Review

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A New Paradigm in Cancer Treatment: Identifying and Targeting Clonal Mutations

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A New Paradigm in Cancer Treatment: Identifying and Targeting Clonal Mutations

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Highlights:

- Clonal mutations are those that are present in all of a given patient's cancer cells.
- TRACERx results suggest that many or most patients have at least one clonal mutation.
- Immunotherapy may not be the best way to exploit clonal mutations.
- "OVERCOME" would exploit clonal mutations with an intracellular microbe.
- OVERCOME may be ideal for solid tumors, at least.

Abstract: Recently concluded, large-scale cancer genomics studies involving multiregion sequencing of primary tumors and paired metastases appear to indicate that many or most cancer patients have one or more "clonal" mutations in their tumors. Clonal mutations are those that are present in all of a patient's cancer cells. Achilles Therapeutics is currently the only company specifically targeting clonal mutations. However, they are doing so with tumor-derived T cells. To address the potential limitations of immunotherapy, I have devised another approach for exploiting clonal mutations, which I call "Oncolytic Vector Efficient Replication Contingent on Omnipresent Mutation Engagement" (OVERCOME). The ideal version of OVERCOME would likely employ a bioengineered facultative intracellular bacterium. The bacterium would initially be attenuated, but (transiently) reverse its attenuation upon clonal mutation detection.

Keywords: multiregion sequencing; multisample sequencing; cell-free circulating tumor DNA; clonal mutations; Achilles Therapeutics; OVERCOME

Introduction

Cancer has plagued multi-cellular organisms since their inception. However, we have only recently begun to develop effective targeted therapies. Most of said therapies have been for blood cancers. Gleevec, the BCR-ABL tyrosine kinase inhibitor, is a prime example of this; it was approved in 2001 for the treatment of chronic myelogenous leukemia[1]. Additionally, immunotherapies such as CAR T-cells have been developed that target T and B cell malignancies[2].

In certain instances, immunotherapies such as anti-PD1 antibodies can help treat melanoma. T-VEC, an FDA-approved oncolytic herpesvirus, is also sometimes effective against melanoma[3]. It is somewhat unclear why melanomas respond so well to immunotherapy and T-VEC as opposed to many other types of cancer.

T-VEC may exert its anti-tumor effects mainly by rendering melanoma lesions immunologically "hot", rather than direct oncolysis[4]. It may also spread more easily through such lesions due to tight endothelial cell-to-cell junctions[5]. Thus, melanoma may simply be particularly amenable to immunotherapy. Perhaps this is because it is often caused at least in part by UV damage-mediated DNA mutations, which can be potently immunogenic[6].

Three other oncolytic viruses have been approved for clinical usage against solid tumors in other areas of the world: Rigvir, Oncorine, and Delytact[7]. Rigvir is an oncolytic enterovirus approved in Latvia for melanoma, Oncorine is a modified adenovirus that is used to treat head and neck cancer, and Delytact is a herpesvirus used to treat malignant gliomas. Rigvir may not be as efficacious as

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T-VEC[8]. Like T-VEC, all three of these vectors appear to exert their oncolytic effects primarily by potentiating the anti-tumor immune response[9–11].

Finally, there is one FDA-approved bacterial vector that is used to treat non-muscle invasive bladder cancer, Bacillus Calmette–Guérin (BCG)[12]. It is a live attenuated strain of *Mycobacterium bovis*. Although it is one of the oldest tumor therapies, its mechanism of action still has not been fully elucidated. As with the aforementioned oncolytic viruses, however, BCG may mainly stimulate an immune response against bladder cancer cells rather than lyse them directly[13].

Regardless, in most instances, the aforementioned therapies for solid tumors are not curative. That is largely because they do not target the tumors with sufficient specificity over normal tissue, and so must be attenuated.

Clonal Mutations

Clonal mutations are defined as mutations that are present in all of a patient's cancer cells. Recently published results from large-scale cancer genomics studies that involve multiregion sequencing of primary tumors and paired metastases, like TRACERx[14], appear to indicate that many or most patients have at least one clonal mutation in their cancers[15–20].

