Peer-Review

Shukla, Ari. 2025. “Multifaceted Functionality of Thymine DNA Glycosylase (TDG) in Tumorigenesis: Implications Regarding Efficacy of TDG-Based Cancer Therapy.” *Journal of High School Science* 9 (3): 516–58. https://doi.org/10.64336/001c.144348.

Although the paper is well researched, it does not satisfy the Journal’s expectations of a review paper as seen here: https://jhss.scholasticahq.com/for-authors , types of manuscripts, review papers. Merely presenting content that is available in the public domain is not enough for publication.
I suggest the following avenue of thought and presentation that will make this manuscript acceptable for publication:
Since, the expression of TDG is context depedent (decreased level=tumor suppressor function and increased level = tumorigenesis promoter), a therapeutic intervention needs to be provided which can
1.detect the level of expression of TDG in tisssue. If less, then make TDG dock/interact with APE1 (or equivalent). If more, then make TDG dock/interact with E3 Ubiquitin Ligase or equivalent. so that TDG level is normalized.
2.The way to do this is using PROTAC, where the capping molecule is itself a TDG degradable ligand.
3.When the PROTAC is injected and TDG level is high, the TDG will uncap the E3Ubiquitin ligase capby degrading it and the resultant interaction of TDG with E3Ubiquitin ligase will degrade TDG., When the TDG level is low, the E3Ubiquitin ligase cap will remain capped, and the PROTAC will enable TDG to interact with the APE1 instead, thereby increasing the activity of TDG.
This solution has not yet been presented in the literature. The solution is unique because it automatically contextually increases or decreases the level of TDG to normalize it regardless of the tissue. PROTAC is stoichiometry independent, meaning the same molecule can have an effect over-and-over again.
If you research this idea and present in in detail in the manuscript along with references, the manuscript will contribute significantly to the existing corpus of knowledge and I can consider it for publication.
Please see the attached file for a schematic of the idea. Please thoroughly search the literature for references, such as the one below:
https://doi.org/10.31635/ccschem.022.202101529

I am very grateful to you and the reviewer for the insightful feedback provided. The implications of PROTAC technology in the context-dependent regulation of TDG expression are intriguing, and the idea presented by the reviewer to incorporate the Pro-PROTAC mechanism was very valuable to my paper. I have carefully considered the comment left by the reviewer and implemented the following change in my manuscript:

I added a section titled “Potential of PROTAC Technology to Correct Dysregulated TDG Expression” (From the bottom of pg. 38 to the top of pg. 46) after the “Discussion and Future Perspectives” section. In this new section, I explain the mechanism of action for the PROTAC drug and explain the rationale, as described by the reviewer, for the utilization of the drug to normalize TDG levels for cancers wherein TDG expression is dysregulated. The section explores the efficacy of PROTAC, Pro-PROTAC as described by Liang et al. in the reference provided by
the reviewer, as well as a Pro-PROTAC molecule where the capping molecule on the E3 Ubiquitin Ligase Ligand is TDG-degradable in decreasing TDG expression in
TDG-overexpressing cancer types. Then, the section proposes a novel sumoylation-targeting chimera (SUMTAC), which potentiates the conjugation of Small Ubiquitin-Like Modifier (SUMO) to TDG. By

doing so, SUMTAC increases enzymatic turnover of TDG, allowing smaller amounts of TDG present in TDG-underexpressing cancers to produce a similar phenotype to that produced by normal TDG expression.

I greatly appreciated the reviewer's suggestion to use the PROTAC structural model to facilitate TDG interaction with APE1 and increase TDG activity. However, I ultimately proposed the SUMTAC mechanism instead for the reason that, due to the similarity of the enzymaticpathways regulating ubiquitination and sumoylation (i.e. involving activating E1, conjugating E2, and ligating E3 enzymes), the existing PROTAC structural model may be more adaptable to a sumoylation context compared to that involving APE1 interaction. Both methods work to
achieve the equivalent result of increasing TDG turnover to mimic the phenotype produced by typical TDG levels despite a lower concentration of TDG. Additionally, although I was intrigued by the reviewer's idea of a PROTAC structure incorporating both a capped ubiquitin ligase ligand and a SUMO ligase ligand (shown in their diagram as APE1 ligand), I did not propose this idea in my paper because the molecular weight of existent PROTAC is already relatively high,
violating Lipinski's Rule of Five. This raises concerns around drug efficacy, which would be intensified if a third ligand were to be added to the base structure of PROTAC. Presumably, screening would occur prior to administration of TDG-targeting PROTAC or SUMTAC to detect the level of TDG in the tissue, reducing the need for solely one drug to regulate TDG levels regardless of whether TDG is overexpressed or underexpressed.

