

Hypothesis

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Hypothesis

Targeting 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 treatable or curable soon, especially if hematopoietic stem cells (HSCs) are harvested and frozen ahead of time for each individual. Alternatively, if sufficient numbers of HSCs can be produced from induced pluripotent stem cells derived from solid cell types, freezing cells ahead of time would not be necessary. Unfortunately, solid tumors are still extremely difficult to treat. Immunotherapy has helped in some instances for solid tumors, *e.g.*, melanoma - and immunotherapy may eventually be able to cure all solid tumors for reasons that are somewhat unclear currently. However, there may be a more direct way to treat solid tumors. 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. There are thousands of clonal point mutations on average in a solid tumor patient's cancer. This may essentially ensure that all of a solid tumor patient's cancer cells could be targeted and eliminated.

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

1. 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 (*e.g.*, obese), it can strike. It also affects the elderly more frequently.

Currently, it would make sense for each person to freeze some of his or her hematopoietic stem cells (HSCs) at an early age, *i.e.*, before any disease. That way, each individual would have autologous HSCs to transplant (non-toxicallyⁱ) back into him or herself in the event of blood cancer later in life. 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 compartmentⁱⁱ. In the future, HSCs could even be produced by induced pluripotent stem cells derived from solid cell types, negating the need for freezing cells ahead of timeⁱⁱⁱ.

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)^{iv,v,vi}. It focuses on truncal, or clonal, mutations as the basis of treatment, and is geared toward solid tumors. 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. 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^{vii,viii,ix}. A facultative intracellular bacterium could possibly be developed that can enter any given human cell based on adhesins and invasins^x 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 approach would allow for 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.

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^{xi,xii,xiii,xiv,xv,xvi,xvii,xviii,xix,xx,xxi}. Passenger^{xxii} 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 point mutations alone in their cancer^{xxiii,xxiv,xxv}. After multi-region or blood sample sequencing^{xxvi,xxvii}, multiplexed targeting of at least several of a patient’s clonal mutations could be sufficient to essentially ensure that the patient’s solid tumor or tumors are eliminated.

The probability that ~5,000 clonal point mutations distributed throughout the genome would be lost in many of the progeny cancer cells is very low, even in advanced cancer cells.

2. 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 Zea or Eno^{xxviii,xxix}. Otherwise, asymmetric division and lysis of one of the progeny cells is also a possibility to release a multitude of guide RNAs^{xxx}. 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 could potentially 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^{xxxi}. Finally, a toxin with a bystander effect could be released once the bacteria reach quorum sensing levels^{xxxii} or after small molecule administration.

3. Conclusions

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.

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