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Cancer vaccines
Are we there yet?

Michael G. Hanna, Jr

Vaccinogen Inc.; Frederick, MD USA

For nearly two decades there has been an abundance of research and clinical development programs underway to develop active specific immunotherapies, to educate the patient's immune response, specifically the T-cell immunity and memory, to recognize and destroy tumor cells by cell-mediated cellular toxicity. While many of these technology platforms achieved promising results in preclinical and clinical phase I and II clinical trials, essentially all but one have failed to achieve FDA market approval as a therapeutic drug product.

This special focus series of commentaries is intended to evaluate the technological and developmental issues of active cancer immunotherapies and will examine and possibly help interpret the enormous number of failures over the last decade, given that there has been only one regulatory success (Dendreon's Provenge for prostate cancer). Many have questioned the value of this product with a minor clinical benefit and a low pharmacoeconomic ratio.

The introductory commentary of this series explores the universe of cancer immunotherapeutics using cancer vaccines and demonstrates that the majority of the technology platforms were conducted in advanced disease, namely patients with solid tumors.[1] These procedures used either autologous/patient-specific vaccines or allogeneic, off-the-shelf antigens. In the former, 3 of the 31 programs are in phase III trials with one approval. In the latter group of the 23 programs, 6 are in phase III clinical trials. The major disease focuses have been on Melanoma and Prostate cancers. Only one advanced development program survives for autologous stage II colon cancer, an adjuvant therapy in an unmet medical need.

In 2007 a major review of active immunotherapies, so called "cancer vaccines," (as distinguished from passive immunotherapy with Monoclonal Antibodies) and the various scientific and business factors that have contributed to the disappointing results in this biotechnology field was presented by Finke et al.[2] The information was based on a review organized by the Sabin Vaccine Institute's Cancer Vaccine Consortium. The review consisted of 9 case studies. The failure of these 9 candidate therapeutics to meet their defined clinical study objectives was attributed to a variety of scientific, clinical and business factors.

Recognizing that the data in this review are over 6 y old, it is interesting that one of the first general considerations was "Select the most informative targets." They point out that ideally the targets should be tumor-specific, and that "it is important to use the intended study population to assess the proportion of tumors that express the target and the proportion of cells within each tumor that express it." This clearly indicates that the review focused on **antigen discovery** and was emphasizing the use of common antigens and presumably was based on the assumption of inter- and intra-tumor **homogeneity**.

According to the first two commentaries of our series, Fidler[3] and Cusnir[4] point out that this is a mistaken assumption and directive, and that this probably was the weakest underlying biologic premise of the past two decades. Cancer is a genetic disease; the genetic sequencing data of tumor cells over the last few years, based on second-generation DNA sequencing technology, clearly reveals that there has been an underestimation of the degree of **heterogeneity** of tumors and tumor cells and their surface antigens. This includes heterogeneity among tumors and within tumors of the same classification and pathological stage. This diversity of tumor cells certainly will affect the immunology of cancer vaccines since antigen discovery must include the products of mutated genes within the cells and the shared mutated genes among the tumor cells.

Fidler states that the major obstacle for the eradication of metastases is the biologic heterogeneity of tumor cells that constitute primary cancers and metastases. Specifically, by the time of diagnosis, malignant neoplasms contain multiple cell populations with diverse biological heterogeneity in growth rate, karyotype, cell surface receptors, antigenicity, immunogenicity, marker enzymes, gene expression, sensitivity to different cytotoxic drugs, invasion, and metastasis. He further states "the implications of tumor cell diversity for the outcome of treatment of cancer metastasis cannot be overstated. The heterogeneous nature of the response of malignant tumor cell subpopulations to cytotoxic drugs and other therapeutic modalities makes it unlikely that a single treatment regimen will be able to kill all the cells in a tumor." In other words, you cannot treat a heterogeneous disease with a homogeneous treatment unless the homogeneous treatment itself is highly polyvalent.

The genomic validation of intratumor heterogeneity was presented by Gerlinger and colleagues[5] this year. They obtained tumor samples from four patients with renal-cell cancer before and after treatment and took multiple samples from each patient's primary and metastatic tumor sites. About two thirds of the mutations that were found in single biopsies were not uniformly detectable throughout all the sampled regions of the same patient's tumor. It is interesting to note that there was a 25-y span

*Correspondence to: Michael G. Hanna Jr; Email: mghannajr@vaccinogeninc.com
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between Fidler's original publications describing intratumor heterogeneity for the metastatic phenotype of transplanted tumors and these genomic studies of Gerlinger.

