Tumor vaccine: Current trends in antigen specific immunotherapy

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Effective cancer treatment to prevent the tumor growth as well as to stop its recurrence is the dream of oncologists. Currently available therapeutic measures like, radiotherapy and chemotherapy, often suffer from severe toxicity and lack of specificity of the drug towards tumor cells. Another promising approach is the 'immunotherapy', in which either the immune system is activated by tumor vaccine to combat the tumor growth or antitumor antibodies can be used. Vaccination can stimulate humoral, cellular and innate immune systems to generate various effector molecules, like antibody, cytotoxic T cells, cytokines etc. In antigen specific immunotherapy, the immune system can be stimulated actively by antigen based tumor vaccine to kill only those tumor cells, having expression of the particular tumor associated antigen. Different experimental, preclinical and clinical studies have proved that generated immune responses are effective to restrict the tumor growth. Useful strategies of antigen specific immunotherapy and outcome of various laboratory and clinic based studies are discussed.

Keywords: Antibody, Antigen, Immunotherapy, Tumor, Vaccine

Effective cancer therapy or prevention has been the dream of clinicians and scientists for many years. Although, the ultimate goal is yet to be achieved, significant progress has already been made in terms of our knowledge and understanding of the process of carcinogenesis. Besides the conventional modes of surgery, chemotherapy and radiotherapy, immunotherapeutic strategies are now accepted as more effective in terms of the requisite specificity that they offer in targeting only tumor cells. On the other hand, chemotherapy and radiotherapy are more general and invasive with several side effects. There are several immunotherapeutic strategies, which are currently under investigation and some are in clinical practice (Table 1). These strategies can be broadly categorized into 'specific antigen based therapy' and 'nonspecific therapy'. Again, specific therapy is either active or passive. In active therapy, patient can be vaccinated with an immunogen to generate an effective immune response. On the contrary, passive therapy utilizes the transfer of effector molecules (antibodies) or cells (CTLs) to patients. In both active and passive therapies, the developed immunity is specific for the immunogen (antigen) only. As tumor cells generally express one or more tumor associated antigen(s) (TAAs) in significantly higher quantity, developed antigen specific immunity can target tumor cell only, without affecting antigen negative normal cells. Such treatment modalities, in general, are termed as 'tumor vaccine'. This review is aimed to discuss different forms of 'tumor vaccine' for antigen specific cancer immunotherapy. Emphasis will be on the principle and results obtained during translation of basic research into clinical set-up.

Antigen specific immunotherapy
Antigen specific immunotherapy holds promise as a complementary approach to chemotherapy, radiotherapy and surgery, for the treatment of cancer patients who are at high risk of relapse or progressive disease. This mode of immunotherapy utilizes various immunogens in the form of TAAs and an adjuvant or

<table>
<thead>
<tr>
<th>Specific (Target tumor antigens)</th>
<th>Nonspecific (Stimulates immune system)</th>
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<tbody>
<tr>
<td>• Tumor Vaccines</td>
<td>• Cytokine Therapy</td>
</tr>
<tr>
<td>• Tumor Lysates</td>
<td>• Interleukin 2</td>
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<tr>
<td>• Tumor cells</td>
<td>• Interferon α2b</td>
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<td>• Tumor cell membranes</td>
<td>• Tumor necrosis factor α</td>
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<tr>
<td>• Purified tumor antigens</td>
<td>• Adjuvant Therapy</td>
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<tr>
<td>• Peptides</td>
<td>• bacille Calmette-Guerin</td>
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<tr>
<td>• Recombinant virus coding tumor antigen</td>
<td>• Corynebacterium parvum</td>
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<td>• Xenogenic tumor antigen</td>
<td>• Detox</td>
</tr>
<tr>
<td>• Anti-idiotypic antibody</td>
<td>• Coley's Toxin</td>
</tr>
<tr>
<td>• DC Vaccines</td>
<td>• DC pulsed with above preparations</td>
</tr>
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DC = dendritic cell

Table 1—Different forms of tumor vaccine

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carrier molecule to promote presentation of the antigen to the immune effector cells. This therapy stimulates the immune system actively, thus, it is also designated as 'active specific immunotherapy'. Its aim is to generate tumor specific antitumor responses through stimulation of the host's immune system by tumor vaccine. Tumor vaccines make use of tumor antigens (Table 2) which in general, are derived from proteins that are produced by tumor and aim to trigger humoral and cellular immune responses. Treatment of cancer patients with autologous tumor cell preparation is considered as the first use of tumor vaccine in human beings\(^1\). Animal studies were done earlier. In subsequent times, a variety of antigen specific immunostimulatory strategies have been applied with some clinical success (Tables 3 & 4). The molecular

<table>
<thead>
<tr>
<th>Class of antigen</th>
<th>TAA*</th>
<th>Expression in cancer**</th>
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<tbody>
<tr>
<td>Overexpressed proteins</td>
<td>CEA</td>
<td>Colon, Breast, Lung, Bladder, Thyroid</td>
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<tr>
<td></td>
<td>Her2/neu</td>
<td>Breast, Ovary</td>
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<td></td>
<td>MUC1</td>
<td>Breast, Ovary, Lung, Colon, Pancreas</td>
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<tr>
<td></td>
<td>PSA</td>
<td>Prostate</td>
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<tr>
<td>Differentiation antigens</td>
<td>MART-1</td>
<td>Melanoma</td>
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<td></td>
<td>gp-100</td>
<td>Melanoma</td>
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<tr>
<td></td>
<td>PSA</td>
<td>Prostate</td>
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<tr>
<td></td>
<td>Tyrosinase</td>
<td>Melanoma</td>
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<tr>
<td></td>
<td>HPV16 E7</td>
<td>Cervix, Oral</td>
</tr>
<tr>
<td>Mutated protein</td>
<td>p53</td>
<td>Colorectum, Breast, Oral, Liver</td>
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<tr>
<td>Cancer testis antigens</td>
<td>MAGE 1</td>
<td>Testis</td>
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<tr>
<td></td>
<td>MAGE 3</td>
<td>Testis, Melanoma, Gastrointestinal tract</td>
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<td></td>
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<tr>
<td>Tumor virus</td>
<td>HPV16 E7</td>
<td>Melanoma, Neuroblastoma, Sarcoma</td>
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<tr>
<td></td>
<td>gp-100</td>
<td>Melanoma, Neuroblastoma, Sarcoma</td>
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<td></td>
<td>PSA</td>
<td>Melanoma, Neuroblastoma, Sarcoma</td>
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<td></td>
<td>Tyrosinase</td>
<td>Melanoma, Neuroblastoma, Sarcoma</td>
</tr>
<tr>
<td></td>
<td>gp-100</td>
<td>Melanoma, Neuroblastoma, Sarcoma</td>
</tr>
<tr>
<td></td>
<td>Telomerase</td>
<td>Colon, Bladder, Oral, Lung, Prostate</td>
</tr>
<tr>
<td>Other antigens</td>
<td>CA125</td>
<td>Breast, Ovary, Lung, Bladder</td>
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<td></td>
<td>CA15-3</td>
<td>Breast, Colon</td>
</tr>
<tr>
<td></td>
<td>CA19-9</td>
<td>Colon, Pancreas, Bladder</td>
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</table>

