Editorial



# A personally tailored RNA cancer immunotherapy

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Modern medicine's scientific basis necessarily requires its practitioners to diagnose and treat based on a pathology's similarities rather than its differences. For a diagnosis of liver cancer, for example, the standard of care has been to treat with surgical or chemotherapeutic approaches for that tumor type. When such standard approaches fail, it is reasonable to ascribe the poor outcome to tumor- and/or patient-specific differences. In the last few decades, genetic, biochemical and informatic data have shown the diversity of cancer types suggesting new paths to treat and perhaps one day cure many cancers.

Let us imagine a forest that contains many trees. Each tree co-exists with its neighbors that collectively share resources, send short- and long-distance signals when danger arises, and even share nutrients with neighboring trees using mycelial networks (1,2). Now imagine one tree begins to get sick. It may be hard to discern what is wrong, and it tries to recover, but inevitably needs to signal its fellow trees that they are in danger. The other trees in response might change some of their biological processes, but it may or may not be enough to curb the danger. Luckily, since this forest is in a protected park, an expert field forest ecologist/ranger, identifies the warning signs, the sawdust-like filings and holes bored into the trunk, that this tree is harboring parasitic beetles. So, to save the rest of the trees, this tree is felled, and its contaminated trunk removed from the forest.

What if a steward of the forest took the beetle's discovery in an inflammatory way? They could have started a fire in response to the beetle's presence, wiping out huge swaths of trees. Replace forest with human, beetle with the insults that may cause oncogenic mutations, forest ecologist with the adaptive immune response, and fire as an indiscriminate chemotherapy, and, well… you get the point. To further the analogy, what if we could train more forest ecologists/rangers in the forest to recognize the sawdust signs of this beetle, or any parasite, early on?

While this metaphor is not support for felling of trees, its point is that we are starting to peer inside cells specifically using the cell's endogenous peptide "sawdust", to look for the molecular roots of cancer, potentially to prevent these malevolent diseases from occurring and halting metastases by combinatorically and rationally designing therapies using all the molecular and physiological information available for each patient.

In this study, Sahin *et al.* (3) design injectable mRNAs which are targeted to dendritic cells for expression and presentation on HLA Class I and HLA Class II complexes; the hope is that this will immunize one's T cells to attack the patient's specific form of melanoma. The patients were in stage III or stage IV of a malignant melanoma and received eight inguinal lymph node injections of the mRNA over the course of 43 days (with some patients continuing the treatment under discretion of the investigator up to 20 vaccine doses). Each of these patients was given mRNAs designed to be "personalized" for their specific form of melanoma.

One of the keys to the success and cost effectiveness of these personalized therapies is the dramatic decrease in the cost of sequencing (sequencing of a single human genome cost ~$2.7 billion US collectively for the human genome project in 2002, versus less than $1,000 per genome in 2017) and the rapid speed at which this data can be generated (hours) and analyzed (hours to a few days). Indeed, this dramatic decrease in cost and time was key

to the study's approach in which sequencing of each patient's tumors played the major role in developing the therapy. Neo-antigens—mutated protein epitopes which are displayed by MHC Class I complexes that arise from single nucleotide polymorphisms of the cancer cells—were discovered by both the DNA sequencing of a tumor biopsy and the frequency of their occurrence measured by mRNA expression level using RNA-Seq. Ten of these neo-antigens were selected for each patient (except for one patient where only five neo-antigens were selected) based primarily on two criteria: (I) predicted affinity of the antigen for the HLA class I and class II complexes; and (II) the high expression of the mutation-encoding RNA based on RNA-seq data. The authors synthesized RNA encoding five 27mer peptides. Each peptide was based on a region of the neo-antigen where the non-synonymous mutation would be present at the 14$^{th}$ position. Glycine-serine linkers flanked these peptide-encoding regions, and the entire construct was further enclosed by sequences on each end for increasing the likelihood of these peptides being routed to HLA class I and II display pathways. Finally, sequences for stabilizing the RNA at the 3' end were added in the plasmid. RNA was *in vitro* transcribed in the presence of a 5' cap analogue, -S-ACA(D1), then purified for delivery. Patients received injections in an inguinal lymph node with ultrasound guidance. In the end, each patient received two RNAs with five of the selected neo-antigens on each.

