Immunotherapies Towards Tumor Initiating Cells and Cancer Stem Cells

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Abstract: Active immunotherapy of cancer is a promising treatment modality by inducing long lived tumor reactive immune effector cells. Several vaccine trials have indicated that its effectiveness is probably best when the tumor burden is low. An attractive population of cells to target by this approach may be tumor initiating cells (TICs). Recent research suggests that such types of cells may be precursors to cancer possibly giving rise to metastatic disease. Although more work is being done to characterize appropriate phenotypic and functional markers, this population of cells would make an ideal target for active immunotherapy in high risk patients, so as to achieve remission over much longer periods of time. This review gives an overview of the potential of immunotherapy and how it may target TICs as a potential treatment option for cancer patients.

INTRODUCTION

Tumor immunology has developed into a distinct entity over the past 2 decades with several key laboratory vaccine concepts being tested as therapies in cancer patients. While several types of cancer vaccines have demonstrated successful eradication of tumors in preclinical models, this promise has not translated as effectively into the clinic. The immune system is believed to have evolved to recognize 'nonself' antigens present on invading pathogens. Cancers, on the other hand, arise from ‘self’ tissue and are not efficient in inducing a strong enough immune response to keep the tumor in check or eradicate them. However, it is well known that tumor immunity exists, perhaps from recognizing epitopes from ‘altered’ self proteins [1]. Examples of spontaneous regressions have been documented in some solid tumors [2] with infiltrating lymphocytes in the tumor bed of ovarian carcinomas correlating with improved prognosis [3]. These examples indicate the presence of immune reactive cells that can potentially reject tumors.

With the results from several pivotal cancer vaccine trials, it has become increasingly evident that a low tumor burden is an important point for vaccines to be effective. Tumor mediated immune suppressive mechanisms such as those seen due to TGF-β and T-reg cells would be low, providing an environment that could be conducive for optimal manipulation of the immune system. Another important aspect is the choice of relevant targets. These may be phenotypic or functional markers on cancer cells, precursors or tumor initiating cells. Among the choice of appropriate targets to treat or prevent a recurrence, an area that is increasingly gaining importance is tumor initiating cells (TICs). Several types of TICs have been described in various studies such as cancer stem cell (CSC) [4], side population and precursor cells, and mature cells [5, 6], which have undergone transformation.

CSCs have been hypothesized in several cancers including leukemias, breast, brain, colon, prostate, pancreas, and are thought to be important players in the incidence of metastases and recurrence of tumors in situ. They are thought to be a quiescent population of cells of up to about 0.01% of a tumor that possibly exhibit chemo- and radio-resistance [7]. In addition to this population, other types of cells such as dominant clones within the tumor may also participate in tumor progression and recurrence [8], and it is probable that most tumors may have combinations of both these cell types which participate in the manifestation of the cancer. Thus any therapy targeting these cells that can initiate a cancer may be an adjunct therapy that will be administered in addition to conventional and novel therapeutics targeting other facets of the tumor. It may also be used as a vaccine in the adjuvant setting to confer long term protection from a recurrence.

For the sake of simplicity in discussing the nature and potential of a population of cells that can give rise to cancer and serve as a potential target of immunotherapy, we will adhere to the term tumor initiating cells (TICs) from now on.

Targeted Immunotherapy of Cancers

The vast majority of chemotherapeutics is indiscriminate in their cytotoxic action on a dividing cell, affecting healthy dividing cells as well as cancer cells. However, as a result of an increasing understanding of how cancer cells work, a handful of new molecules on cancer cells were identified and targeted, so as to minimize unwanted side effects. In addition to existing targeted chemotherapies, a few biologics have also been developed. In passive immunotherapy, antibodies targeting specific cell surface markers on cancer cells or receptor ligands are administered (described below). The effect of the therapeutic is short lived, making repeated treatments necessary. In contrast, active immunotherapy or vaccines engage the immune system to specifically target antigen expressing tumors. They can create a memory pool of immune effectors that will potentially allow for long lived surveillance of new tumors bearing the same targets. Below is a short description showing the potential of both passive and
active immunotherapies of cancer in treatment and prevention settings.