*The list of TAAs in the Table is not complete. Several other antigens are available with potential to use in tumor vaccine. Except ganglioside antigens, most of these TAAs are protein/glycoprotein
**Apart from the organ names referred, expression may occur in other organs during carcinogenesis

<table>
<thead>
<tr>
<th>Table 3—Milestones in the development of antigen specific immunotherapy</th>
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<tbody>
<tr>
<td>1800-1950</td>
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<td>1950-1970</td>
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<td>1970-1990</td>
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<tr>
<td>1990-present</td>
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<tr>
<td>1895 1957</td>
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<tr>
<td>First use of heteroimmune sera in patients</td>
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<tr>
<td>Start of tumor immunization studies in inbred mice</td>
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<tr>
<td>Development of monoclonal antibody technology</td>
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<tr>
<td>Discovery of first MHC-restricted tumor antigen</td>
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<td>1902 1960</td>
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<tr>
<td>First therapeutic use of autologous cancer cells</td>
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<tr>
<td>Transfer of antitumor immunity with lymphocytes</td>
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<tr>
<td>1975 C. parvum potentiation of antitumor responses</td>
</tr>
<tr>
<td>1993 Definition of MHC class II structure</td>
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<tr>
<td>1943 1963</td>
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<tr>
<td>Demonstration of antitumor immunity in mice</td>
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<tr>
<td>Initial clinical studies with lymphocytes from cured patients</td>
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<tr>
<td>1988 TIL cell + IL-2 therapy</td>
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<tr>
<td>1995 Peptide vaccine trial</td>
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<tr>
<td>1990 Start of humanized monoclonal antibody trials</td>
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<tr>
<td>1996 Achievement of in vitro growth of dendritic cells</td>
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<tr>
<td>1997 Dendritic cell tumor antigen trials</td>
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</tbody>
</table>
BARAL: ANTIGEN SPECIFIC THERAPY OF TUMORS

Table 4—Examples of clinical trials targeting TAAs by antigen specific immunotherapy

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Immunogen</th>
<th>Cancer</th>
<th>Phase of trial</th>
<th>Clinical outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous tumor Cell</td>
<td>Autologous Tumor</td>
<td>Colorectal Cancer</td>
<td>III</td>
<td>Reduced Risk of Recurrence</td>
<td>Vermorken et al.</td>
</tr>
<tr>
<td>Cell vaccines</td>
<td>Lysate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allogenic tumor Cell</td>
<td>Vaccinia Virus</td>
<td>Melanoma</td>
<td>III</td>
<td>Increase in Overall Survival</td>
<td>Wallack et al.</td>
</tr>
<tr>
<td>Cell vaccines</td>
<td>Oncolyase</td>
<td></td>
<td>II</td>
<td>Disease Free Survival of Patients More Than 90</td>
<td>Morton &amp; Barth</td>
</tr>
<tr>
<td></td>
<td>Polyvalent Whole Cell Vaccine</td>
<td>Melanoma</td>
<td></td>
<td>Months</td>
<td></td>
</tr>
<tr>
<td>Peptide Vaccines</td>
<td>MAGE-3 Peptide</td>
<td>Melanoma</td>
<td>Pilot</td>
<td>Response in 33% patients</td>
<td>Marchand et al.</td>
</tr>
<tr>
<td></td>
<td>Tyrosinase Peptide</td>
<td>Melanoma</td>
<td>II</td>
<td>Stable Disease in 2 Patients</td>
<td>Scheibenbogen et al.</td>
</tr>
<tr>
<td></td>
<td>gp100 Peptide</td>
<td>Melanoma</td>
<td>II</td>
<td>Immune Response to Peptides in 20 patients among 24</td>
<td>Weber et al.</td>
</tr>
<tr>
<td>Dendritic Cell (DC)</td>
<td>PSM-P1/P2 pulsed DC</td>
<td>Prostate</td>
<td>II</td>
<td>Complete Response in 2 &amp; Partial response in 7 patients among 33 Proliferative Response to Idiotype protein in 5/6 patients</td>
<td>Murphy et al.</td>
</tr>
<tr>
<td>vaccines</td>
<td>Idiotype Protein</td>
<td>Myeloma</td>
<td>Pilot</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>gp100 &amp; Tyrosinase</td>
<td>Melanoma</td>
<td>I</td>
<td>Response in 50% Patients</td>
<td>Lim &amp; Baileywood</td>
</tr>
<tr>
<td></td>
<td>pulsed DC</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Humanized MAb</td>
<td>Anti-HER2 MAb + Chemotherapy</td>
<td>Metastatic Breast Cancer</td>
<td>III</td>
<td>Response Rate 62% with MAb &amp; 36% without MAb Patients</td>
<td>Cobleigh et al.</td>
</tr>
<tr>
<td>Radiolabeled MAb</td>
<td>Anti-CD20 MAb 90Y- AntiCD20</td>
<td>NHL Relapsed</td>
<td>II</td>
<td>Response Rate 48%, Complete Response 26%</td>
<td>McLaughlin et al.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>I/II</td>
<td></td>
<td>Witzig et al.</td>
</tr>
<tr>
<td>Anti-idiotypic MAb</td>
<td>MAb 3H1 (mimicking CEA) +5-FU</td>
<td>Colorectal Cancer</td>
<td>II</td>
<td>Complete Response in 1 patient &amp; stable disease in 12 patients among 47 stage III patients</td>
<td>Foon et al.</td>
</tr>
<tr>
<td></td>
<td>MAb 1A7 (mimicking GD2) +5-FU</td>
<td>Melanoma</td>
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Characterization of novel human TAAs and increased understanding of the immunological pathways involved in tumor immunity have paved the way to design a promising tumor immune vaccine. TAAs are readily available now and several ways to deliver original or surrogate antigen have been developed. A rigorous autotumorlytic immune response can be induced in cancer patients by immunization with TAAs. This immunization results in the generation of antigen specific antibody response to mediate antibody dependent cellular cytotoxicity and other effector responses, including antigen specific T cell responses and objective clinical responses on cancer patients. The most important and widely used approaches consist of whole tumor cell vaccine, purified TAA vaccine, peptide vaccine, anti-idiotypic vaccine, dendritic cell based vaccine etc. These vaccine forms were evaluated in experimental system as well as in clinical trials. Important work is presently underway to correlate the antigen specific, anti-tumor humoral and cellular immune responses with the clinical response. Detected anti-tumor immunity may not always be reflected in clinical response and in most of the cases tumor load appears to be a primary limiting factor. Beside antigen specific immunotherapy, potential of nonspecific immunotherapy was also examined.