References 93 to 102 (Pages 62 to 63) were identified from the literature and added to the review to support and inform the TDG/PROTAC section. I hope that the additional section presenting the concept and structure for the novel TDG targeting PROTAC and SUMTAC drugs sufficiently addresses the reviewer's concerns and ensures that the review contributes significantly to the existing corpus of knowledge. Thank you again for the time and effort given by the reviewer to outline this fascinating TDG/PROTAC mechanism and your consideration for publication in the Journal of High School Science.

Thank you very much for trying to address my comments. Sincerely appreciated. However, you have not addressed this comment to my satisfaction.
You state "…..Presumably, screening would occur prior to administration of TDG-targeting PROTAC or SUMTAC to detect
the level of TDG in the tissue, reducing the need for solely one drug to regulate TDG levels regardless of whether TDG is overexpressed or underexpressed.
References 93 to 102 (Pages 62 to 63) were identified from……."
That was not the point of my suggestion. The point was that the molecule would 'adjust' so as to correct underexpression of TDG in one tissue/organ and or overexpression of TDG in another tissue/organ to normalize TDG levels throughout the tumor regardless of whether underexpressed or over-expressed. Please see my post on
Linkedin: https://www.linkedin.com/posts/shireeshpapte_enzyme-catalyzed-activation-of-pro-protac-activity-7347328294255566848-JU55?
utm_source=share&utm_medium=member_desktop&rcm=ACoAAAKRCSwBPS07qJ1AhFaB4ri7l46H9i1Esic
While I appreciate the author's m.w concern, this attribute by itself is not a deal-breaker. Please pursue the linkedin blurb and incorporate into your manuscript. If you still think the mw may end up being too large, you can always put this down as a limitation.
You can of course leave your PROTAC section in.

I look forward to your response.

Thank you so much for your insightful feedback and clarification regarding the justification for a trifunctional chimera in the normalization of dysregulated TDG expression. I appreciated the reference to your post on LinkedIn, which certainly augmented my understanding of the structural concept proposed. To address this comment, I implemented the following change to my manuscript:

Original comment: "That was not the point of my suggestion. The point was that the molecule would 'adjust' so as to correct underexpression of TDG in one tissue/organ and or overexpression of TDG in another tissue/organ to normalize TDG levels throughout the tumor regardless of whether underexpressed or over-expressed…While I appreciate the author's m.w concern, this attribute by itself is not a deal-breaker. Please pursue the linkedin blurb and incorporate into your manuscript. If you still think the mw may end up being too large, you can always put this down as a limitation."

Response: I have added four paragraphs ("To normalize TDG expression throughout the entirety of a tumor which may…be necessary to ensure TriCHIM efficacy as described in this section.", pgs. 30 to 32) to the end of my "Potential of Chimeric Technology to Correct Dysregulated TDG Expression" section proposing the trifunctional chimera, abbreviated as "TriCHIM"; explaining its structural model, and describing its potential effects within tissues both overexpressing and underexpressing TDG. I have also added Figure 8 on pg. 31 to assist the reader in visualizing the TriCHIM molecule and function. As suggested, I included limitations regarding molecular weight at the end of the section as a limitation. I hope that my response addresses this comment sufficiently and to your satisfaction. Thank you again for your time and effort to review my manuscript and provide such a valuable explanation of the TriCHIM mechanism. I look forward to receiving your response.

Thank you for addressing my comments. The manuscript is much improved. I have some last formatting comments before I accept.
1.Please upload a word doc version of the manuscript. Must be single column, Times New Roman, 12 Font and meet formatting requirements of the Journal.
2.References must be in APA format. Please number references manucally - do not use the software's functionality to number references.
3.Remove the Abbreviations list and instead, make sure all the words in the list are listed in their full forms in the first instance they appear in the text.
4.Please add a space between paragraphs and do not indent the first line of the paragraph.
5.Remove the inverted commas around the keywords.

Thank you for your additional comments regarding formatting. I have implemented the following changes in my manuscript:
1. Please upload a word doc version of the manuscript. Must be single column, Times New Roman, 12 Font and meet formatting requirements of the Journal.

I have converted the file to a word doc, and ensured the document is written in 12 point Times New Roman font in a single column.

2. References must be in APA format. Please number references manucally - do not use the software's functionality to number references.

I have reformatted my references to APA style. Reference numbers are done manually.

3. Remove the Abbreviations list and instead, make sure all the words in the list are listed in their full forms in the first instance they appear in the text.

The abbreviations list has been removed. All abbreviations are listed in full form at their earliest use in the text.

4. Please add a space between paragraphs and do not indent the first line of the paragraph.

I have reformatted the paragraphs in alignment with the above comment, removing indentations and inserting a space between paragraphs.

5. Remove the inverted commas around the keywords.

The inverted commas around the keywords have been removed. Thank you again for your valuable feedback, and your consideration for publication in the Journal of High School Science.

Thank you for addressing my concerns. Accepted.