

Hypothesis

Not peer-reviewed version

Targeting Clonal Mutations in Solid Tumors with Personalized Oncolytic Microbes

[Michael Renteln](#)*

Posted Date: 17 December 2025

doi: 10.20944/preprints202512.0624.v2

Keywords: blood cancer; solid tumors; clonal mutations; hematopoietic stem cells; facultative intracellular bacterium; Craspase



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a [Creative Commons CC BY 4.0 license](#), which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Hypothesis

Targeting Clonal Mutations in Solid Tumors with Personalized Oncolytic Microbes

Michael Renteln

Independent Researcher; mrenteln@gmail.com

Abstract

Immunotherapy has shown much promise for blood cancers, which may all be treatable or curable soon, especially if hematopoietic stem cells are harvested and frozen ahead of time for each individual. However, solid tumors are still extremely difficult to treat. Immunotherapy has helped in some instances for solid tumors, e.g., melanoma, and may eventually be able to cure all solid tumors for reasons that are a bit unclear currently. There may be a more direct way to treat solid tumors, though. I have written multiple articles about targeting truncal, i.e., clonal, mutations in solid tumors as a means of eliminating them. This would be a treatment specific to each patient. However, in my earlier work, I was under the impression that there may only be a handful of clonal mutations in an average solid tumor patient. After further investigation, it seems that instead of just several, there could be thousands of clonal mutations on average in a solid tumor patient. This may essentially ensure that all of a solid tumor patient's cancer cells could be targeted.

Keywords: blood cancer; solid tumors; clonal mutations; hematopoietic stem cells; facultative intracellular bacterium; Craspase

Introduction

Cancer has been the bane of multi-cellular organisms since their inception. That is why our cells have evolved over billions of years to have strict gene pathways that help prevent cancer. Still, however, especially for those who smoke and are otherwise unhealthy, it can strike. It also affects the elderly more frequently.

It would make sense for us all to freeze some of our hematopoietic stem cells (HSCs) at an early age, i.e., before any disease. That way, each individual would have autologous HSCs to transplant (non-toxically [1]) back into him or herself in the event of blood cancer down the line. The HSCs would be multiplex epitope-edited to protect them from antibody-drug conjugates that would be administered to wipe out the entirety of their old white blood cell compartment [2].

Advanced solid tumors are, unfortunately, still very deadly. I came up with an approach called "Oncolytic Vector Efficient Replication Contingent on Omnipresent Mutation Engagement" (OVERCOME) [3–5]. It focuses on truncal, or clonal, mutations as the basis of treatment. Importantly, all types of mutations could be targeted via OVERCOME, including driver, passenger, coding, and non-coding mutations.

For solid tumors, OVERCOME first involves multi-region sequencing and/or circulating tumor cell/DNA analysis. This would identify the clonal mutations of the given patient. Ideally, there would be many dispersed throughout the genome. Next, a facultative intracellular bacterium like *Listeria monocytogenes* or *Salmonella Typhimurium* would be programmed to export multiplexed molecular sensors that trigger transient replication and hyper-virulence when they detect one or more of the patient's clonal mutations. A stay-on variant of Craspase may be the best sensor in this context, as it can be easily multiplexed and is sensitive to point mutations, perhaps especially when combined with synthetic mismatches [6–8]. A facultative intracellular bacterium could possibly be developed that can enter any given human cell based on adhesins and invasins [9] that bind to ubiquitously expressed cell surface proteins. Multiplexed CRISPR-based transcriptional activation (CRISPRa)

could be used to ensure that there are mutated transcript 'targets', with detection of the transcript at a non-mutated site being the basis for ceasing CRISPRa. This would be a self-limiting amplification system to protect normal, non-cancerous cells as much as possible. It may be prudent to have CRISPRa on a "timer" for each target transcript using a temporal promoter cascade - to give the bacterium time to detect if a given transcript is already present before upregulating it. Still, upregulating so many genes at once may be prohibitively problematic for non-cancerous cells. To avoid this, the best strategy would be to mark nascent transcripts generated by the bacterial enzyme so that they are not translated into proteins. dCas9 targeted to the given gene promoter provides specificity, which can be theoretically combined with an RNA editor that marks nascent transcripts generated by the bacterial dCas9 [10].

While I have already written three articles about this approach, earlier I was unaware of just how many clonal mutations an average solid tumor patient has [11–21]. Passenger [22] and non-coding mutations may not be targetable by traditional therapies, but they are via direct mutation detection. It seems as though the average solid tumor patient may have ~5,000 clonal single nucleotide variants (SNVs) alone in their cancer [23–25]. After multi-region or blood sample sequencing [26,27], multiplexed detection of many or all of a patient's clonal mutations could be sufficient to essentially ensure that all of his or her tumor cells are eliminated. The probability that ~5,000 clonal SNVs would be lost in many of the progeny cancer cells is very low. This is because at most, only ~0.01% of the genome is likely to be mutated in any given cancer cell, even those in the late stage of the cancer's development [28]. If we assume that 5,000 SNVs are at least somewhat randomly distributed throughout the genome, then one can see that the odds are very low that they would all be lost.