**Nonspecific immunotherapy**

Immunostimulation by selective 'Biological Response Modifiers' and various cytokines is also a wide area of investigation. One of the earliest experiences in immunotherapy arose from the application of Coley's toxins, which stimulate
nonspecifically the immune system, in patients with unresectable sarcoma. Other bacterial agents such as bacilli Calmette-Guerin (BCG), Staphylococcus pyogenes and Corynebacterium parvum are believed to function similarly. In 1992, the Federal Drug Administration (FDA), USA approved IL-2, as first immunotherapeutic agent in cancer treatment of patients with metastatic renal cell carcinoma. Partial clinical responses were reported in renal cell carcinoma and metastatic melanoma. Among interferons, IFN-α2b has had the most significant effect in human cancer immunotherapy. Several clinical studies have already been conducted with IFN-α2b and in a Phase III trial IFN-α2b has proved to be effective in prolonging overall survival and disease free survival in melanoma patients who are at high risk of disease recurrence following surgery. Presently, IFN-α2b is a FDA approved immunotherapeutic agent being used in the adjuvant treatment of cancer. Other cytokines, like, TNFα, IL-4, IL-12 and GMCSF, have some clinical significance in the treatment of cancer. NK cells, LAK cells and macrophages act as effectors in cytokine network of nonspecific immunotherapy. However, this area is beyond the scope of present review.

Tumor associated antigen, the key for vaccine development

Process of tumorigenesis is associated with the alteration in gene sequences and expression levels of various protein antigens. These changes lead to the expression of several tumor associated antigens (TAAs), e.g., carcinoembryonic antigen (CEA), prostate specific antigen (PSA), MAGE, Her2/neu, MUC1, HPV16-E7 etc. (Table 2). Such TAAs could be useful in detecting cancer, determining prognosis, monitoring disease progression or therapeutic response. Continuous efforts are on to discover new TAAs by screening strategies such as the SEREX programme, comparative proteome analysis or gene expression profiling. TAA, generally a protein molecule, derived from tumors, can be either tumor specific, expressed exclusively in tumor tissue, or tumor associated, highly overexpressed in tumors but can also be found in normal tissues. Most important tumor specific antigens are: cancer testis antigens, e.g., MAGE, protein variants that are created by somatic mutations within tumor cells, e.g., CDK4, and proteins from tumor viruses, e.g., HPV16E7. These are found only in tumor tissues and therefore do not attract the risk of autoimmune reactions during immunotherapy. Some TAAs are, differentiation antigen, like, tyrosinase and overexpressed proteins, e.g., Her2/neu, CEA, PSA etc. Some antigens are shared among different forms of tumors and include telomerase and survivin. On the other hand, expression of some antigens is restricted within a small percentage of tumors, such as, the MAGE gene family. Altered genetic events during or before carcinogenesis, like, gene mutation, amplification, rearrangement, translocation etc, are reflected by the overexpression of TAA. These genetic events may be initiated by some unknown factor(s), termed as etiological factor. TAAs, after their expression on cancer cell surface, play a definite role in favour of tumor cells, specifically by decreasing the cellular adhesion and promoting metastasis. Based on the tumor specificity and widespread expression, TAAs are being selected as a candidate for vaccine development. Vaccination with TAA, in its different form, may elicit antibody response that is generally specific for the immunizing antigen. Adequate amount of these antibodies with high avidity are expected to bind the same antigen present on tumor cell surface to induce cell lysis. Tumor cell lysis may include antibody dependent cellular cytotoxicity and complement mediated cytotoxicity. Most of these TAAs, except T independent antigens like ganglioside antigens, can elicit T cell response and promote cytotoxic T cell (CTL) mediated tumor cell lysis.

Tumor vaccine in various forms

The first and most obvious type of vaccine is tumor cell preparation or membrane preparation from tumor cells, as a source of TAA. With the advancement of molecular biologic approaches, gene modified tumor cells expressing TAA have also been used. In addition, purified antigen from tumor cell preparation, recombinant tumor antigen, DNA-encoding protein antigens and protein derived peptides have been tested, and some of them are also evaluated in clinical trials. Several investigators were successful in obtaining some active antitumor immune responses with these immunotherapeutic approaches. However, major problem using tumor material for immunization is that TAAs are typically weakly immunogenic due to tolerance. An effective method to break the tolerance is to present the critical epitope in a different molecular environment to the
tolerized host. While this can be done with well-defined antigens, it is impossible with most TAAs because they are chemically ill-defined and difficult to purify. Another elegant approach for active specific immunotherapy is anti-idiotypic approach based on Jerne’s network hypothesis. Using this approach, immunization can be done by anti-idiotypic antibody (mirror image of TAA) instead of original antigen, which is also effective to break the immune tolerance.

