

## Immunological Approaches for Treatment of Advanced Stage Cancers Invariably Refractory to Drugs

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### Abstract

Worldwide, deaths due to cancers are taking an increasing toll. Invariably over time cancer cells become refractory to available drugs. At this stage, the tumor is largely metastasized and not amenable to radical surgery or focal radiations. This review seeks to bring out the existence of heterogeneity of cell types in each cancer, and proposes adoption of a combined approach employing more than one therapeutic agent for a more lasting treatment. Also proposed is the use of monoclonal therapeutic antibodies and vaccines against ectopically expressed key molecules for killing of cancer cells and prevention of their multiplication. Focus is on androgen-independent carcinoma of prostate and a variety of cancers expressing ectopically human chorionic gonadotropin (hCG) and/or Carcino-embryonic antigen (CEA). The utility of using antibodies directed against cell membrane located epitopes for homing and delivery of a safe anti-cancerous compound Curcumin to cancer cells is also described.

**Keywords:** Carcinoma prostate; LHRH vaccine; Therapeutic monoclonals; Vaccines against hCG; CEA; Targeted delivery of curcumin

### Introduction

Vaccines were traditionally made against communicable diseases caused by infectious micro-organisms. Their introduction in children's immunization programs brought down drastically deaths occurring in olden days due to infections in many countries. Life span increased significantly. Deaths are now caused increasingly by cardio-vascular ailments and cancers. Cancers detected early are amenable to radical surgical removal. Relapses however occur. A variety of chemotherapeutic drugs combined with radiations and surgery lengthen the life of the patient. However in most cancers, a stage is reached when the cancers are resistant to the available drugs. At this stage, the tumor has metastasized widely and is no longer amenable to surgical removal. Palliative care is given to the patient, which is all the doctors can do, but the death of the patient is inevitable. This article reviews the possible utility of employing immunological therapies for coping with cancers at this stage for lengthening the survival of the patient. Most leads are at present based on laboratory research and observations in experimental animals, but have the potential of clinical application. Also a few clinical trials have been carried out.

### **Carcinoma of Prostate**

Prostate carcinoma is a major cancer of males and accounts for the largest or second largest number of deaths of males due to cancer in many countries. In USA, there were an estimated 2,707,821 men living with prostate cancer in 2011. About 233,000 new cases of prostate cancer will be diagnosed in 2014, which is nearly 14% of all new cancer cases (http://seer.cancer.gov/statfacts/html/prost.html). Upto a stage, its growth is sparked by the male hormone, testosterone (T<sub>4</sub>). Drugs based on counteracting T<sub>4</sub> take care of the patient, but a stage arrives

when it becomes independent of androgens. At both stages, immunological approaches can be employed. At the former hormone dependent stage, vaccination can save considerably the cost of drugs and the frequency of their intake. At the androgen-independent stage, *therapeutic antibodies* offer intervention in a situation where no alternate effective drugs are currently available, in addition to the anti-LHRH vaccine to cope with the fraction of cancer cells dependent on testosterone.

#### A vaccine against LHRH for blocking testosterone

LHRH (Luteinizing-Hormone-Releasing Hormone) is a decapeptide made in hypothalamus. It travels through the portal circulation to the pituitary, where it induces the formation and secretion of the gonadotropins, Follicle Stimulating Hormone (FSH) and Luteinizing-Hormone (LH). These in turn act on gonads to make sperm and testosterone. Blocking LHRH by bio-effective antibodies blocks the entire pathway, akin to non-surgical orchiectomy. Testes & prostate shrink (Figure 1), testosterone falls (Figure 2).



Figure 1: Effect of anti-LHRH vaccine on rat prostate.





Immunological castration is however superior to surgical orchiectomy. It is reversible in contrast to the latter, which is permanent. On decline of antibodies, testes grow again in size and functionality. Thus employing anti-LHRH vaccine, the patient can benefit from retaining normal genital anatomy, while cutting off testosterone during the period that antibodies are in circulation.



**Figure 3: (A)** Amino acid sequence of LHRH-DT Vaccine [1]. **(B)** Structure of LHRH modeled by a knowledge based approach. The molecule takes a bend in the middle to proximate N and C terminal amino acids. Superimposed ribbon drawing highlights the type III b-turn in the hormone.

LHRH, being a "self" molecule, the immune system is tolerant to it. It has to be linked to a carrier to render it immunogenic. We created a linkage site by replacing glycine at position 6 by D-lysine, which was then linked via a spacer with either tetanus or diphtheria toxoid (DT) [1]. This strategy retained the native conformation of the molecule, bringing C and N terminals of LHRH to adjacent positions with a fold in the middle (Figure 3). LHRH-TT/DT thus made was fairly

immunogenic. Adsorbed on alum, it elicited antibodies causing the decline of testosterone to almost castration level (Figure 2) [2].

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## Clinical evaluation of immunizing against LHRH in patients of carcinoma of prostate

After obtaining permission from Regulatory Agencies and approval of Ethics committees, clinical trials were conducted with the LHRH vaccine in 28 patients of carcinoma of prostate, 12 each at the All India Institute of Medical Sciences, New Delhi and at Post Graduate Institute of Medical Research and Education, Chandigarh and 4 patients at Urologische Zentrum Salzburg, Austria. Figure 4 shows the clearance of prostate mass in a patient in Chandigarh. Figure 5 shows the effect in a patient in Austria, where vaccination, causing the formation of anti-LHRH antibodies brought down the testosterone and PSA (Prostatic Specific Antigen). On decline of antibodies, there was a tendency to reversal, which was effectively counter checked by a booster injection.