Clonal mutations would be ideal targets for personalized therapy. Some tumors are in anatomical locales that are difficult or dangerous to biopsy, however. A non-invasive option for identifying a patient's mutational spectrum, which is becoming increasingly feasible in terms of clinical application, would be to analyze circulating tumor cells[21] or circulating cell-free tumor DNA in the blood or cerebrospinal fluid[22–27]. Although it possible to determine clonal mutations, targeting these mutations is not very facile at present.

Dr. Charles Swanton, Chief Investigator of the TRACERx study, co-founded a company called Achilles Therapeutics in 2016; it is currently the only company specifically targeting clonal mutations. However, they are leveraging an immunotherapy tactic to do so, specifically tumor-derived T cells[28]. From a mechanistic perspective, immunotherapy may not be the best way to exploit clonal mutations. Firstly, many mutations affect intracellular antigens. While MHC class I complexes can display intracellular peptides derived from mutated proteins, 40-90% of human cancers downregulate said complexes[29]. Secondly, even if a mutant protein is on the cell's surface, some of the patient's cancer cells may evolve to downregulate the production of that mutant protein. The latter point applies to the display of peptides derived from mutant intracellular proteins via MHC class I complexes as well.

Recently, I devised an approach for exploiting clonal mutations in solid tumors at least that can theoretically circumvent these issues, which I call "Oncolytic Vector Efficient Replication Contingent on Omnipresent Mutation Engagement" (OVERCOME)[30].

OVERCOME

The general idea of OVERCOME is to use an oncolytic virus or intracellular bacterium with the broadest possible tropism that is either programmed not to replicate or attenuated until it detects one or more clonal mutations via molecular "switches"[30–37]. Moreover, many hyper-virulence modules could be triggered by clonal mutation detection[38–41]. Somewhat similar strategies have been proposed before with oncolytic viruses, but replication was not made dependent on mutation detection[42].

Crucially, with such a vector, clonally mutated genes can be forcibly upregulated via transcriptional activators to essentially ensure a detection signal.

Clonally mutated intergenic regions could also theoretically be targeted by DNA-binding switches[43,44]. However, if the DNA is targeted, an enzymatic cascade may be required for sufficiently rapid amplification of the mutation "signal"[45]. Such a cascade might increase vector off-target activity. In the near future, induced transcription of any intergenic region might be possible, which could lead to less off-target activity than an enzymatic cascade-based mechanism.

Ideally, the vector would target all of a patient's clonal mutations simultaneously, transcriptionally upregulate any clonally mutated genes, and conditionally become hyper-virulent in

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many ways. Such sophisticated bioengineering may require a lot of extra packaging space, however. Given the essentially unlimited packaging space of bacteria, an intracellular bacterium may be the best oncolytic vector in this context.

While I did not specify this in my previous article, as opposed to viruses, the restoration of intracellular bacterial replication potential or attenuation reversal may need to be transient in order to avoid systemic infections.

Various attenuated bacterial species can be intravenously injected in humans with minimal side effects[46–48]. Notably, bacteria naturally colonize tumors when injected intravenously[49]. Moreover, some bacteria at least are able to cross the blood-brain barrier after intravenous injection, which is a very helpful characteristic for treating central nervous system tumors like glioblastoma[50,51].

The three intracellular bacterial species that are best studied in the context of cancer are *Salmonella* Typhimurium[52], *Listeria monocytogenes*[53], and *Shigella flexneri*[54]. I previously suggested the possible use of *Vibrio natriegens* as a vector because of its rapid replication rate[55] and the fact that only two genes are required for extracellular bacterial entry into mammalian cells[56], but it does not seem to survive in the cytoplasm of human cells[57]. A prophage-free strain of *V. natriegens* may be more applicable here[58].

A possible benefit of using a facultative intracellular bacterium over an obligate intracellular bacterium would be that it may not need to invade very many cancer cells; activated vectors could transmit the detection signal to extracellular bacteria via AI-1, a membrane-permeable quorum sensing molecule[59].

Additionally, for neuron-based cancer, at least, *Toxoplasma gondii* could eventually be helpful[60].

Finally, it is theoretically possible that some number of patients may have no clonal mutations in their cancers.

In this unlikely scenario, a small set of subclonal mutations that together are present in all of their cancer cells could be targeted.

