

# Tumor cells - poacher turned gamekeeper?

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The oncologists' dream is to be able to destroy cancer cells using the body's own immune system. A number of strategies have been used in the development of cancer vaccines, and some have been more successful than others. Dalglish and Whelan review some of the problems associated with the creation of an active cancer vaccine, together with some of the novel therapies in development.

Creating a vaccine to target cancer is a huge challenge since a number of factors conspire against the immune system to prevent it mounting an effective response against cancer cells. Perhaps the most important of these is 'self-tolerance' where the immune system has to overcome an understandably inherent reticence to attacking the body's own cells.

Despite this reluctance to target 'self', anecdotal evidence going back over a century suggests that certain immunological stimuli can lead to tumor regression. This provides the rationale for attempting to use the immune system to overcome cancer. Vaccines derived from tumors, proteins and peptide epitopes have all been used to induce a clinical response. Encouraging early results, however, have unfortunately often been followed by disappointing clinical results, leading to a loss of faith in the approach.

Today, new insights into basic immunology and molecular oncology have generated a resurgence of interest. Big pharmaceutical and start-up biotech companies are both currently developing new approaches to the design of cancer vaccines.

## The story so far

Evidence from numerous, mostly non-randomized clinical trials that have used several different approaches to a variety of tumors, support the following conclusions:

- Small-volume disease is much more likely to benefit from vaccination than bulky end-

stage disease. Patients who produce a measurable immune response survive longer than those who do not, even if no clinical response is seen.

- Unlike vaccines designed to prevent infectious diseases, vaccines designed against cancer have to be given repeatedly, regardless of whether there is large volume or minimal residual disease.

- Tumor antigens are similar to 'self' and hence there is a need to break tolerance. One strategy is to employ powerful adjuvants, which induce a cell-mediated response, for example, as used in the BCG (*Bacille Calmette Guerin*) vaccine.

- Personalized vaccines (eg, based on autologous cell-based systems) are too impractical to use in large-scale studies.

## The use of adjuvants

It has been suggested for many years that it may be possible to use bacterial products to 'switch-on' the immune system. This concept has been refined as the eponymous 'danger theory', in which it is suggested that the immune system will only be activated by a threat. Bacterial and viral products represent such a threat and are ideal as adjuvants for use with specific antigens. Vaccination with non-specific stimulants such as the BCG tuberculosis vaccine, or its derivative, purified protein derivative (PPD), have both been reported to induce clinical responses in a restricted number of tumors, especially when injected directly into the tumor.

Many adjuvants are still derived from bacteria and viruses, for example *Mycobacterium vaccae* and CpG. A variety of non-microbial adjuvants also exist, including QS21, which is derived from tree bark and is exceptionally effective in driving antibody production. Interestingly, the only commercially licensed product is alum, an aluminium salt, which is thought to act as a reservoir for antigen release. It is especially effective in stimulating antibodies. For cancer therapies, it would appear that a Th1 cytokine environment would be most beneficial and any adjuvant that can achieve this may be highly appropriate for coupling with a cancer vaccine.

## Cancer antigens

Cancers are essentially 'self' cells that have by-passed normal homeostatic regulation mechanisms. Consequently, the immune system has difficulty differentiating them from non-malignant cells. Fortunately, there are a number of markers that may be employed to specifically identify, and attack, tumor cells. These may be roughly divided into four major classes:

- *Tumor specific antigens (TSA)* - a relatively small group of antigens exemplified by the cancer-testis antigens. These genes are completely silent in normal tissue, but are expressed by cancerous cells, so making them highly specific markers of disease. Examples include the MAGE antigens in melanoma.

- *Tumor associated antigens (TAA)* - largely differentiation antigens, expressed by normal cells but massively over-expressed in cancerous tissue. Many targets initially thought to be tumor-specific are widely spread over many tumors such as the gangliosides and mucin antigens.
- *Mutational antigen* - point mutations are common in many cancers. An example is the common mutation of the p53 oncogene.
- *Viral antigens* - some virus have been shown to be oncogenic and examples are the E6 and E7 oncogenes from human papilloma virus.