**Tumor cell as a vaccine**

The most straightforward means of immunization is the use of whole tumor cell preparation (either autologous or allogenic tumor cells). The advantage to this approach is that all potential tumor antigens can be presented to the immune system for processing and presentations to the appropriate T-cell precursors. In addition, steps involved in the purification of antigen from tumor material can be avoided. The difficulty with this approach lies in the availability of fresh autologous tumor material and in the scarcity of well characterized long term tumor cell lines that are HLA-typed and expressed high levels of MHC antigens. This approach was examined in animal models and also in clinics. In a randomized trial, 98 patients of colorectal cancer were treated by resection alone or resection plus irradiated autologous tumor cells. A significant improvement in overall and disease free survival was seen in the patients received active specific immunotherapy.

Morton and Barth have performed an extensive work on tumor cell vaccine on metastatic melanoma. They conducted phase I/II trials of active immunotherapy using an irradiated preparation of whole cells from three allogenic human melanoma cell lines that express at least 15 tumor associated antigens. Fifteen to 20% of patients who received this polyvalent melanoma cell vaccine (PMCV) for treatment of stage IV melanoma had partial or complete regression of distant metastases and significantly longer survival than control patients. Interestingly, patients who received PMCV after curative surgical resection had median survival duration of 38 months, compared with the postoperative median survival time of only 13 to 18 months.

**Purified TAA as a tumor vaccine**

Purified antigen can be used as an immunogen in vaccine with an objective to induce an antibody response against a TAA and to generate antigen specific cytotoxic T cells (CTLs). Human TAAs are non-self in animal system and highly immunogenic. Thus, results obtained from experimental system have shown a strong antigen-specific immune response against the tumor. However, these antigens are poorly immunogenic in human system as most of these tumor antigens are self-antigen. As a consequence, positive results from animal system may not be always applicable in humans. Transgenic animal model, which expresses human TAA and, thereby, reflects the human situation would help to understand the fate of TAA vaccine and to find out the ways to overcome the problem of tolerance.

Irrespective of its limitations, this approach is tested in human system, specifically in melanoma using different ganglioside preparations. An altered pattern of cell surface ganglioside (GD3, GD2, GM2 etc) expression was observed in malignant melanoma and other cancers of neuroectodermal origin. Overexpression of gangliosides on the surfaces of these cancer cells, makes them attractive target for immunotherapy. GM2 vaccination with BCG in melanoma patients have been reported to induce IgM antibodies to GM2 in most patients and disease free survival was extended in patients producing high titer of anti-GM2 antibodies. These antibodies are principally IgM, but inconsistent IgG titer was also detected. GD3 and GD2 vaccines were also tested, but overall results are not very much promising. To improve the clinical outcome, these gangliosides were conjugated with keyhole limpet hemocyanin (KLH) and injected in combination with various adjuvant preparations, resulting in increased immunogenicity and better tolerance to patients.

A tumor associated protein, GA-733, has also been used as a vaccine for the treatment of colon cancer. This vaccine can reduce the tumor growth by a specific cell and antibody mediated immune response. Recently, Biomira, Inc. (Edmonton, Alberta, Canada) introduced a tumor vaccine using a mucin type carbohydrate antigen (Sialyl-Tn). In this vaccine, Sialyl-Tn was conjugated to the carrier molecule, KLH. This Sialyl-Tn-KLH vaccine has been developed for immunizing patients with mucin-expressing tumors. In murine and human studies, the vaccine has been shown to stimulate anti-Sialyl-Tn antibodies and mucin-specific T-cell responses. The immune response was reported to be augmented by pretreatment with intravenous cyclophosphamide that...
serves to inhibit suppressor T-cells. Survival benefit was reported for breast cancer patients who received the Theratope vaccine after intravenous cyclophosphamide. A multinational phase III study tested this vaccine in patients with metastatic breast cancer. Better clinical response or stability of the disease was observed in this trial. Other malignancies for which the vaccine may be applicable include ovarian and gastrointestinal cancers.

**Peptide vaccine as a substitute of whole TAA vaccine**

Instead of whole TAA vaccine, peptides generated from the sequence information of a TAA were also tested as vaccine. These peptides can produce antibody response and cellular immune response directed against the active epitope of TAA. An advantage of peptide vaccine is that they can be synthetically generated in a reproducible fashion. In a study, 63 patients were vaccinated with a 105-amino acid polypeptide that included 5 repetitions of the entire conserved tandem repeat of the MUC1 peptide. In these patients, delayed type hypersensitivity (DTH) reactions were evaluated at 48 hr and intense T-cell infiltration was reported in majority of patients. A limited number of patients had a 2-4 fold increase in mucin specific CTL precursors in the peripheral blood after vaccination. A 9-mer peptide spanning the MUC1 tandem repeat with an HLA-All MHC class I restriction was identified. CTLs specific for this peptide were also identified from peripheral blood.

**Recombinant vaccinia virus in tumor vaccine**

The ability to engineer recombinant viruses encoding TAAAs, coupled with the intrinsic immunogenicity of viruses, has engendered broad interest in recombinant viral vaccines. Currently, members of the pox-, retro-, herpes-, and aden-associated viruses have been used as vehicles in recombinant viral vaccine construction. Adenovirus, encoding MART-1, gp100 and tyrosinase, induced both humoral and cellular immune responses when used to vaccinate melanoma patients. Schlom *et al.* at National Cancer Institute, Bethesda, MD, tested this approach. Vaccinia virus has been safely and successfully used as a live vaccine for the prevention and eradication of smallpox. These viruses are highly immunogenic and stimulate both humoral and cell mediated responses. A recombinant vaccinia virus expressing human CEA (rV-CEA) was reported to stimulate the T-cell response and strong anti-CEA antibody response in animal species, including nonhuman primates. Vaccination of mice with rV-CEA rendered them resistant to the growth of subcutaneously transplanted CEA-expressing tumors. In addition to CEA, this model was also effective for melanoma antigen p97. Upon immunization, mice were found to be protected from the challenge with melanoma cells, which was mediated by the generation of strong immune response. Recombinant vaccinia virus constructs with other candidate human TAAs have also been developed and tested in animal models. To improve this vaccinia-virus based immunotherapy, low dose IL-2 or GMCSF was administered in a murine tumor model with rV-CEA. Recently, Greiner *et al.* reported that combination of rV-CEA with costimulatory molecules (B7.1, ICAM-1, LFA-3) along with biological adjuvant GMCSF reduced the number of intestinal tumors in CEA-transgenic mouse model.