Conclusion

It is clear that effective therapies for solid tumors are urgently needed. While immunotherapy has had much success in the realm of blood cancers, it is unclear whether it will end up being similarly efficacious for solid tumors. From a mechanistic standpoint, targeting cell surface antigens certainly seems like a less promising strategy than targeting mutated nucleic acids or proteins from the interior of the cell. Thus, the development of a facultative intracellular bacterial vector that can surmount these mechanistic challenges could be crucial to curing solid tumors.

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References

1. Hochhaus A, Larson RA, Guilhot F, Radich JP, Branford S, Hughes TP, et al. Long-Term Outcomes of Imatinib Treatment for Chronic Myeloid Leukemia. New England Journal of Medicine 2017;376:917–27. https://doi.org/10.1056/NEJMoa1609324.
2. De Marco RC, Monzo HJ, Ojala PM. CAR T Cell Therapy: A Versatile Living Drug. Int J Mol Sci 2023;24:6300. https://doi.org/10.3390/ijms24076300.
3. Sun L, Funchain P, Song JM, Rayman P, Tannenbaum C, Ko J, et al. Talimogene Laherparepvec combined with anti-PD-1 based immunotherapy for unresectable stage III-IV melanoma: a case series. J Immunother Cancer 2018;6:36. https://doi.org/10.1186/s40425-018-0337-7.
4. Ferrucci PF, Pala L, Conforti F, Cocorocchio E. Talimogene Laherparepvec (T-VEC): An Intralesional Cancer Immunotherapy for Advanced Melanoma. Cancers (Basel) 2021;13:1383. https://doi.org/10.3390/cancers13061383.