### The immune response to cancers

Empirical observation has shown that tumors often become infiltrated with cells, most notably, T-cells. Animal models have shown that immunodeficient mice have a higher propensity for tumor development and so it seems reasonable that the acquired immune system can play a role in the control of cancer. It is also true to say that the innate system may play its part since non-specific cells such as natural killers (NK), macrophages and eosinophils are often detected within tumors.

The picture is further complicated by the dendritic cell (DC), which seems to play a pivotal role in connecting the innate and adaptive immune responses. DCs are activated by a number of receptors for bacterial and viral antigens, which leads to their maturation into full antigen presenting cells (APC). Once they mature, DCs can then drive the adaptive immune response. It is clear that these cells play a major role in reconciling the innate killing response with the more specific adaptive response. DCs have the unique ability to 'cross-prime' which means that they can take antigen from an exogenous source and present it via MHC class I, rather than the more conventional class II pathway. This ability is key to the development of effective cancer vaccines since it allows class I restricted antigens to be administered and give rise to the CD8 cytotoxic T-lymphocytes (CTL) so often detected within tumors.



**Targeting drugs to specifically kill cancer cells has long been the dream of the oncologist. Are magic bullets really the answer? Perhaps the most promising therapy encompasses the use of cancer cells as vaccines - surely a case of poacher turned gamekeeper?**

The role of antibodies within cancer is still largely unknown, although it is clear that very large antibody responses are often detected. The antigen NY-ESO-1, for example, was detected by SEREX, a technique that involves screening an antigen library with patient serum. The perceived wisdom is that a Th1 immune response, exemplified by elevated interferon- $\gamma$  and interleukin-12 (IL-12) levels and promoting a cellular immune response, may be preferable to a Th2 background, with higher levels of IL-4 and IL-10, which is more permissive of antibody-mediated responses. It should be noted that this is

far from proven and may yet turn out to be incorrect. To date, no clinical trial has completely correlated cytokine profile with disease prognosis.

### Clinical trials

Current technologies in trial vary from basic non-specific stimulators designed to induce strong cell-mediated (Th1) responses such as *Mycobacterium vaccae*, through to high-tech derived peptide, dendritic cell, gene therapy (GT) and DNA vaccine approaches.

It is worth noting that some GT trials focus on a single antigen or peptide epitope in a pseudo-vaccination setting.

## The range of vaccines under study

- *Recombinant protein* - both TSA and TAA have been used as single protein vaccines. Proteins on their own are largely ineffective but, when administered with a potent adjuvant, can give rise to both cellular and antibody responses. Given the specificity of these markers, it is possible that potent anti-tumor responses will be generated.
- *Peptide vaccines* - a refinement of the above is to use individual epitopes from specific proteins. These short peptides are the specific sequences known to bind to particular HLA restricting elements. This type of vaccine is limited to patients expressing particular HLA genes, but has been refined to include several epitopes binding both HLA class I and class II alleles.
- *Dendritic cell therapy* - given the huge potential of dendritic cells to activate the immune system, several strategies to employ these cells have been attempted. A major hurdle to overcome is that reliable DC cell lines are rare and thus need to be made freshly for each patient. Considerable effort has been made in attempts to generate protocols for freezing DCs to avoid repeated venesection of the patient. DCs can be loaded in a variety of ways, including specific peptide epitopes, whole peptides, or even lysates of whole tumors. Given the high potency of DCs, relatively small numbers of cells are needed for each vaccination.
- *Antibody vaccines* - a number of interesting antibody approaches exist and can roughly be divided into three major areas: drug targeting, antibody-mediated lysis and anti-idiotypic vaccines.
  - (i) In drug targeting, antibodies to specific disease markers are conjugated to either a chemo- or radio-therapeutic agent so drugs are delivered directly to the cancerous tissue. This is possibly the closest approximation to the elusive 'magic bullet' sought for so long by oncologists.
  - (ii) The second approach is very similar. Rather than conjugating the antibodies it uses complement-fixing antibody isotypes. Again, targeting is specific and lysis is achieved by the activation of the complement pathway.
  - (iii) The third approach is considerably more complex, and relies on the subtle ability of antibodies to discern 3-dimensional structures. In conventional antibodies, the complementary determining region (CDR) mutates such that it becomes a perfect match to its potential target. However, antibodies are proteins and, hence, are themselves immunogenic. If another antibody is directed against the CDR, it follows that it will be a perfect copy of the original target. This approach is useful for generating immune responses to difficult-to-clone molecules, and has the further advantage of being able to break immune tolerance, thus overcoming the reticence of the immune system to attack 'self' molecules.
- *DNA vaccines* - the observation that sequences of naked DNA can be used to generate potent immune responses offers an interesting alternative therapy. Essentially a sequence of DNA encoding a specific tumor antigen is injected, usually intramuscularly, which is transcribed into protein and a powerful immune response generated. While this approach is simple and effective in the mouse, without a protein component, it is far from effective in the human setting.
- *Gene therapy* - at the most basic level, gene therapy is the replacement of a defective gene *in vivo*. Most gene therapy approaches in cancer are effectively high-tech vaccines with cytokines and co-stimulatory molecules transfected into cell lines or tumors in order to enhance their immunogenicity. While still in its infancy, this is an interesting approach and a number of examples exist.
- *Autologous vaccines* - this approach involves removing a tumor, *in vitro* manipulation of cancer cells, and the reinfusion of a non-proliferative vaccine derived from the original cancer.
- *Allogeneic vaccines* - a similar approach has been employed using tumors derived from HLA-mismatched individuals. This approach relies on the fact the cancers often share antigens and as the allogeneic tumor is rejected, the immune system is vaccinated against the shared antigens. This is attractive from a clinical perspective since large numbers of cells can be generated without recourse to the patient. The enhanced immunogenicity of a mismatched allogeneic cell means that unlike autologous cell vaccines, there is little benefit from transfecting cytokines into these cell lines.