**Tumor cells engineered with genes of immunostimulatory molecules**

Another important strategy in active specific immunotherapy is the development of engineered tumor cells, where gene or protein of different immunostimulatory molecules are transferred into the cells, expressing TAAs. Tumor escape from immune effectors is most often caused by weak immunogenecity of TAAs, antigen masking or overall immunosuppression. To compensate these problems, cytokine genes, foreign HLA genes, TAA genes and genes of co-stimulatory molecules were introduced into tumor cells to make these as an efficient vaccine. In a study, IL-2 gene was introduced to
lymphoma and melanoma using diptheria toxin. When these engineered cells were injected into mice, an active immune response was reported to prevent the tumor growth. Vaccination with membranes modified by protein transfer to express glycosylphosphatidylinositol (GPI)-linked B7.1 (CD80), induces protective immunity in mice and allogenic antitumor T-cell proliferation in humans in vitro. GPI was also used to express IL-12 on tumor cell surface, which prevented tumor growth in mice in a highly tumorigenic murine mastocytoma model. B7-transfected melanoma cells stimulate directly CD8+ T cells for tumor rejection. In some cases, after transfection with the B7-1 and B7-2 genes, poorly immunogenic tumors fail to elicit an effective immune response. However, such tumor was reported to be immunogenic after transfection of the genes encoding murine B7-1 together with CD48.

Vaccines for human papilloma virus (HPV) associated tumors

Human papilloma virus (HPV) has been found to be closely associated with several forms of cancer, particularly with cervical carcinoma. More than 100 HPV types have been characterized until now, among them 11 have been considered as high risk types and detected in tumor tissue. In recognition of the association of cervical cancer with this viral infection, substantial interest has arisen to develop effective prophylactic and therapeutic vaccines. Prophylactic strategies currently under investigation focus on the induction of effective humoral and cellular immune responses, which are potentially protective against subsequent HPV infection. Papillomavirus-like particles have been synthesized to induce neutralizing antibody responses, and impressive immunoprophylactic effects have been demonstrated in both animals and humans. The therapeutic vaccines were constructed utilizing two nonstructural early proteins coded by HPV (products of their E6 and E7 oncogenes). These E6 and E7 proteins are very much essential for the pathogenesis of HPV associated tumors and, thus, several attempts have been made to target these oncoproteins. Active immunotherapy with E7 protein along with CpG oligonucleotide in a HPV associated murine tumor model, resulted in a very promising outcome in relation to the slower tumor growth and extended survival. This vaccination protocol was able to induce significant humoral and T cell response to combat the tumor growth.

Immunization with xenogenic TAA

Priming adaptive immunity against self-antigens is potentially a difficult task. Presentation of altered self to the immune system is a strategy to elicit immunity against poorly immunogenic antigens. Immunization with conserved paralogues of TAA can induce adaptive immunity against self-antigens expressed by cancer. Cancer immunity induced by xenogenic immunization follows multiple and alternative pathways. The effector phase of tumor immunity can be mediated by cytotoxic T cells or macrophages and perhaps natural killer cells for antibody-dependent immunity. Helper CD4+ T cells are also required to generate such immunity. Using this strategy, rats were immunized with human Her2/neu protein and developed significant antibody and T-cell responses that were specific for both human and rat Her2/neu. Immunization with human glioma protein causes tumor growth inhibition in rat glioma model. This tumor inhibitory effect is due to the antigen specific IgG immune response and CTL response. Guevara-Patio et al. have shown that xenogenic DNA vaccination can elicit tumor immunity through T cell and antibody-dependent effector mechanisms. They have tested xenogenic DNA vaccines for melanoma differentiation antigens, tyrosinase-related protein 2 and gp100 and prostate-specific membrane antigen in association with CTLA-4 blockade.

Anti-idiotypic antibody as a vaccine

The idiotype network hypothesis of Lindenmann and Jerne offers an elegant approach to transform epitope structures into idiotypic determinants. According to this concept, immunization with an antibody (Ab1) against an antigen (TAA) can generate several types of anti-Id antibodies (Ab2) in the injected host. One set of these anti-Id antibodies, designated Ab2β, acts as ‘internal image’ of the antigen and mimics its molecular features. Thus, injection of Ab2β to the new host will elicit a new set of antibodies, which, besides binding to the Ab2β (Ab3 response), will also bind to the nominal antigen (Ab1’ response). The efficacy of anti-Id antibodies as cancer vaccine has been demonstrated in a number of preclinical and clinical studies.

Anti-Id vaccine and conventional TAA vaccine both stimulate the adaptive immune system in an active specific manner through T- and B-cell receptors. Priming adaptive immunity against self-antigens is potentially a difficult task. To overcome
this particular problem, anti-Id vaccine has advantages over conventional antigen vaccine. Firstly, TAAs are often a part of “self” and usually evoke a very poor immune response in tumor bearing hosts due to tolerance to the antigen. Following immunization with a TAA, generation of immune response may be obstructed by either central or peripheral tolerance. In central tolerance, no anti-TAA antibody is generated after injection of TAA due to absence of responsible immunocytes (clonal deletion). Another possibility is that the antibody generating immune cells remain silenced following challenge with TAA (clonal anergy). In peripheral tolerance, some antibody is initially produced, but then, because of the expression of suppressive mechanisms (suppressor T cells, antibodies, immune complexes etc.), antibody production diminishes or ceases entirely. For example, cancer patients are immunologically tolerant to CEA, which is likely to be related to its oncofetal origin. An effective method to break the tolerance is to present the critical epitope in a different molecular environment to the tolerated host. Anti-Id antibody is the mirror image of the nominal antigen and behaves as a foreign molecule in patients due to its heterologous nature. Mirror image anti-Ids mimic the three-dimensional structures of antigens and, thus are genetically unrestricted and effective across the species barrier because heterologous antigens are better immunogens. Anti-Id vaccines are proteins, hence, will have to be MHC restricted theoretically. Anti-Ids can generate a significant amount of antibody (Ab3) response, which has ability to recognize the TAA after breaking the tolerance imparted by self-TAA vaccine. Anti-Id vaccine, 3H1, is reported to induce helper CD4+ T cell response instead of suppressor T cells. This may be another way to avoid the self tolerance. Secondly, most of the TAAs are poorly characterized and difficult to purify. On the other hand, using hybridoma technology anti-Id MAb can be produced in large quantity, which will be more economical. Anti-Id vaccine also excludes the possibility of some undesired side effects which are sometime associated with conventional antigen vaccine. As a biological molecule, it has no toxic effect except causing little fever or swelling at the site of injection. Moreover, anti-Id vaccine is nothing but an immunoglobulin, it can be manipulated at protein or gene level.