5. Xu B, Ma R, Russell L, Yoo JY, Han J, Cui H, et al. An oncolytic herpesvirus expressing E-cadherin improves survival in mouse models of glioblastoma. Nat Biotechnol 2019;37:45–54. https://doi.org/10.1038/nbt.4302.
6. Pham TV, Boichard A, Goodman A, Riviere P, Yeerna H, Tamayo P, et al. Role of ultraviolet mutational signature versus tumor mutation burden in predicting response to immunotherapy. Mol Oncol 2020;14:1680–94. https://doi.org/10.1002/1878-0261.12748.
7. Su Y, Su C, Qin L. Current landscape and perspective of oncolytic viruses and their combination therapies. Transl Oncol 2022;25:101530. https://doi.org/10.1016/j.tranon.2022.101530.
8. Su Y, Su C, Qin L. Current landscape and perspective of oncolytic viruses and their combination therapies. Transl Oncol 2022;25:101530. https://doi.org/10.1016/j.tranon.2022.101530.
9. Alberts P, Tilgase A, Rasa A, Bandere K, Venskus D. The advent of oncolytic virotherapy in oncology: The Rigvir® story. Eur J Pharmacol 2018;837:117–26. https://doi.org/10.1016/j.ejphar.2018.08.042.
10. Zhang Q, Li Y, Zhao Q, Tian M, Chen L, Miao L, et al. Recombinant human adenovirus type 5 (Oncorine) reverses resistance to immune checkpoint inhibitor in a patient with recurrent non-small cell lung cancer: A case report. Thorac Cancer 2021;12:1617–9. https://doi.org/10.1111/1759-7714.13947.
11. Sugawara K, Iwai M, Ito H, Tanaka M, Seto Y, Todo T. Oncolytic herpes virus G47Δ works synergistically with CTLA-4 inhibition via dynamic intratumoral immune modulation. Mol Ther Oncolytics 2021;22:129–42. https://doi.org/10.1016/j.omto.2021.05.004.
12. Katims AB, Tallman J, Vertosick E, Porwal S, Dalbagni G, Cha EK, et al. Response to 2 Induction Courses of Bacillus Calmette-Guèrin Therapy Among Patients With High-Risk Non-Muscle-Invasive Bladder Cancer: 5-year Follow-Up of a Phase 2 Clinical Trial. JAMA Oncol 2024:e236804. https://doi.org/10.1001/jamaoncol.2023.6804.
13. Antonelli AC, Binyamin A, Hohl TM, Glickman MS, Redelman-Sidi G. Bacterial immunotherapy for cancer induces CD4-dependent tumor-specific immunity through tumor-intrinsic interferon-γ signaling. Proc Natl Acad Sci U S A 2020;117:18627–37. https://doi.org/10.1073/pnas.2004421117.
14. Hayes TK, Meyerson M. Molecular portraits of lung cancer evolution. Nature 2023;616:435–6. https://doi.org/10.1038/d41586-023-00934-0.
15. Yachida S, Jones S, Bozic I, Antal T, Leary R, Fu B, et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature 2010;467:1114–7. https://doi.org/10.1038/nature09515.
16. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. New England Journal of Medicine 2012;366:883–92.
17. Schrijver WA, Selenica P, Lee JY, Ng CKY, Burke KA, Piscuoglio S, et al. Mutation profiling of key cancer genes in primary breast cancers and their distant metastases. Cancer Res 2018;78:3112–21. https://doi.org/10.1158/0008-5472.CAN-17-2310.
18. Mitchell TJ, Turajlic S, Rowan A, Nicol D, Farmery JHR, O'Brien T, et al. Timing the Landmark Events in the Evolution of Clear Cell Renal Cell Cancer: TRACERx Renal. Cell 2018;173:611-623.e17. https://doi.org/10.1016/j.cell.2018.02.020.
19. Spain L, Coulton A, Lobon I, Rowan A, Schnidrig D, Shepherd STC, et al. Late-Stage Metastatic Melanoma Emerges through a Diversity of Evolutionary Pathways. Cancer Discov 2023;13:1364–85. https://doi.org/10.1158/2159-8290.CD-22-1427.
20. Frankell AM, Dietzen M, Al Bakir M, Lim EL, Karasaki T, Ward S, et al. The evolution of lung cancer and impact of subclonal selection in TRACERx. Nature 2023;616:525–33. https://doi.org/10.1038/s41586-023-05783-5.
21. Thiele J-A, Bethel K, Králíčková M, Kuhn P. Circulating Tumor Cells: Fluid Surrogates of Solid Tumors. Annu Rev Pathol 2017;12:419–47. https://doi.org/10.1146/annurev-pathol-052016-100256.
22. Murtaza M, Dawson S-J, Pogrebniak K, Rueda OM, Provenzano E, Grant J, et al. Multifocal clonal evolution characterized using circulating tumour DNA in a case of metastatic breast cancer. Nat Commun 2015;6:8760. https://doi.org/10.1038/ncomms9760.
23. Pereira B, Chen CT, Goyal L, Walmsley C, Pinto CJ, Baiev I, et al. Cell-free DNA captures tumor heterogeneity and driver alterations in rapid autopsies with pre-treated metastatic cancer. Nat Commun 2021;12:3199. https://doi.org/10.1038/s41467-021-23394-4.
24. Li S, Hu R, Small C, Kang T-Y, Liu C-C, Zhou XJ, et al. cfSNV: a software tool for the sensitive detection of somatic mutations from cell-free DNA. Nat Protoc 2023;18:1563–83. https://doi.org/10.1038/s41596-023-00807-w.