**Passive Immunotherapy**

Targeted immunotherapies have been successfully developed in the antibody realm. So far, they have been restricted to the development of antibodies to various cancer and stromal associated molecules. Over the past decade, several monoclonal antibodies have been approved as passive immunotherapeutics. These antibodies are being used in treatment regimens either singly or in combination with other targeted chemotherapies. Largely, they are those that bind to members of the growth factor receptor family. Examples are Erbitux® (Imclone Systems, Inc) and Herceptin (Genentech, Inc) where they bind the extracellular domain of the receptor and prevent its ligand from engaging the receptor to induce downstream signaling and proliferation. Erbitux® is used to treat advanced colon cancer and acts by targeting the EGF receptor (EGFR) either as a single agent or in combination with irinotecan. Herceptin® is the other targeted antibody used in the treatment of HER2-positive breast cancer in women with stage II, III or IV also as a single agent or in combination with paclitaxel. Both EGFR and HER2 are found to be overexpressed in colon carcinomas and breast cancer, respectively, and therefore make suitable targets. Avastin®, on the other hand, targets circulating VEGF ligand thereby reducing the availability of the ligand and the probability of engaging VEGFR to initiate angiogenesis. In combination with 5-fluorouracil chemotherapy Avastin® is used in the treatment of metastatic colon and rectal carcinoma. Radiolabelled antibodies have also been successful in targeting B-cell lymphomas. Noteworthy ones are Bexxar™ (Corixa, Inc/Glaxo-Smith Kline, Inc) and Zevalin® (Biogen-Idec, Inc) which are radiolabelled monoclonal antibodies to CD20, to treat relapsed or refractory low grade, follicular or transformed B-cell non-Hodgkin's lymphoma, while Rituxan™ (Biogen-Idec, Inc) is an unlabelled monoclonal used to target CD20 among positive, refractory non-Hodgkin's Lymphoma (NHL) patients. In the case of radiolabeled antibodies, the attack on the tumor cell is probably two-pronged. The antibody carries the radiisotope to the cancer where it is destroyed by radiation. In addition, some of these antibodies may also exhibit a cytotoxic activity on the cancer cell.

**Active Immunotherapy**

Active immunotherapies or cancer vaccines have not seen the same level of success as antibodies. However, there is still widespread belief for the potential and utility of harnessing the patient's immune system to recognize and keep the cancer from recurring. With over half a dozen pivotal trials that have not been successful in the recent past, several criteria pertaining to all spheres of research and development have come to light providing guidelines that may help in the progress of generating a successful cancer vaccine [9-14]. A list showing a variety of vaccines that were clinically tried (and some ongoing) are shown in Table 1. This is not a complete list of all cancer vaccines that have been tried or are ongoing, but one that shows a variety of vaccines that have reached a pivotal trial [15-36]. Of all the vaccines to date, the furthest along that may be close to licensure is Provenge®, an autologous vaccine pulsed with PAP for treatment of asymptomatic, metastatic, androgen-independent prostate cancer. At the time of filing a BLA for Provenge®, the study showed that vaccinated patients showed a survival time of about 4.5 months longer than the placebo treated control group with a 41% overall reduction in the risk of death. However, the FDA requested additional clinical data in support of the BLA filing. The planned interim results of this trial just received indicated a 20% reduction in the risk of death in the vaccine arm relative to the placebo arm (http://investor.dendreon.com/Re...). There were no safety concerns and the trial is expected to continue to its final analysis.

Several factors have emerged from these trials that may be considered for further development of vaccines and these may be scientific, clinical, or logistical in nature. In the preclinical area, identification of relevant target antigens (both on the tumor and stroma) that are specific and relevant to biology of the tumor should be considered. A variety of antigens have been identified to which either spontaneous or induced immune responses have been reported in several studies [37]. Although immunization with these antigens have been known to protect mice from subsequent syngeneic tumor challenge, as well as induce a detectable T cell or antibody response in vaccinated patients, these data have not correlated with a desired clinical outcome. Lessons from some of the successful therapies have taught us that targeting those molecules crucial for signaling/proliferation and cell survival, or those that are overexpressed may be one method of arresting tumor spread. Examples of these are tyrosine kinase and proteosome inhibitors (Gleevec, Iressa and Tarceva, and Velcade, respectively) as chemotherapy, and antibodies to members of the growth factor receptor family (EGFR and HER2) and angiogenesis inducing ligand (VEGF). Erbitux, a chimeric monoclonal antibody, binds to the extracellular domain of the receptor and blocks binding of the ligand EGF to block downstream signaling and consequently growth and proliferation. Herceptin, a humanized monoclonal antibody, inhibits tumor growth by arresting cell cycle at the G1 phase as well as by disrupting downstream signaling, also leading to cell cycle arrest. Other factors to consider may be inclusion of suitable adjuvants in the vaccine regimen, other T cell co-stimulants, T-reg inhibitors and methods to down-regulate tumor suppressor factors [38]. The idea is to achieve a magnitude of anti-tumor response as that seen in an infectious disease setting. The greatest chance of achieving this success is most likely when some of these immunological agents are given in combination. However, it has become increasingly clear that this is also more achievable only when the tumor burden is minimal or there is no evidence of disease. In this scenario, there is less tumor induced immune suppression that could dampen an effective anti-tumor response.