Apart from these beneficial effects, anti-Id vaccines also have some potential complications. It is unknown how long anti-Id immunity will last. After vaccination with murine anti-Id antibodies, an individual may generate a human anti mouse antibody (HAMA) response that can neutralize the vaccine. Humanization of the anti-Id antibodies may be necessary to overcome the problem. Moreover, care must be taken in choosing the antibody for the vaccination, since different anti-Id antibodies generated against the same idiotope can have different or even opposite physiological effects. However, several clinical evidences suggest that these limitations could have never been a major problem to restrict their clinical use.

Progress in the field of anti-idiotypic vaccine

Basic information on B- and T-cell induced responses using the anti-Id approach was obtained from studies in a mouse leukemia model (L1210). A number of anti-Id hybridomas against MAb to the L1210 tumor were generated. These anti-Id MAbs have been reported to inhibit the tumor growth by the induction of tumor specific DTH, CTL, antibodies and T helper cells in the system. In the same system, 100% cure of established tumors was reported by combining anti-Id vaccine with cyclophosphamide, whereas, 50% cure rate was obtained with anti-Id therapy alone. Using an anti-Id vaccine mimicking CEA, significant tumor growth restriction and survival benefit were noted in C57BL/6 mice challenged with lethal dose of CEA positive murine tumor. This anti-Id vaccine was also effective in CEA-transgenic mouse model bearing murine colon tumor. This model is closer to the human system, as gastrointestinal tract of these transgenic mice expresses human CEA. Potential of anti-idiotypic antibodies for the regression of murine tumor growth was reported by several groups. Based on promising results from animal experiments, preclinical and clinical studies have already been performed with anti-Id antibodies.

An Ab2β anti-Id antibody to a murine MAb was generated that identifies a highly restricted T-cell antigen designated gp37. This antigen is present in 70% of acute T-cell lymphoblastic leukemia and 30% of T-cell lymphoma. Following administration of this anti-Id antibody, 3 of 4 patients developed humoral and idiotypic cell mediated response. One among 4 patients had complete remission of the tumor. Another TAA is human milk fat globule membrane antigen expressed in breast cancer. An
Anti-Id MAb targeting this TAA was developed that can induce specific antitumor immune responses in mice, rabbit and monkeys. In human, this antibody can generate high titer of antibody response and T-cell response, but clinical response is not always correlated with the immune response. This antibody was also evaluated in breast cancer patients with bone marrow transplantation. Among several TAAs, CEA has received prime importance in cancer immunotherapy. A number of investigators have generated anti-Id antibodies in rats, mice, baboons and humans that mimic CEA. An anti-Id MAb, designated as 3H1, that mimics a specific epitope of CEA has been developed and characterized. In a Phase I clinical trial with 3H1, development of anti-CEA immunity causing ADCC of CEA positive cells was reported. In another study, this anti-Id therapy was combined with standard chemotherapy, where no adverse effect of 5-fluorouracil on 3R1-induced immune response was observed. Moreover, this vaccine increases overall survival of treated patients. In addition to anti-CEA antibody response, 3H1 may stimulate Th1 type helper T cells to secrete cytokines, like, IFN-γ and IL-2, which in turn, induce cytotoxic T cells. cDNA encoding the variable heavy and light chain of 3R1 has been cloned and sequenced. Sequence information was utilized to develop anti-idiotypic single chain vaccine and to design peptide vaccine to obtain an anti-CEA antibody response.

The human anti-Id antibody mimicking GA733-2 antigen, induced long lasting antigen specific T-cell immunity and specific IgG antibody response against target antigen. Similarly, monoclonal anti-Id antibodies have been developed that mimic the gp72 antigen. Administration of aluminium hydroxide precipitated antibody in 13 patients with advanced colorectal cancer produced blastogenic responses to gp72 antigen in 9 patients. Cryopreserved lymphocytes from this anti-Id immunized patient could kill autologous tumor cells.

Ganglioside antigens are generally overexpressed in cancers of neuroectodermal origin. Several attempts have been made to target these antigens using anti-Id approach. An anti-Id antibody (BEC-2), which mimics disialoganglioside GD3, was examined in a Phase III trial. Another promising anti-Id antibody is 1A7 that mimics T cell independent antigen GD2. Excellent anti-GD2 humoral response was detected using this 1A7 vaccine, which was observed to be reflected in the increment of overall survival of patients. An additional survival benefit was observed after combination of high dose IFN-α2b with this vaccine (unpublished data). Anti-Id antibody mimicking different epitopes of human Her2/neu was reported. This antibody may have therapeutic efficacy for Her2/neu positive cancer patients, which is yet to be tested in clinical trials.

