

Hypothesis

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Targeting Both Driver and Passenger Clonal Mutations in Solid Tumors with Personalized Oncolytic Microbes

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Hypothesis

Targeting Both Driver and Passenger Clonal Mutations in Solid Tumors with Personalized Oncolytic Microbes

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Abstract

Immunotherapy has shown much promise for blood cancers, which may all be 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. For blood cancers, the entire white blood cell compartment can be eliminated and replenished by cells generated by pristine, edited hematopoietic stem cells. Obviously, solid organs cannot be eliminated in a similar fashion. Immunotherapy has still 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. However, I don't think we should fully rely on that possibility – or at least wait for it if we do not have to. I have written multiple pieces about targeting truncal, i.e., clonal, mutations in cancer as a means of curing it. This would be a treatment specific to each patient. However, in my earlier work, I misunderstood the quantities of clonal mutations in most solid tumors. Although there are only a handful of clonal 'driver' mutations in most solid tumor patients, there are also at least a handful of clonal 'passenger' mutations. While traditional therapies cannot effectively target passenger mutations, directly detecting mutated transcripts allows for them to become targets.

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-toxicity[1]) back into him or herself if the individual were to get 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've thought for over a decade about how we could cure solid tumors. Many years ago, Dr. Aubrey de Grey sent me Dr. Alexander Varshavsky's paper about "targeting the absence"[3]. It detailed a very exciting approach wherein homozygous deletions in cancer were used as the basis for treatment, representing 0 vs. 1 in Boolean logic. The problem with the approach is that not all the cancer cells may have a given homozygous deletion, i.e., it may be subclonal. His approach was called "deletion-specific targeting".

However, this introduced me to the concept of detecting mutations in cancer directly with molecular "switches". I then sought to understand if the most early occurring mutations in solid tumors are generally maintained throughout development of the primary tumor and any metastases. It seemed as though that was often the case.

I came up with an approach called “Oncolytic Vector Efficient Replication Contingent on Omnipresent Mutation Engagement” (OVERCOME)[4–6]. It focuses on truncal, or clonal, mutations as the basis of treatment. Unfortunately, it is not guaranteed that every one of the patient’s cancer cells will contain the targeted mutation. I formerly believed that there were only a handful of clonal mutations in each patient’s solid tumor(s). Multiplexing seemed like the best option, but it seemed as though sometimes there would not be any clonal mutations in a patient’s solid tumors. Technically, one could target a set of subclonal mutations that together were predicted to cover all of a patient’s solid tumor cells. But that was not ideal.

However, I recently realized that I was simply looking at clonal ‘driver’ mutations, not mutations in general – which includes ‘passenger’ mutations. Some tumor types may feature more clonal drivers than passengers, but there would likely still be some in most cases[7]. Passenger mutations may not be targetable by traditional therapies, but they are via direct mutation detection. When taking both types of clonal mutations into account, it seems as though the vast majority of solid tumor patients have at least 5-10 clonal mutations in their cancer[8–18]. After multi-region or blood sample sequencing[19,20], multiplexed detection of all the possible clonal mutations – perhaps ideally scattered in different areas of the genome – could be sufficient to essentially ensure that all of a patient’s solid tumor cells are eliminated.

Anti-Cancer Bacterial Microbots:

Thus, we could theoretically program a facultative intracellular bacterium like *Listeria monocytogenes* or *Salmonella* Typhimurium to export multiplexed Craspase and trigger transient replication and hyper-virulence when it detects one or more clonal mutations. A facultative intracellular bacterium could possibly be developed that can enter any given human cell based on adhesins and invasins[21] that bind to ubiquitously expressed cell surface proteins and search for the patient’s clonal mutations, wherein transient attenuation reversal was made dependent on the detection of at least one of those mutations. Multiplexed 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. In this way, solid tumors could theoretically be cured.

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 Zea or Eno[22,23]. Otherwise, asymmetric division and lysis of one of the progeny cells is also a possibility to release a multitude of guide RNAs[24]. 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[25]. Finally, a toxin with a bystander effect could be released once the bacteria reach quorum sensing levels[26] or after small molecule administration.

Conclusion:

While this strategy 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.

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