Cusnir elucidates the now-recognized aspect of intertumor heterogeneity and the impact on tumor biology, diagnostics and therapy. This article teaches that is not unthinkable that in the near future, besides just dividing tumors by their organ of origin and then proceeding with staging, tumors will first be classified based on their genetic and antigenic markers—and then decide whether a traditional 4-stage system will even be needed, since tumors with very good prognosis might behave equally after a curative resection despite being a traditional stage I or III tumor. By the same token it is also within reach that treatments will be designed specifically for each patient based on their metabolism and tumor biology, allowing for truly personalized medicine.

To further expand on this topic of heterogeneity Wood et al.[6] asked the question "how many genes are mutated in a human tumor?." They analyzed this question in breast and colorectal cancers; they reported there are ~80 DNA mutations that alter amino acids in a typical cancer; thus these are all candidates for unique markers or tumor-specific antigens. Examining the overall distribution of these mutations in different cancers of the same type leads to a new view of cancer genome landscapes, namely they are composed of a handful of commonly mutated genes found in the majority of tumors "mountains," but are dominated by a much larger number of infrequently mutated genes "hills." This study included an analysis of the sequences of 20,857 transcripts from 18,191 human genes, including the great majority of those that encode proteins. The genes were sequenced in 11 breast and 11 colorectal cancers. Any gene that was mutated in the tumor but not in normal tissue from the same patient was analyzed in 24 additional tumors. Selected genes were further analyzed in another 96 colorectal cancers to better define their mutation frequency and aid subsequent bioinformatic analyses. Statistical analyses suggested that of the ~80 mutations in an individual tumor, < 15 were likely to be responsible for driving the initiation, progression, or maintenance of the tumor. Although the numbers of mutant genes in breast and colorectal cancers were similar, the particular genes that were mutated were quite different, as were the type of mutations found. The surprising finding that has an enormous impact on cancer vaccines, or specific immunotherapy, is that of the ~80 mutations only ~3 were common and thus would be shared antigens. Thus with polyvalent cancer vaccines, a robust and therapeutic immune response would not be provided by allogeneic cells or even a relatively minor component of "off the shelf" common antigens. Based on these results, the antigen discovery aspect of cancer vaccines takes on a whole new level of complexity and is fraught with new hurdles. Autologous tumor cell vaccines become a much more technologically and immunologically sound approach to cancer vaccines. This, for active immunotherapy cancer treatment advocates, is not all bad. The findings confirm that the genetic lesions that are unique to the original tumor cells, "the trunk of the evolutionally tree," are consistently expressed.[7]

The Finke paper from the 2006 Cancer Vaccine Consortium (CVC) along with the published "Guidance for Industry, Clinical Considerations for Therapeutic Cancer Vaccines," while failing to recognize tumor heterogeneity in their reports, did outline clinical trial design and manufacturing guidance, most of which would not be of value with allogeneic or nonfunctional tumor antigen vaccines. There were some points made in these reports as well as in our technology platforms commentaries that seem to have been relevant themes for the failed studies. These were:

1. **Flawed trial design**: Patients not stratified properly and the evidence for baseline shifts in the studied population. The underlying heterogeneity of many of the studied cancers and the types of patients selected may have masked efficacy in certain subgroups—the specific types of patients that showed promise in phase I/II studies. By using historical data, instead of randomized control data in phase II settings, to identify these groups, many of these trials experienced a baseline shift from the original trial design and were unable to achieve statistical significance for the enrolled cohort as a whole. The companies were unable to obtain the money to perform another trial to treat the specific subgroups that showed promise. Moreover, some of the subgroups were too small to gain statistical significance in a second trial, and so there was not a rationale for pursuing these a priori. This situation was demonstrated in the technical manuscript by Reitsma and Combest[8] with respect to the late stage development of Oncophage.

2. **Moving goal line**: Historical data didn't help in trial design when with later-stage disease, conventional treatments were improving throughout the duration of these trials, making it less probable for their drugs to show significant improvement over the control group. It is interesting that one of the primary questions that can be raised and has been answered is - in adjuvant studies where treatment follows curative surgery, have there been technological improvements in surgical procedures that have changed the baseline? The answer is no. While laparoscopic resections are being used more and more compared with open surgical procedures, Nelson and Sargent[9] published a comparative analysis showing no baseline changes, at least in colorectal cancer.

3. **Late-stage disease as a treatment group**: Financial decisions likely drove many of these companies to ignore occult or early-stage disease patients and take on late-stage disease as they could achieve events more quickly. They wanted the fastest return on investment that could meet their endpoint. But as a realistic issue, even large companies with a high level of resources would be unwilling to invest in a trial in early-stage disease that of its own nature would be of long duration, very expensive and speculative, relative to other R&D project opportunities readily available in a large company. This strategy goes against the tenets of vaccinology, and the briefer observation period did not allow enough time for the vaccine to work.