**Adjuvants to enhance the antigen specific immunity**

Modern vaccines are increasingly reliant upon administration with additional substances, termed adjuvant, in order to stimulate effective immune responses against an antigen. An adjuvant can stimulate humoral or cellular or both wings of the immune system by activating B cells, T cells, NK cells, monocytes/macrophages or dendritic cells. Potentiation of the active immune response by an adjuvant also involves the production of various cytokines that work in an integrated fashion both locally and systemically. The use of the same antigen with different adjuvants has been shown to elicit significantly different responses from the immune system. For example, comparison of immunization of mice with killed Schistosomula from *Schistosoma mansoni* with the adjuvant BCG, pertussis, *bacterium parvum*, tetanus toxoid, LPS, yeast glucan, aluminium hydroxide and saponin showed that only the animals immunized with BCG or saponin were protected from live *S. mansoni* challenge. In addition to the efficacy of the adjuvant for eliciting an antigen specific protective immune response, the issue of toxicity of the adjuvant needs further consideration. Freund's adjuvant, considered as a gold standard among all adjuvants, helps to produce excellent humoral and cell mediated immunity, but this water-in-oil emulsion is unsuitable for the human and veterinary uses because of the imparted local and systemic toxicity. Similarly lipopolysaccharide, which is also a strong adjuvant, is highly toxic. At present, aluminium hydroxide (Alugel) precipitated vaccines are approved by Food and Drug Administration, USA for use in tumor immunotherapy. Beside Alugel, some other adjuvants, like, QS-21, are currently being used in clinical trials. An immunostimulatory molecule CpG oligodeoxynucleotide (CpGODN) is also useful as immune adjuvant in tumor antigen immunization. This adjuvant enhances the immune response.
response to vaccine strategies involving T-independent antigen by using CpGODN encapsulated in liposomes. In Trypanosoma cruzi immunization model, this CpG adjuvant is proved as a Th1 promoting adjuvant. This adjuvant is also useful in passive therapy with a MAb in lymphoma. This CpGODN was also used as an adjuvant during vaccination with anti-Id MAb 3H1, mimicking CEA. This vaccination significantly increases overall survival of tumor bearing mice, possibly by induction of ADCC and cytotoxic T lymphocytes. Recombinant cytokines also play a certain role as adjuvant. In multiple myeloma, therapeutic efficacy of idiotype vaccine is disappointing. Use of GM-CSF and IL-2 along with vaccine can increase the antitumor activity. These cytokines enhance the antitumoral response possibly by enhancing the NK and CD8+ T cell activity. An antigen specific immune response and increase in the secretion of IFN-γ, GM-CSF and TNF-α were observed to be elevated in resected melanoma patients when peptide vaccine (derived from tumor antigen gp100 and tyrosinase) was combined with GM-CSF as an adjuvant. A suitable adjuvant that can induce activation of various T cell subsets and production of various cytokines is thought to be more effective and less toxic than immunization with external cytokine as adjuvant. In a recent study, liposomes containing monophosphoryl lipid A coated with alum is proved to be effective to enhance immune response directed against 30KDa secretory protein of Mycobacterium tuberculosis H37Ra. Interestingly, this vaccine formulation along with this neoadjuvant exhibited a Th1 shift in terms of higher IgG2a response over IgG1. Neem leaf preparation was used as an immune adjuvant to increase the immunogenecity of B16melanoma antigen and breast tumor associated antigen (unpublished data). This neem preparation induces the secretion of IL-12 and IFN-γ in vitro (unpublished data). Studies are in progress to define the mode of action of this promising adjuvant.

Dendritic cells (DCs) in tumor vaccine

Dendritic cells (DCs) constitute a family of antigen presenting cells defined by their morphology and their unique capacity to initiate a primary immune response. They originate from bone marrow and are present as immature antigen presenting cells in nonlymphoid tissues. On activation by antigenic challenge and/or inflammation, the DCs mature and migrate to the secondary lymphoid tissues where they present the processed antigen to T cells. As DC has unique T cell stimulatory property, small numbers of DCs are sufficient to induce an effective T cell response. In addition to the activating T cells, DCs may directly modulate growth and differentiation of B cells, and, thus, regulate humoral responses. Because of this unique immunostimulatory properties, several attempts have been made to enhance active specific immunity by using DCs pulsed with vaccine. Different investigators have proved that antigen-pulsed DCs are more effective than general antigen vaccine. As a consequence, DCs are pulsed with TAAs in their different forms, e.g., tumor cell lysate, peptide, proteins, etc. and excellent antitumor effects are reported in vitro and experimental tumor models. Recently, Saha et al. reported that dendritic cells pulsed with 3H1 (an anti-idiotypic antibody mimicking CEA) can induce antigen specific protective immunity. Based on the accumulated evidence, it is suggested that DCs can be considered as a good candidate in designing antigen specific tumor vaccines.

Mechanism of action of tumor vaccine – Role of T cells

In active specific immunotherapy immunization with tumor vaccine results in the generation of anti-TAA antibodies. This antibody may interact with TAA, present on tumor cell surface, to induce ADCC and CDC. Secondly, tumor vaccine is endocytosed by antigen presenting cells, like, dendritic cells and degraded to 14-25 mer peptides to be presented by MHC class II antigens to activate CD4+ helper T cells. Activated CD4+ T cells, secretes Th2 cytokines, like IL-4 and stimulates B cells for antibody production. CD4+ T cells also secrete Th1 cytokines like IL-2 and IFN-γ that activate CD8+ T cells, NK cells and macrophages. On the other hand, endocytosed tumor vaccine (TAA) may be degraded into 9-10 mer peptides to be presented in the context of MHC class I antigen to activate CD8+ cytotoxic T cells. These cells can directly kill tumor cells by different mechanism, e.g., perforin mediated lysis, activation of Fas ligand etc. On the basis of recognition of antigens by T cells, antigens can be divided into two groups, e.g., T dependent and T independent. Most of the protein antigens are T dependent antigen and vaccination with this type of antigen receives T cell help for the tumor.
growth restriction. On the other hand, T independent antigens, like, carbohydrate antigens, mediate their antitumor effect generally through humoral immune system\textsuperscript{31}. Livingstone and his group have used several T independent carbohydrate antigens as a tumor vaccine after forming various antigen-conjugates\textsuperscript{156,157}. Use of adjuvant also helps to increase the potency of such antigens\textsuperscript{158}.

**Progress in tumor vaccine research in India**

Several research groups from India have studied various aspects of TAA biology, however, tumor vaccine research is still in infancy in India. Success in designing and application of tumor vaccine partly depends on the acquired knowledge on TAA or tumors. Expression of TAAs, particularly on different etiologically distinct cancers (oral cancer, cervical cancer etc.), from Indian patient population has prime importance to make a good platform for tumor vaccine research in India. A good database helps to select an antigen for targeting by tumor vaccine.