There are also a considerable number of trials using both autologous and allogeneic tumor cells as vaccine on a single antigen or peptide epitope. Many of these studies use cells transfected with cytokines such as IL-2, IL-12, GM-CSF, or co-stimulators such as B7 to increase the potency of the vaccine.

Outcomes of large phase II studies using allogeneic cell lines to treat metastatic melanoma, pioneered by Dr Donald Morton at the John Wayne Cancer Institute, have been robust enough to lead to very large randomized double-blind trials. Unlike drugs, however, large vaccine trials may have more opportunities to fail and may explain the failure of other phase II studies to translate into successful phase III data. These include the inherent difficulty of quality control of large volume scale-up of biological materials, the need to get the optimum adjuvant for the vaccine (some successful phase II studies have a different adjuvant to the larger phase III studies), and the requirement for the multicenter trialists to appreciate the need to continue the vaccine course and not stop due to minor toxicity or disease progression. The route of administration, in addition, can be vital to efficacy with subcutaneous vaccination being no substitute for intradermal vaccination in the case of BCG or *Mycobacterium vaccae*.

### A whole cell approach

It is clear that that no one method has shown outstanding effectiveness. It may well be that this is because the major cancer-causing epitopes may change throughout the course of disease, thus rendering single proteins, peptides and epitopes ineffective. This leads us inexorably back to whole cell vaccines, which must contain the whole gamut of cancerous antigens. The drawback of autologous vaccination is that when a tumor is large enough to be excised and made into a vaccine, it may already be too late for the patient. This is further complicated by the time constraints of carrying out *in vitro* manipulation – making this approach inherently limited. A close approximation is to use allogeneic whole cells.

Allogeneic whole cells can be grown to large numbers and thus are a viable form of vaccine. It has been shown that tumors share antigens regardless of their MHC haplotypes. It is feasible to begin clinical trials with essentially an unlimited source of vaccine.

Initial studies carried out by Onyvx in a phase I trial were encouraging and have recently been expanded into a phase II trial using an allogeneic whole cell vaccine to prostate cancer. Interim data are expected in 2003.

### Poacher turned gamekeeper?

Immunological cancer vaccines have long been regarded as unpromising, but recent advances in basic immunology have prompted their re-examination. As a result, immunotherapy has grown rapidly and has achieved remarkable advances in a relatively short time. Cancer, however, comprises a diverse range of antigenic targets and a single antigen approach is unlikely to succeed. Possible solutions to this problem include revisiting the use of cancer cells as vaccines – surely a case of poacher turned gamekeeper?

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