Anti-Cancer Bacterial Microbots

While rapidly sequencing a patient's cancer and bioengineering a facultative intracellular bacterium with these features would be very difficult for each new patient, e.g., making sure all the molecular switches have exceptionally high on/off target ratios, it should be possible.

RNA export from *L. monocytogenes* could potentially be effected using the proteins Zee or Eno [29,30]. Otherwise, asymmetric division and lysis of one of the progeny cells is also a possibility to release a multitude of guide RNAs [31]. However, perhaps the best possibility would be to figure out how to co-opt a type 4 secretion system (T4SS), which usually is able to export DNA and proteins.

There may still be some cancer cells that lack all of the target mutations. However, OVERCOME may be able to account for this in a few ways. Transient reversal of attenuation could be prolonged somewhat, so that replication and hyper-virulence is triggered for a longer period of time. Also, an activation signal could be transmitted from intracellular bacteria that have found a target to nearby extra and intracellular bacteria using the cell-permeable quorum sensing small molecule, AI-1 [32]. Finally, a toxin with a bystander effect could be released once the bacteria reach quorum sensing levels [33] or after small molecule administration.

Conclusion

While OVERCOME is very involved, and it would require rapid sequencing and bioengineering for each patient – DNA sequencing costs have been dropping dramatically, and we are at the point in synthetic biology that such bioengineering is feasible.

I think that multiplexed targeting of patient clonal mutations could eliminate solid tumors with essentially no side effects, and that we should look into this possibility immediately.

Author Contributions: M.R. wrote the article.

Funding: N/A.

Conflicts of Interest: The author declares no conflicts of interest.

References

1. Palchaudhuri R, Saez B, Hoggatt J, et al. Non-genotoxic conditioning for hematopoietic stem cell transplantation using a hematopoietic-cell-specific internalizing immunotoxin. *Nat Biotechnol* 2016;34(7):738–745; doi: 10.1038/nbt.3584.

2. Garaudé S, Marone R, Lepore R, et al. Selective haematological cancer eradication with preserved haematopoiesis. *Nature* 2024;630(8017):728–735; doi: 10.1038/s41586-024-07456-3.

3. Renteln M. Conditional replication of oncolytic viruses based on detection of oncogenic mRNA. *Gene Ther* 2018;25(1):1–3; doi: 10.1038/gt.2017.99.

4. Renteln M. Promoting Oncolytic Vector Replication with Switches that Detect Ubiquitous Mutations. *Current Cancer Therapy Reviews* 2024;20(1):40–52; doi: 10.2174/1573394719666230502110244.

5. Renteln M. Targeting clonal mutations with synthetic microbes. *Critical Reviews in Oncology/Hematology* 2025;206:104572; doi: 10.1016/j.critrevonc.2024.104572.

6. Hu C, van Beljouw SPB, Nam KH, et al. Craspase is a CRISPR RNA-guided, RNA-activated protease. *Science* 2022;377(6612):1278–1285; doi: 10.1126/science.add5064.

7. Xiao Y, Chen J, Hou X, et al. A NanoLock-enabled, Craspase-based strategy for highly sensitive RNA detection. *Nucleic Acids Res* 2025;53(17):gkaf907; doi: 10.1093/nar/gkaf907.

8. Shembrey C, Yang R, Casan J, et al. Principles of CRISPR-Cas13 mismatch intolerance enable selective silencing of point-mutated oncogenic RNA with single-base precision. *Science Advances* 2024;10(51):eadl0731; doi: 10.1126/sciadv.adl0731.

9. Niemann HH, Schubert W-D, Heinz DW. Adhesins and invasins of pathogenic bacteria: a structural view. *Microbes and Infection* 2004;6(1):101–112; doi: 10.1016/j.micinf.2003.11.001.

10. Goldberg GW, Kogenaru M, Keegan S, et al. Engineered transcription-associated Cas9 targeting in eukaryotic cells. *Nat Commun* 2024;15:10287; doi: 10.1038/s41467-024-54629-9.

11. Vogelstein B, Papadopoulos N, Velculescu VE, et al. Cancer Genome Landscapes. *Science* 2013;339(6127):1546–1558; doi: 10.1126/science.1235122.

12. Mroz EA, Rocco JW. The challenges of tumor genetic diversity. *Cancer* 2017;123(6):917–927; doi: 10.1002/cncr.30430.

13. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing. *N Engl J Med* 2012;366(10):883–892; doi: 10.1056/NEJMoa1113205.