Studies on TAA, also called tumor marker, can be divided into two groups. Firstly, search for a new antigen and its characterization. Secondly, screening of an established marker in different regions of India. In relation to the search of new TAA, Chattopadhyay and co-workers made significant contribution. A tumor antigen with mol. wt. 83 kDa (MTAA) was isolated from murine mammary tumor virus (MuMTV) induced tumors in mammary gland of C3H/Jax mice\textsuperscript{159} and circulating antibody against MTAA was detected in the serum of breast cancer patients\textsuperscript{160}. Another glycoprotein antigen (mol. wt. 85 kDa) was also isolated and characterized from breast tumor. Diagnostic significance of this antigen is also reported\textsuperscript{161,162}. Investigation on antigenic changes in cervical epithelium either from normal (premenopausal and postmenopausal) or cancer bearing women\textsuperscript{163} demonstrated a 67 kDa antigen from cervical cancerous tissues, which was observed to be cross reactive with serum from cancer cervix and other gynecological patients\textsuperscript{164}. Diagnostic potential of these three antigens was evaluated in a panel of patients, suffering from various types of cancer\textsuperscript{165} and these are reported to be potential diagnostic tool for breast and cervical cancer\textsuperscript{161,165}.

Some efforts have been made to assess the status of various established antigens among Indian patient population. Bhatnagar \textit{et al.} compared the level of CEA in tissue and serum of colon cancer patients. They concluded that determination of tumor CEA could be a useful adjunct for the clinical management of colon carcinoma\textsuperscript{166}. Another group assessed the level of prolactin, CA15.3 and TPA in breast carcinomas\textsuperscript{167}. Muthuswamy and Raste measured CA15.3 in association with CEA. Based on the experimental results, they concluded that CA15.3 reflects tumor burden in a better way than CEA\textsuperscript{168}. Alpha-fetoprotein was found to be increased in hepatoma patients from South India\textsuperscript{169}. A high prevalence of circulating p53 antibodies was observed in 60\% esophageal squamous cell carcinoma patients, indicating its potential as a marker in the screening of high risk population\textsuperscript{170}. P53 was also studied in oral premalignant and malignant lesions in association with p-glycoprotein\textsuperscript{171}. Das \textit{et al.} studied extensively p53 gene in various forms of cancer having high incidence in Eastern India\textsuperscript{172,177}. A 62-64 kDa protein TAA was identified in human oral carcinomas xenografted in nude mice\textsuperscript{178}. This TAA was targeted to lyse oral cancer cells by antibody (3F8E3) dependent cellular cytotoxicity after modulation with cytokines\textsuperscript{179}. Shedding of this TAA limits its use as a vaccine target, however, modulation by rHu-IFN alpha increases firm anchorage of TAA on the cancer cells, thereby, increasing its potential as vaccine target\textsuperscript{180}. Abdulkader \textit{et al.} studied several TAAs and tumor associated viral antigens in oral and cervical cancer patients\textsuperscript{181}, e.g., hepatitis B surface antigen\textsuperscript{182}, herpes simplex virus type 1 antigen\textsuperscript{183}, adenoviral antigen\textsuperscript{184} and p53\textsuperscript{185}. These studies will help to design the immunotherapeutic strategy in our country. However, further elaborate study on other markers expressed in various forms of cancer in different geographical regions of India is essential.

Little is known on tumor vaccine research from India. Talwar \textit{et al.} developed two vaccines against hCG and GnRH for fertility control\textsuperscript{186}. In addition to their effect in fertility control, these vaccines were also utilized in cancer\textsuperscript{186}. A particular type of lung cancer secretes hCG as a growth factor. Anti-hCG vaccine was reported to be effective to combat this particular type of cancer\textsuperscript{187}. On the other hand, anti-GnRH vaccine was used for hormone dependent prostate carcinoma patients\textsuperscript{187}, which may act by inhibiting blood testosterone level.

In 1970s, Ray \textit{et al.}\textsuperscript{188} used neuraminidase treated tumor cells as 'tumor vaccine' and encouraging results were reported. They observed that neuraminidase treated 3-methylcholanthrene induced
fibrosarcoma grew less well in C3H mice compared with the untreated tumor. In DBBN induced fibrosarcoma model reduced transplantability was noted if tumor cells were pretreated with neuraminidase. Several studies in this direction on various tumor models proved the efficacy of neuraminidase treated tumor cells as a ‘tumor vaccine’\textsuperscript{188}. They hypothesized that target cells may be killed directly by immune lymphocyte and macrophages and neuraminidases may alter certain cellular contacts.

Baral \textit{et al.} in their studies, have vaccinated mice with different tumor antigens to examine antibody as well as T cell responses. In these studies, a neem leaf preparation was observed to be effective as an immune adjuvant to enhance the antigen specific immunity (unpublished data). This neem preparation stimulated the murine immune system and activated immune system is able to restrict the \textit{in vivo} growth of murine carcinoma and melanoma\textsuperscript{189}.

**Conclusions and future prospects**

Active specific immunotherapy is a new hope in cancer treatment to avoid the toxicities imparted by chemotherapy and radiotherapy. This approach is effective to kill tumor cells in a specific manner, thus normal cells remain unaffected. Identification of antigen expressed on tumor cells, preferably in early stage, is the primary criteria to design an immunotherapeutic strategy. After getting idea on antigenic status, the antigen in its suitable form is presented \textit{in vivo} to initiate optimum antibody and T-cell responses. The journey on tumor vaccine research was initiated from whole tumor cell lysate vaccine and in subsequent time, several developments have been made including DC based vaccine. Some of these vaccines were tested in clinical trials with some encouraging results (Table 4). This mode of therapy is particularly effective to prevent recurrences after surgical removal of primary tumor and is proved to be safe. Unlike vaccines used for the prevention of different diseases, scope of prophylactic vaccine in cancer is limited, due to unavailability of a universal TAA, till date. This field is still open to find out a solution. Describing new developments of tumor vaccine research, hundreds of papers are published in each year with promising views. The day is not too far when the outcome of extensive tumor vaccine research will be utilized in clinic for the benefit of cancer patients along with conventional therapies. To utilize the full benefit of this treatment, particularly in our country, early detection programme and screening of patients for the appearance of TAA (marker) need to be strengthen in the meantime.

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