Review

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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Abstract: Over the last several years, large-scale cancer genomics studies involving multi region, multi-sample sequencing indicate that most or at least many cancer patients may have one or more "clonal" mutations in their tumors. Clonal mutations are those that are present in all of a patient's cancer cells. They can be identified via multiregion, multisample biopsies—or circulating tumor cells/cell-free DNA. Achilles Therapeutics is currently the only company targeting patient-specific, clonal mutations. They have opted to utilize an immunotherapy approach. However, I recently devised another approach for exploiting clonal mutations called "Oncolytic Vector Replication Contingent on Omnipresent Mutation Engagement" (OVERCOME). It is based on the identification of patient-specific, clonal mutations and targeting them using a bioengineered facultative intracellular bacterium. It would be initially non-replicating, but transiently regain the ability to replicate (and also transiently become hyper-virulent) upon mutation detection via molecular switches.

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 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 oncolytic bacterial vector that is used to treat (early-stage) non-muscle invasive bladder cancer, Bacillus Calmette–Guérin (BCG) [12]. It is an attenuated strain

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of *Mycobacterium*. As with the aforementioned oncolytic viruses, BCG also appears to mainly stimulate an immune response against the tumors that it is injected into [13].

Regardless, in most instances, the aforementioned therapies for solid tumors are not curative. That is largely because the solid tumors cannot be targeted with sufficient specificity over normal tissue. However, through the work of many dedicated cancer researchers over the last decade or so, it has started to become clear that many patients may have one or more clonal mutations in their tumors [14–19].

Clonal mutations are those that occurred earliest in a given patient's primary tumor and are retained in all sequenced regions of the primary tumor and any metastases. Additionally, the remainder of patients may at least have a small set of subclonal mutations that together cover all sequenced regions of their tumor or tumors. In order to identify a patient's mutational signature, multiregion, multisample sequencing can be employed. Unfortunately, tumors in certain anatomical regions are not easy to biopsy - especially in a multiregion fashion. A less invasive option would be for clinicians to analyze circulating tumor cells (CTCs) or circulating cell-free tumor DNA (ctDNA) instead as a means of identifying clonal mutations [20–25]. Of note, a software tool was recently developed that streamlines the mutational analysis of ctDNA [xxii], as well as a method for enhancing the yield of ctDNA from the blood [xxiv].

Although we have the ability to determine clonal mutations through various methods, targeting these mutations is not very facile at present. Dr. Charles Swanton, the eminent cancer genomics researcher, has done pioneering work in determining the evolutionary trajectory of human tumors, especially in the context of lung malignancies [xvii, xviii, xix, xxiii]. His work on the TracerX project helped establish the concept of clonal mutations being present in most (or at least many) patients' cancers.

Dr. Swanton co-founded a company called Achilles Therapeutics, which has already begun to target clonal mutations using immunotherapy. Unfortunately, at least 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 [26]. 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.

I propose that we instead utilize an intracellular vector to target clonal mutations. If the replication of an oncolytic virus were made dependent on or enhanced by clonal mutation detection, it could specifically replicate within and autonomously spread throughout a patient tumor or tumors. The same is true for an intracellular bacterial vector, although restoration of its replication potential should be transient. In either case, hyper-virulence could also be induced by clonal mutation detection.

I call this strategy "Oncolytic Vector Efficient Replication Contingent on Omnipresent Mutation Engagement" (OVERCOME) [27].

Overcome

While it is true that an oncolytic virus such as a herpesvirus could be of use to detect patient-specific clonal mutations, viral vectors have limited packaging space. It has been said that half of the HSV-1 genes are non-essential, but in reality that does not seem to be the case. The reason for this is that individually the genes may be non-essential, but when deleted in combination, it can lead to abortive infections [28]. Additionally, some genes that are deemed non-essential may have immunosuppressive effects that are required for productive *in vivo* infections.

In contrast, the vaccinia virus can package up to 25 kb of extra DNA while still maintaining replication potential *in vitro* [29]. *In vivo*, however, this number may be much smaller; too much extra DNA may interfere with cellular infection. Additionally, it is somewhat unclear if RNA molecules or proteins large enough to allow for clonal mutation-dependent viral replication can readily escape the viral factory (and if necessary, re-enter the factory).

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Given the essentially unlimited packaging space of bacteria, a facultative intracellular bacterium may be the best oncolytic vector in this context. Facultative intracellular bacteria, at least Gram-negative vectors with an *msbB* mutation, can be intravenously injected with minimal side effects [30–33]. Moreover, bacteria 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 [34,35].

The three facultative intracellular bacterial species that are best studied in the context of cancer are *Salmonella* Typhimurium [36], *Listeria monocytogenes* [37], and *Shigella flexneri* [38]. I suggested the possible use of *Vibrio natriegens* as a vector in this context because of its rapid replication rate, but it does not seem to survive in the cytoplasm of human cells [39]. Additionally, for neuron-based cancer, at least, *Toxoplasma gondii* could eventually be helpful [40].

Importantly, a bacterial vector could secrete a multitude of TALE-activators or zinc finger protein (ZFP)-activators that target the promoters of mutant genes to essentially ensure they are sufficiently upregulated. However, these proteins would also be secreted in infected noncancerous cells, which might be problematic.

Thus, in addition to switches that target the mutated part of the unregulated transcript or protein, it might be ideal to also express switches that detect it at one or more non-mutated sites. When the latter switches activate, further secretion of the TALE- or ZFP-activators would be halted.

Furthermore, clonally mutated intergenic regions can also theoretically be targeted by DNA-binding proteins. However, because the DNA is being targeted, an enzymatic cascade may be required for sufficiently rapid amplification of the mutation "signal" [41]. Such a cascade might increase vector off-target activity, i.e., replication within noncancerous cells, though. 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.

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 cover all sequenced regions of their tumor or tumors 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. Additionally, some blood cancers may recur when treated with CAR T-cells [42]. Perhaps future cell-based immunotherapies can prevent cancer cell escape variants by expressing a multitude of orthogonal cell surface receptors that bind different ubiquitous cell surface proteins of the given white blood cell type [43].

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