4. **Unrealistic efficacy expectations**: Overly aggressive projections of benefit compared with conventional drugs. Just because cancer vaccines are less toxic and better tolerated did not mean that they were magic. Some experts believe that all the failures have led to more realistic expectations on the part of the FDA for what might be achievable in a phase II or III clinical trial for a cancer vaccine.

5. **Prior evidence of clinical activity is a requirement for progressing to a phase III trial**: Lacking enough validation of the concept from basic research and early-phase clinical trials is a track toward failure.

6. **Relevant potency and identity quality control assays**: Not being capable of developing sufficient potency and identity assays of the final drug product to prove that the product as characterized was safe and correlates to clinical efficacy, is a problem in manufacturing quality control. While everyone agrees upon and understands potency and identity assays, the nature of some cancer vaccines can make it difficult to prove that the selected potency assay and identity assay are appropriate for the given product. As an example, a highly polyvalent product such as an allogeneic tumor cell vaccine may not lend itself easily to the definition of such assays. Furthermore, even if such assays are developed, setting accurate and meaningful control limits can be challenging.

7. **Pharmaceutical process alterations**: Using business decisions to modify the manufacturing process for economics and convenience have led many platforms to lose the essence of what worked originally in early studies with the product. However, the challenge is that early-stage processes are usually not scalable or robust enough to support commercial manufacturing and in the early stage of product development before clinical proof of principle, it is difficult to justify the resources necessary to have a scalable and robust process. On the other hand, when a manufacturing process is modified for commercial development, a company may lack the time and resources to perform a clinical bridging study prior to a phase III clinical trial in order to show that the early and commercial processes give the same product, and product assays might not be specific enough to detect differences in the products of these processes.

8. **Uncertainty of dosage level**: Testing in late-stage disease patients and not having enough clinical research to optimize the dose and regimen of the vaccine to achieve the most robust immune response has been a problem. The primary failing is that, unlike chemotherapeutic drugs, a tenant of biological therapy is "more is not always better and is often worse", given that dose-response curves are often bell-shaped in nature. Furthermore, even if such trials are performed, in lieu of knowledge of a surrogate assays for efficacy, one might not know which immunological readout to follow in order to determine dosage level. This situation was demonstrated in the technical manuscript by Reitsma and Combest[8] with respect to the late stage development of Oncophage.

Antigen Discovery

Now that we are aware of the genetic diversity of cancer with both intratumor and intertumor antigen heterogeneity, the past process of antigen discovery becomes a prime suspect for the failures of cancer vaccines. It is not enough to have a presumptive antigen but more relevant to have and use functional, tumor-specific antigens. It is understandable why none of the allogeneic tumor cell vaccines have provided significant clinical benefit. There is a paucity of functional tumor antigens that are universally expressed, strongly immunogenic, and cancer-specific, unless the antigen discovery involves autologous tumor cells. It is improbable to select any one of the 50 or so common marker proteins that may be expressed in some but not all tumors, or the rare possibly shared antigens. When evaluating all of the antigen discovery activity thus far, it is clear that there has been a paucity of shared tumor-associated antigens that would provide a homogeneous immunity to antigenically heterogeneous tumors. A further complication is that even if a cocktail of antigens were identified that could provide broad coverage, multiple companies might control the intellectual property associated with these antigens, and suitable business agreements can be hard to achieve, especially if more than two companies are involved. In addition, the development of a robust cytotoxic, cell-mediated immune response with any single protein that can successfully destroy antigenically polyvalent tumor cells is unlikely to achieve major clinical benefit.

Therefore, it is essential, with all that we have learned at both the tumor genomic and immunotherapeutic level, to reevaluate the antigen discovery process and the consideration of a universal or even comprehensive immunological target(s). Here we can offer several, albeit not exhaustive, considerations.

Autologous tumor cell vaccines. The use of the patient's own tumor to a robust tumor-specific immune response is the purest approach to deal with tumor cell antigen heterogeneity. The FDA recognizes autologous tumor cell vaccines in the Guidance for Industry: Clinical Considerations for Therapeutic Cancer Vaccines (in review, 2012).

Their guidance in this area was likely not prompted by the recent tumor cell genomic diversity data. Their guidance is more likely based on the use of dendritic cells which involves a process of expansion in cell culture, rather than using the patients' own tumor cells.

They point out the following: "Design of studies using autologous vaccine products that are derived from the patients' own tumors poses unique challenges and deserves some special considerations. Manufacturing such vaccines can take a considerable period of time and in some instances, may take up to several months. If complete remission or stable disease is eligibility criteria, the time required for manufacture may mean that, some trial subjects may not remain eligible because of disease recurrence or progression."