14. Kumar S, Warrell J, Li S, et al. Passenger mutations in 2500 cancer genomes: Overall molecular functional impact and consequences. *Cell* 2020;180(5):915–927.e16; doi: 10.1016/j.cell.2020.01.032.

15. Frankell AM, Dietzen M, Al Bakir M, et al. The evolution of lung cancer and impact of subclonal selection in TRACERx. *Nature* 2023;616(7957):525–533; doi: 10.1038/s41586-023-05783-5.

16. Sottoriva A, Kang H, Ma Z, et al. A Big Bang model of human colorectal tumor growth. *Nat Genet* 2015;47(3):209–216; doi: 10.1038/ng.3214.

17. Aaltonen LA, Abascal F, Abeshouse A, et al. Pan-cancer analysis of whole genomes. *Nature* 2020;578(7793):82–93; doi: 10.1038/s41586-020-1969-6.

18. Priestley P, Baber J, Lolkema MP, et al. Pan-cancer whole-genome analyses of metastatic solid tumours. *Nature* 2019;575(7781):210–216; doi: 10.1038/s41586-019-1689-y.

19. Spain L, Coulton A, Lobon I, et al. Late-Stage Metastatic Melanoma Emerges through a Diversity of Evolutionary Pathways. *Cancer Discov* 2023;13(6):1364–1385; doi: 10.1158/2159-8290.CD-22-1427.

20. Loeb LA, Kohn BF, Loubet-Seneor KJ, et al. Extensive subclonal mutational diversity in human colorectal cancer and its significance. *Proceedings of the National Academy of Sciences* 2019;116(52):26863–26872; doi: 10.1073/pnas.1910301116.

21. Schrijver WA, Selenica P, Lee JY, et al. Mutation profiling of key cancer genes in primary breast cancers and their distant metastases. *Cancer Res* 2018;78(12):3112–3121; doi: 10.1158/0008-5472.CAN-17-2310.

22. Hao J-J, Lin D-C, Dinh HQ, et al. Spatial intratumor heterogeneity of genetic, epigenetic alterations and temporal clonal evolution in esophageal squamous cell carcinoma. *Nat Genet* 2016;48(12):1500–1507; doi: 10.1038/ng.3683.

23. Fang Y, Chan-Seng-Yue M, Ng K, et al. The subclonal footprint of pervasive early dissemination in pancreatic cancer. 2025. bioRxiv preprint doi: <https://doi.org/10.1101/2025.11.27.690823>.

24. Dentre SC, Leshchiner I, Haase K, et al. Characterizing genetic intra-tumor heterogeneity across 2,658 human cancer genomes. *Cell* 2021;184(8):2239–2254.e39; doi: 10.1016/j.cell.2021.03.009.
25. Gerstung M, Jolly C, Leshchiner I, et al. The evolutionary history of 2,658 cancers. *Nature* 2020;578(7793):122–128; doi: 10.1038/s41586-019-1907-7.
26. Li S, Hu R, Small C, et al. cfSNV: a software tool for the sensitive detection of somatic mutations from cell-free DNA. *Nat Protoc* 2023;18(5):1563–1583; doi: 10.1038/s41596-023-00807-w.
27. Martin-Alonso C, Tabrizi S, Xiong K, et al. Priming agents transiently reduce the clearance of cell-free DNA to improve liquid biopsies. *Science* 2024;383(6680):eadf2341; doi: 10.1126/science.adf2341.
28. Scott RJ, Ziolkowski A, Mossman D, et al. Tumour mutational burden using a targeted panel approach for comprehensive tumour profiling focusing on colorectal cancer. *Hered Cancer Clin Pract* 2025;23(1):10; doi: 10.1186/s13053-025-00308-9.
29. Pagliuso A, Tham TN, Allemand E, et al. An RNA-Binding Protein Secreted by a Bacterial Pathogen Modulates RIG-I Signaling. *Cell Host & Microbe* 2019;26(6):823–835.e11; doi: 10.1016/j.chom.2019.10.004.
30. Teubner L, Frantz R, Pietra LL, et al. SecA2 Associates with Translating Ribosomes and Contributes to the Secretion of Potent IFN- β Inducing RNAs. *International Journal of Molecular Sciences* 2022;23(23); doi: 10.3390/ijms232315021.
31. Lin D-W, Liu Y, Lee Y-Q, et al. Construction of intracellular asymmetry and asymmetric division in *Escherichia coli*. *Nat Commun* 2021;12(1):888; doi: 10.1038/s41467-021-21135-1.
32. Kamaraju K, Smith J, Wang J, et al. Effects on membrane lateral pressure suggest permeation mechanisms for bacterial quorum signaling molecules. *Biochemistry* 2011;50(32):6983–6993; doi: 10.1021/bi200684z.
33. Din MO, Danino T, Prindle A, et al. Synchronized cycles of bacterial lysis for in vivo delivery. *Nature* 2016;536(7614):81–85; doi: 10.1038/nature18930.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.