25. Abbosh C, Frankell AM, Harrison T, Kisistok J, Garnett A, Johnson L, et al. Tracking early lung cancer metastatic dissemination in TRACERx using ctDNA. Nature 2023;616:553–62. https://doi.org/10.1038/s41586-023-05776-4.
26. Martin-Alonso C, Tabrizi S, Xiong K, Blewett T, Sridhar S, Crnjac A, et al. Priming agents transiently reduce the clearance of cell-free DNA to improve liquid biopsies. Science 2024;383:eadf2341. https://doi.org/10.1126/science.adf2341.
27. Escudero L, Martínez-Ricarte F, Seoane J. ctDNA-Based Liquid Biopsy of Cerebrospinal Fluid in Brain Cancer. Cancers (Basel) 2021;13:1989. https://doi.org/10.3390/cancers13091989.
28. Robertson J, Salm M, Dangl M. Adoptive cell therapy with tumour-infiltrating lymphocytes: the emerging importance of clonal neoantigen targets for next-generation products in non-small cell lung cancer. Immunooncol Technol 2019;3:1–7. https://doi.org/10.1016/j.iotech.2019.09.003.
29. Bubeník J. Tumour MHC class I downregulation and immunotherapy (Review). Oncol Rep 2003;10:2005–8.
30. Renteln MA. Promoting Oncolytic Vector Replication with Switches that Detect Ubiquitous Mutations. CCTR 2024;20:40–52. https://doi.org/10.2174/1573394719666230502110244.
31. Adamala KP, Martin-Alarcon DA, Boyden ES. Programmable RNA-binding protein composed of repeats of a single modular unit. Proceedings of the National Academy of Sciences 2016;201519368; doi: 10.1073/pnas.1519368113.
32. Kim SJ, Kim JH, Yang B, et al. Specific and Efficient Regression of Cancers Harboring KRAS Mutation by Targeted RNA Replacement. Molecular Therapy 2017;25(2):356–367; doi: 10.1016/j.ymthe.2016.11.005.
33. Azhar Mohd, Phutela R, Kumar M, et al. Rapid and accurate nucleobase detection using FnCas9 and its application in COVID-19 diagnosis. Biosensors and Bioelectronics 2021;183:113207; doi: 10.1016/j.bios.2021.113207.
34. Langan RA, Boyken SE, Ng AH, et al. De novo design of bioactive protein switches. Nature 2019;572(7768):205–210; doi: 10.1038/s41586-019-1432-8.
35. Qian Y, Li J, Zhao S, et al. Programmable RNA sensing for cell monitoring and manipulation. Nature 2022;610(7933):713–721; doi: 10.1038/s41586-022-05280-1.
36. Brink KR, Hunt MG, Mu AM, et al. An E. coli display method for characterization of peptide-sensor kinase interactions. Nat Chem Biol 2023;19(4):451–459; doi: 10.1038/s41589-022-01207-z.
37. Sørensen OE, Follin P, Johnsen AH, et al. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. Blood 2001;97(12):3951–3959; doi: 10.1182/blood.v97.12.3951.
38. McKee TD, Grandi P, Mok W, et al. Degradation of Fibrillar Collagen in a Human Melanoma Xenograft Improves the Efficacy of an Oncolytic Herpes Simplex Virus Vector. Cancer Research 2006;66(5):2509–2513; doi: 10.1158/0008-5472.CAN-05-2242.
39. Rauschhuber C, Mueck-Haeusl M, Zhang W, et al. RNAi suppressor P19 can be broadly exploited for enhanced adenovirus replication and microRNA knockdown experiments. Sci Rep 2013;3:1363; doi: 10.1038/srep01363.
40. Toesca IJ, French CT, Miller JF. The Type VI Secretion System Spike Protein VgrG5 Mediates Membrane Fusion during Intercellular Spread by Pseudomallei Group Burkholderia Species. Infection and Immunity 2014;82(4):1436–1444; doi: 10.1128/iai.01367-13.
41. Sette P, Amankulor N, Li A, et al. GBM-Targeted oHSV Armed with Matrix Metalloproteinase 9 Enhances Anti-tumor Activity and Animal Survival. Molecular Therapy - Oncolytics 2019;15:214–222; doi: 10.1016/j.omto.2019.10.005.
42. Huang H, Liu Y, Liao W, Cao Y, Liu Q, Guo Y, et al. Oncolytic adenovirus programmed by synthetic gene circuit for cancer immunotherapy. Nat Commun 2019;10:4801. https://doi.org/10.1038/s41467-019-12794-2.
43. Varshavsky A. Targeting the absence: homozygous DNA deletions as immutable signposts for cancer therapy. Proc Natl Acad Sci USA 2007;104(38):14935–14940; doi: 10.1073/pnas.0706546104.
44. Slomovic S, Collins JJ. DNA sense-and-respond protein modules for mammalian cells. Nature Methods 2015;12(11):1085–1090; doi: 10.1038/nmeth.3585.
45. Fink T, Lonzarić J, Praznik A, Plaper T, Merljak E, Leben K, et al. Design of fast proteolysis-based signaling and logic circuits in mammalian cells. Nat Chem Biol 2019;15:115–22. https://doi.org/10.1038/s41589-018-0181-6.