While the above suggestions are broad indications for a successful cancer vaccine, a few of these can be more readily applied to that of a TIC setting. These will be addressed in the section below.

**Tumor Initiating Cells as Potential Target for Immunotherapy**

Cancer stem cells are one of the TICs which could be targets for vaccines. CSCs should possess the properties of proliferation and differentiation at least into the fates of the
tissue in which the tumor occurs. In addition serial transplantation into mouse xenograft models, which should result in formation of tumors which are similar to the parental tumor, is considered a functional assay for CSC. By these definitions, there have been several papers in the recent past which report that CSCs have been isolated from tumors of blood (specifically leukemias), brain, breast, colon, pancreas and prostate [39-47]. Table 2 lists the surface markers which have been used for this identification. The markers which have been used for isolation of these CSCs are not exclusive for CSCs, but are also found on normal stem cells, or even in other tissues. Furthermore clear markers identifying other types of TICs are also in the process of being defined.

Given the dearth of identifying markers on TICs, there is increasing reliance on functional properties for isolation and definition. Two frequently used experimental protocols used are the sphere forming assay and the xenograft assay. The sphere forming assay is reminiscent of the neurosphere assay which is one of the methods used to proliferate neural stem cells. As it selects for a population of proliferating cells which can grow without a substratum; these cells could potentially include any of the proliferative cells of the tumor, in addition to the CSCs. The xenograft model has served to prove that tumors can be serially passaged within mice, in order to demonstrate that tumors of similar histology can be propagated with putative CSCs. However, the propagation of human cells in mice might include a selection step where only selected cells could survive and proliferate in an alien atmosphere of a xenogeneic species. In addition, cells within the fraction thought to exclude CSCs have also been shown to cause tumors in the case of brain and colon CSCs [48, 49].

### Table 1. Examples of Cancer Vaccine Trials that are Either Discontinued or Ongoing

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Nature of Vaccine</th>
<th>Disease</th>
<th>Trial Status</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melacine® (Corixa)</td>
<td>Allogeneic whole cell tumor lysate+DETOX</td>
<td>Melanoma - Stage III, Stage IV</td>
<td>Discontd.</td>
<td>[15, 16]</td>
</tr>
<tr>
<td>PANVAC-VF (Therion)</td>
<td>Inactivated fowl pox virus encoding for costimulatory</td>
<td>Pancreatic cancer</td>
<td>Discontd.</td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td>molecules ICAM-1, B-7.1, LFA-3, and CEA and MUC-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bec2 (Imclone)</td>
<td>Anti-Id to GD3</td>
<td>SCLC</td>
<td>Discontd.</td>
<td>[18]</td>
</tr>
<tr>
<td>Canvacin™ (Cancer-Vax)</td>
<td>Allogeneic whole cell vaccine</td>
<td>Melanoma - Stage IV; Stage III</td>
<td>Discontd.</td>
<td>[19, 20]</td>
</tr>
<tr>
<td>Oncophage® (Antigenics)</td>
<td>Hsp96 (kidney cancer cells)</td>
<td>Renal cell carcinoma - Stage IV</td>
<td>Discontd.</td>
<td>[21]</td>
</tr>
<tr>
<td>Theratope® (Biomira)</td>
<td>Sn-KLH (MUC1)</td>
<td>Metastatic (Stage IV) breast cancer</td>
<td>Discontd.</td>
<td>[22]</td>
</tr>
<tr>
<td>MyVax (Genitope)</td>
<td>Patient specific recombinant idiootype protein coupled with KLH and injected with GM-CSF</td>
<td>Follicular non-Hodgkin’s lymphoma</td>
<td>Discontd.</td>
<td>[23]</td>
</tr>
<tr>
<td>GVAX (Cell Genesys) – VITAL 2</td>
<td>Allogeneic prostate tumor cells transfected with GM-CSF</td>
<td>Hormone resistant prostate cancer (GVAX immunotherapy in combination with Taxotere chemotherapy vs. Taxotere and prednisone)</td>
<td>Discontd.</td>
<td>[24, 25]</td>
</tr>
<tr>
<td>GVAX (Cell Genesys) – VITAL 1</td>
<td>Allogeneic prostate tumor cells transfected with GM-CSF</td>
<td>Hormone resistant prostate cancer (GVAX immunotherapy vs. Taxotere chemotherapy and prednisone)</td>
<td>Ongoing</td>
<td>[26]</td>
</tr>
<tr>
<td>Lucanix™ (NovaRx)</td>
<td>Allogeneic tumor cells transfected with antisense TGFβ</td>
<td>Non small cell lung cancer</td>
<td>Ongoing</td>
<td>[27, 28]</td>
</tr>
<tr>
<td>Proteinase 3 PR1 peptide (The Vaccine Company)</td>
<td>Peptide + GM-CSF</td>
<td>Acute Myeloid Leukemia (AML)</td>
<td>Ongoing</td>
<td>[29-31]</td>
</tr>
<tr>
<td>Sipuleucel-T (Dendreon)</td>
<td>Autologous CD54+ cells/PAP</td>
<td>Metastatic, hormone refractory, asymptomatic prostate cancer</td>
<td>Ongoing; interim results in Oct 2008</td>
<td>[32-36]</td>
</tr>
</tbody>
</table>