This is probably true if tissue culture is used to expand cells. However in our experience, the time required for dissociation of parenchymal stage II and III colon tumor cells, into a sterile live-cell drug product is ~6 h. The quality control takes 21 d, primarily to include the sterility test. The agency also recognizes this by recommending, "…consideration should be given to optimization of the vaccine manufacturing process prior to late phase clinical trials in an effort to increase the proportion of the patients who are randomized to the treatment arm and receive the active product." This is clearly good advice and should be used in any technology platform.

Nevertheless, the use of live, metabolically active, yet nontumorigenic. autologous tumor cells is a new paradigm in vaccine manufacturing and has more logistical and regulatory aspects than have been engaged in past processes. However, one has to

choose the route that complies with the available tumor biology and immunotherapeutic data. In our case in the beginning the logistics was a glass half empty. However, it has now become a glass half full and rising.

It was earlier noted that the only FDA approved cancer vaccine product is an autologous vaccine using expanded dendritic cells carrying a tumor-associated peptide PA2024, the recombinant fusion protein of human PAP with GM-CSF, prior to reinfusion to the metastatic castration-resistant prostate cancer patient. The drug "Provenge (Sipuleucel-T) provides patients approximately nine weeks in median overall survival improvement. The $93,000 per treatment cost makes for an unfavorable pharmacoeconomic analysis, $270,000 dollars per life-year gained. This is a simple reality for this particular product and its label.

As new autologous vaccine processes develop, the logistics will be improved and the cost/benefit ratio should also improve as seen with many new and developing drug products.

Cancer stem cell-like cells (CSC-like cells) and circulating tumor cells (CTC). Another source of antigenically responsible autologous tumor cells can be the circulating tumor cells (CTC), a distinct population of cancer cells found in circulation in blood. The CTCs can be considered as progenitors of relapse. Also, there is speculation that within the CTCs there are cancer stem cells (CSC), which have the ability to give rise to metastatic tumors. More and more data suggest that CSC play a significant role in tumor evasion of the several standard cancer treatment modalities. From an active specific immunotherapeutic point of view, the cancer stem cells should possess not only the variety of intertumor diversity but also the tumor-specific antigens, which would induce a broad tumor-specific immune response in the host. We could question why this does not happen naturally, the plausible reasons can be dosage, route of exposure to the immune system, requirement for a strong immunostimulant for homing of effector cells.

To provide more accurate evidence for the CSC within CTCs, several approaches will need to be mounted; these would be techniques based on cell culture, flow cytometry, and molecular-based techniques. The latter is based on the fact that the CSC phenotype is defined by multiple markers which would also include gene expression analysis. These studies are being applied to various solid tumors such as breast cancer, pancreatic cancer, lung, melanoma, sarcoma and other tumors (Tang).[10]

Finally, there are other considerations that need to be studied and correlated with the knowledge of heterogeneity in order to develop effective and beneficial cancer vaccines. These would involve understanding and extending the relationship of the MHC class 1 regulatory factors associated with cell-membrane-localized tumor antigens.

Conclusion

A superficial analysis of the cancer vaccine immunotherapy field, considering the enormous number of failures and the relatively minimal benefits of the one registered autologous product may seem to cast doubt on the future of this clinical approach to treating solid tumors. However, there is a light at the end of the tunnel. New sophisticated genomic studies have better characterized the molecular and genomic biology of tumor cells beyond the homogenous profile proffered by routine pathologic and histochemical classifications.

Understanding the genetic basis of antigenic heterogeneity is a very important realization to immunologists. No longer can we expect to treat a heterogeneous disease with homogeneous therapies. Clearly patient-specific therapies such as immunotherapy need to be conducted with the relevant, functional antigens/targets. It is now recognized that the more relevant clinical setting for cancer vaccines is in adjuvant setting in occult-disease patients. There is a great deal of potential yet to be realized using relevant cancer vaccines alone and in combination therapies with non-immunosuppressive cytotoxic drug therapies.

It is a matter of fact that the greatest advances in the development of successful clinical treatments have been achieved based on a more precise understanding of the functional biologic basis of the particular disease. We have realized for over 30 y that cancer is a genetic disease and that malignancy, or unregulated growth, is a consequence of mutations. In fact the impetus for the search for "Oncogenes" was a natural consequence of this knowledge, and the failure to find such a universal cancer-specific gene(s) should have been an indication that there was greater diversity or heterogeneity than originally expected. The explosion of the DNA sequencing technology and implementation has certainly validated the current perception of tumor cell biology, as possessing both intertumor and intratumor heterogeneity. From an immunologic viewpoint, this makes the previous concepts of antigen discovery for cancer vaccines impractical and problematic, with the implication that one of the soundest approaches would employ autologous tumor cells. This new paradigm for patient-specific immunotherapy will be more complex, and while there will be greater benefit and safety, this approach may require a retrofitting that is at odds with current clinical oncology and pharmaceutical standards and methodologies. This, however, is the beginning of greater progress in the area of patient-specific, active immunotherapy.

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