The thirteen patients in the study had non-synonymous mutations discovered by exome and RNA sequencing of tumors with comparison to healthy blood cells. The response to these neo-antigen determinants was measured by blood sample before and after vaccination. Of the 125 epitopes delivered representing the sequenced neo-antigens 60% resulted in measurable CD4+ and CD8+ T cell responses. Of those responses, 57% of the epitopes triggered T helper (CD4+) cell proliferation only, 17% gave CD8+ responses and 25% were of both types of T cells.

These presentations could be optimized/adjusted as an increased understanding of the variety of HLA class I and class II complexes comes to light. Specifically, if we understand the relative binding affinities of each patient's receptors better, scientists and medical practitioners can get a better handle on designing optimum epitopes and routing sequences. This will be key to improving the cancer immunotherapy toolset as it expands in the future to be better optimized for each individual patient.

To probe for T cell receptor function, T cell receptors were sequenced and cloned and then transfected into naive T cells *in vitro*. A K562 cell line expressing patient-specific HLA alleles was used to study *in vitro* CD8 target specific interactions. These interactions were also probed via ELISpot (IFN-gamma) and multimer staining. For 13 of the measured T Cell receptor reactivities, minimal sequences spanning the 27mer peptides containing the single amino acid mutations were identified. Further, for two of the patients, eight TCRs from CD4+ and CD8+ cells were cloned and blood from these patients were deep sequenced before and after the vaccine was delivered, and these TCR sequences were not detectable in the pre-vaccinated blood, but measurably present 50 days post-vaccination.

Following the process of neo-antigen discovery and construction of the personalized RNA therapy, the median time to start of the vaccine delivery was 103 days. Of the thirteen patients treated, eight had no radiologically detectable tumors at the beginning of the vaccine treatments, had measurable immune responses and showed no signs of tumors in the follow-up period of 12–23 months. The remaining five patients upon being accepted into the treatment program, relapsed prior to the vaccine administration. Two of these tumor-bearing patients exhibited a marked response to the vaccine treatments. Unfortunately, one of these patients, believed to be tumor-free based on MRI scans before the first administration of the vaccine, was scanned again following initiation of vaccine treatment and new metastases were discovered. Why the failure? The researchers measured a lack of HLA class I function from the melanoma cells isolated from a resectate of this patient. Furthering on this, they did detect transcripts of the HLA class I protein but not of 2-microglobulin, the co-factor that dimerizes with HLA class I molecule to form a complete HLA class I complex, suggesting a reason for resistance to the treatment because of the lack of functional HLA class I complexes in these melanoma cells.

A small human trial published concomitantly in *Nature* followed a similar strategy (4). The researchers immunized melanoma patients with patient-derived neo-antigen epitopes in peptide form; this treatment offers another path forward for future combinatorial cancer immunotherapies, and potentially these two treatments could have use cases where the combination of the two may be advantageous. As the RNA mutanome trial, in its near future, expands the number of patients, the effectiveness can be furthered quantified, and improved. There is a large parameter space available to optimize these RNA therapies. First, the RNA sequence could be further improved, adding in sequences that might abet

 

peptide presentation, translational efficiency, or stability. Second, further understanding optimum peptide sequences for binding to the broad type distribution of HLA class I and class II complexes will help better select epitopes for design. Third, given the rapidly advancing understanding of the evolutionary trajectories of cancer, scientists and clinicians could update the RNA vaccine epitopes as better targets emerge and/or others desist in frequency in a patient's tumors, involving multiple sequencing measurements through time. Fourth, optimizing delivery may be improved with wrapping the RNA with lipids, complexation agents or virus like particles. Fifth, as the cost of sequencing continues to decrease, with some claims of reagent prices even approaching $100 for whole genome sequencing in the next few years, we expect this kind of treatment and RNA vaccine development to become more popular and easily adoptable by medical practitioners and insurance providers. Finally, by combining these treatments with orthogonal strategies, including PD-1/PD-L1 effectors (5,6) that do not depend on functional HLA class I or class II display to be efficacious, we expect to avoid some of the resistance mechanisms that arose during this work.

Can we train our immune cells to better recognize our specific forms of cancer? The answer seems to be yes. Like the intensive training of a forest ecologist, our cells can be trained to assimilate knowledge that humans have gained from the scientific information deluge of the past century, providing further hope and potential solutions to the many classes of cancer.

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