 70 | Change "most" to "many" |
| 70 | Replace "it they publish potential artifacts or faulty research data" with "that they publish potentially artifactual or faulty research results" |
| 80 | Include that the authors should use in addition to the cell line name, the RRID/Cellosaurus identification number." As an example, CVCL_0534 for SMMC-7721. |
| 98 | The word "falsified" implies in this context an intentional falsification of data. Consider saying "...with false, non-reproducible research results." Or "...unreliable, potentially meaningless research results." Or something similar. |
| 102 | It seems that the reference "[1,2-28]" refers to all 28 of the references listed. Is that intended? |
| 186 | Because different countries use different date conventions, which can be confusing, please spell out the date 6.3.2023 so it is March 6, 2023 or 6 March 2023. |
| Table 1 | In the title of this table, change it to read: "Twenty-one misidentified... ". Thus, readers do not need to count the number of entries in the table. Also change the date as mentioned above for line 186. |

Reply 3: I am extremely grateful for your many your suggestions for improving grammar and spelling.

Changes in the text: All corrections were done.

Reviewer C

Comment 1: Nice review article to bring more awareness of misidentified cell lines to the community. My only comment is in line 102, the references listed are [1, 2-28], I think it should read [1, 26-28] so that it identifies the ICLAC and the Cellosaurus references.

Reply 1: Many thanks for the time you spent in reading my manuscript. I have corrected the number of citations.

Changes in the text: Citation corrected.

Reviewer D

Comment 1: The authors provide a valuable, but quite brief Editorial Commentary regarding the ongoing problem of misidentification of cell lines resulting from: a) poor culture practices that lead to cross contamination or b) poor naming and labeling practices resulting in misidentification.

The article would be improved if the authors focused more on the "...More than Just a Recommendation" aspect that was mentioned in the article title and provide readers with a set of tools detailing how (and how often) to appropriately authenticate cell lines. Focus areas could include requirements from funding agencies and research journals. (For example: Cell Line Checklist for Manuscripts and Grant Applications – ICLAC).

Reply 1: Many thanks for the time you spent in reading my manuscript. As suggested, I have added some brief statements on the requirements from funding and research journals.

Changes in the text: Addition of a new paragraph at the end of the text.

Comment 2: This manuscript would be improved by mentioning specific notices published by the National Institutes of Health (NIH), describing the importance of cell line authentication. For example: Notice Number: NOT-OD-08-017 and NOT-OD-15-103 and infographic_2 (nih.gov) are useful to grant applicants.

It would also be helpful to include an example of the template used by peer reviewers of NIH (or other non-US funding agency) grant applications to illustrate the current requirements for providing information/data on cell line authentication.

The Editors in Chief of scientific journals should be responsible for providing clear guidelines to each peer reviewer requiring the inclusion of data demonstrating authentication results. For example, the requirement to include capillary electrophoresis data and analysis indicating the cells have been appropriately authenticated.

Reply 2: These are great suggestions. Accordingly, I have added some sentences in which I highlight the things you mentioned.

Changes in the text: Addition of some sentences in the main text.

Comment 3: Other recommendations:

Although more tools are becoming available for authentication of cell lines from other species, those reagents remain limited and the focus remains on reagents and tools to authenticate human cell lines. It would be helpful to build awareness of that issue.

The Assay Guidance Manual is an open access e-book in the National Library of Medicine that contains a recent chapter published in 2023 describing details regarding cell line authentication. This is a valuable resource for this topic area.

Reply 3: Thanks for this hint. I have cited this important chapter in the revised version and added some comments on its

Changes in the text: Addition of a short paragraph and one additional reference.

Comment 4: There seems to be a flaw in logic in Lines ~52-58. It is not clear if there is evidence that the cells used in reference 22 were misidentified or cross contaminated. Just because one source of a cell line has been identified as contaminated, it doesn't automatically mean that all cells of that designation are contaminated. Each source needs to be independently authenticated to determine whether or not it is contaminated. For example, cells may be contaminated in an individual academic lab; but, the same cells from ATCC may be authentic.

Reply 4: Many thanks for your attention. I have added a sentence for clarification.

Changes in text: One sentence was added to increase the clarity of what is meant.

Comment 5: There are grammar, spelling, and singular/plural errors remaining in the manuscript. Some are listed below.

Line 23 ... , but later be shown ? (but later shown)

Line 33 that has not derived (that was not derived)

Line 45 (do not start sentences with numbers; spell them out.)

Line 53 Albumin (should not be capatilized)

Line 76 is an effective mean (is an effective means)

Line 98-102 run-on sentence needs to be re-written for clarification.

Line 185 Data is (should be Data are)...the word data is plural

Reply 5: Thank you very much for pointing me to mistakes in grammar and spelling. I have corrected the things you mentioned.

Changes in text: Correction of mentioned errors in grammar and spelling in the text.