Table 1: A list of various types of cancer vaccines in pivotal trials that has provided valuable indications for the successful development of future vaccines (Discontd.; Discontinued)
These recent conflicting observations relating to CSCs and their properties, does leave some room for evolution in current methods of identification of CSCs.

Markers specific for TICs are being sought for purposes of identification, and they will also prove useful for targeting these cells. Several other methods may be used to identify such surface markers including differential expression, genomics and proteomics approaches. In another approach it was determined whether markers known to occur in certain cancers are present in CSCs isolated from those specific tissues [50]. MUC1, a well known tumor marker, was present in cells selected as the ‘side population cells’ from MCF7, a breast cancer cell line. The ‘side population’ includes cells which group as a population of smaller cells with increased efficiency of dye efflux and which are thought to co-isolate with the CSC population. In another study of multiple myeloma (MM) immunity to the SOX2 antigen was expressed in patients with a precursor condition to MM called monoclonal gammopathy of undetermined significance (MGUS). This immunity was lost in patients where the disease had progressed although SOX2 positive cells remained suggesting a T-cell malfunction [51]. However, this study does suggest that SOX2 is a possible marker for some precursors.

In spite of the limitations noted above, the concept of TICs is an extremely attractive one to factor into cancer therapeutics. For effective use in developing an immunotheraphy targeting these cells, identification of cells which could give rise to tumor mass is perhaps more important rather than specifying whether they are CSCs or precursors. Immunotheraphies may be used to advantage in scenarios such as choosing relevant targets, in order to develop cancer vaccines. Targeting TICs using such approach becomes very attractive towards a promising promising option for cancer.

### CONCLUSIONS AND PROJECTIONS

Cancer patients who have been treated and have no evidence of disease are still considered to be at a risk for recurrence for up to a few years, potentially from residual cells which include TICs, which may give rise to metastatic tumors. While a great deal of information is not available on identifying TICs, the scenario to target these cells is very attractive and more effort is needed in characterizing these cells which could give rise to tumors. Depending on cancer type, markers may be more or less unique to the TIC or shared with the mature tumor as in the MUC1 instance. Active immunotheraphy is potentially a means to induce long lived protection from a recurrence among cancer patients. Application of some of the aforementioned considerations such as choosing relevant targets, combination therapies to enhance immune efficacy, applying these approaches to a patient population with a low tumor burden or where they can be screened for known precursors of cancer, may help to effectively develop cancer vaccines. Targeting TICs using this approach becomes very attractive towards a promising promising option for cancer.

### LIST OF ABBREVIATIONS

CSCs = Cancer Stem Cells

TIC = Tumor Initiating Cells

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Immunotherapy and Tumor Initiating Cells


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