46. Toso JF, Gill VJ, Hwu P, Marincola FM, Restifo NP, Schwartzentruber DJ, et al. Phase I Study of the Intravenous Administration of Attenuated Salmonella typhimurium to Patients With Metastatic Melanoma. J Clin Oncol 2002;20:142–52.
47. Heimann DM, Rosenberg SA. Continuous Intravenous Administration of Live Genetically Modified Salmonella Typhimurium in Patients With Metastatic Melanoma. J Immunother 2003;26:179–80.
48. Le DT, Picozzi VJ, Ko AH, Wainberg ZA, Kindler H, Wang-Gillam A, et al. Results from a Phase IIb, Randomized, Multicenter Study of GVAX Pancreas and CRS-207 Compared with Chemotherapy in Adults with Previously Treated Metastatic Pancreatic Adenocarcinoma (ECLIPSE Study). Clin Cancer Res 2019;25:5493–502. https://doi.org/10.1158/1078-0432.CCR-18-2992.
49. Duong MT-Q, Qin Y, You S-H, Min J-J. Bacteria-cancer interactions: bacteria-based cancer therapy. Exp Mol Med 2019;51:1–15. https://doi.org/10.1038/s12276-019-0297-0.
50. Sun R, Liu M, Lu J, Chu B, Yang Y, Song B, et al. Bacteria loaded with glucose polymer and photosensitive ICG silicon-nanoparticles for glioblastoma photothermal immunotherapy. Nat Commun 2022;13:5127. https://doi.org/10.1038/s41467-022-32837-5.
51. Mi Z, Yao Q, Qi Y, Zheng J, Liu J, Liu Z, et al. *Salmonella*-mediated blood–brain barrier penetration, tumor homing and tumor microenvironment regulation for enhanced chemo/bacterial glioma therapy. Acta Pharmaceutica Sinica B 2023;13:819–33. https://doi.org/10.1016/j.apsb.2022.09.016.
52. Raman V, Van Dessel N, Hall CL, Wetherby VE, Whitney SA, Kolewe EL, et al. Intracellular delivery of protein drugs with an autonomously lysing bacterial system reduces tumor growth and metastases. Nat Commun 2021;12:6116. https://doi.org/10.1038/s41467-021-26367-9.
53. Ding Y-D, Shu L-Z, He R-S, Chen K-Y, Deng Y-J, Zhou Z-B, et al. Listeria monocytogenes: a promising vector for tumor immunotherapy. Front Immunol 2023;14:1278011. https://doi.org/10.3389/fimmu.2023.1278011.
54. Shipley A, Frampton G, Davies BW, Umlauf BJ. Generating Shigella that internalize into glioblastoma cells. Front Oncol 2023;13. https://doi.org/10.3389/fonc.2023.1229747.
55. Xu J, Yang S, Yang L. Vibrio natriegens as a host for rapid biotechnology. Trends Biotechnol 2022;40:381–4. https://doi.org/10.1016/j.tibtech.2021.10.007.
56. Grillot-Courvalin C, Goussard S, Huetz F, Ojcius DM, Courvalin P. Functional gene transfer from intracellular bacteria to mammalian cells. Nat Biotechnol 1998;16:862–6. https://doi.org/10.1038/nbt0998-862.
57. Gäbelein CG, Reiter MA, Ernst C, Giger GH, Vorholt JA. Engineering Endosymbiotic Growth of E. coli in Mammalian Cells. ACS Synth Biol 2022;11:3388–96. https://doi.org/10.1021/acssynbio.2c00292.
58. Pfeifer E, Michniewski S, Gätgens C, Münch E, Müller F, Polen T, et al. Generation of a Prophage-Free Variant of the Fast-Growing Bacterium Vibrio natriegens. Appl Environ Microbiol 2019;85:e00853-19. https://doi.org/10.1128/AEM.00853-19.
59. Kamaraju K, Smith J, Wang J, Roy V, Sintim HO, Bentley WE, et al. Effects on membrane lateral pressure suggest permeation mechanisms for bacterial quorum signaling molecules. Biochemistry 2011;50:6983–93. https://doi.org/10.1021/bi200684z.
60. Bracha S, Hassi K, Ross PD, Cobb S, Sheiner L, Rechavi O. Engineering Brain Parasites for Intracellular Delivery of Therapeutic Proteins 2018:481192. https://doi.org/10.1101/481192.