**Figure 4:** Nephrostograms showing the noticeable reduction of prostatic tissue mass at various stages of immunization with anti-LHRH vaccine.



**Figure 5:** Effect of anti-LHRH vaccine on a patient with advanced carcinoma of prostate. With the generation of antibodies, testosterone and prostatic specific antigen (PSA) levels fall and stay low for several months [2].

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Table 1 summarizes the observations on 12 patients immunized with the LHRH vaccine at AIIMS, 6 patients received a dose of 200  $\mu$ g of the vaccine per injection and 6,400  $\mu$ g. These observations point out to the benefit that vaccination with LHRH vaccine can give to such patients [2].

Effect of immunization	Dose Level	
	200 µg (n=6)	400 µg (n=6)
Clinically Stable/Improvement in Symptoms	4	5
Reduction in Prostatic Size/Hardness	1	3
Reduction in Acid Phosphatases	1	4

**Table 1:** Observations in clinical trials conducted at AIIMS in patients of carcinoma of prostate after immunization with either 200  $\mu$ g or 400  $\mu$ g of anti-LHRH vaccine. Vaccine was administered as 3 primary injections at monthly interval followed by a booster at 8th month [2].

## Androgen independent carcinoma of prostate

**Therapeutic monoclonal antibodies; Combination therapy:** Many years back [3], we developed a monoclonal antibody (MoAb730), which killed in presence of Complement, both DU 145 and PC 3 cells derived from patients dying of androgen independent prostatic carcinoma. The killing was dose dependent, but plateaued at 70-80% of cell death at saturating levels (Figure 6).



Castration-Resistant Prostate Cancer cells, DU145 [3].

This observation was of interest indicating the possibility of developing monoclonal therapeutic antibodies for this stage of the cancer. The fact that one can kill only 70-80% of the cancer cells and not all, pointed to the existence of heterogeneity of cell types in cancers, thereby demanding the requirement of additional antibodies targeting alternate epitopes. Three more monoclonal antibodies were developed. Employing a combination of these enabled the killing of nearly 98% of DU 145 cells (Figure 7).



**Figure 7:** Synergistic Cytotoxic action of MoAbs on Androgen-Independent Castration-Resistant Prostate Cancer cells, DU145. Cytotoxicity was determined by MTT assay [1=7B2G10; 2=730; 3=3C8D4; 4=730+3C8D4; 5=7B2G10+3C8D4].

Thus the combination of antibodies did succeed in killing almost all cancer cells. The lysis of cancer cells by these antibodies involves Complement activation following the binding of the antibodies to the epitopes on membranes of DU145 and PC3 cells. It is assumed that the functions of the Complement system do not diminish in cancer patients.

**Vaccines against advanced prostate cancer:** Two vaccines have shown encouraging results in "metastatic castration-resistant" prostate cancer (mCRPC) patients in recent years. One of them is directed against Prostatic Acid Phosphatase (PAP). It is generated in autologous dendritic cells harvested from patient himself, which are infected with a fusion protein consisting of PAP and GM-CSF. By doing so, it is assumed that the cells become competent to present PAP to the host immune system, with the hope that it will respond by both cell mediated immunity and antibody response. The vaccine has received USFDA approval for use in mCRPC patients. It is reported that this vaccine extends the survival of the patient by a median of 4.1 months. This deduction is made on the basis of a large Phase III trial randomized with controls in 512 patients with minimal disabling symptom of mCRPC. The vaccine made from dendritic cells removed from patients, is given intravenously thrice over a month [4,5].

The second vaccine is a recombinant vaccine built in attenuated strains of vaccinia and fowlpox viruses termed as ProstVac-VF. Both the recombinant vaccinia and fowlpox vectors carry 4 genes: Prostate Specific Antigen (PSA) and 3 T-cell co-stimulatory molecules (TRICOM), which are (i) Leukocyte function-associated antigen-3 (LFA-3), (ii) Intracellular adhesion molecule-1 (ICAM-1), (iii) B7.1. The primary immunization is carried out with Vaccinia based recombinant vaccine (ProstVac-V) and boosters are given with recombinant fowlpox virus vaccine (ProstVac-F) carrying the PSA and TRICOM. The vaccine has undergone randomized, double blind Phase II placebo controlled trials. Each patient received vaccinia based recombinant vaccine for primary immunization followed by booster injections of fowlpox based vaccine. Each is given with GM-CSF as adjuvant. The vaccine treated group had a median overall survival of 25.1 months versus 16.6 months in the control group [6].

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## Antibodies for Homing and Targeted Delivery of Safe Anti-Cancerous Compounds to Cancer Cells

Antibodies have the ability to "home" specifically to discrete epitopes on the antigen, be these on membranes of cells or elsewhere. Could the antibodies be employed to deliver a "Drug" directly to the cancer cell? We tested this possibility for MOLT-4, a cell line developed from a T lymphoblastic leukemia patient in relapse. These cells express ectopically hCG but anti-hCG antibodies do not kill these cells with or without Complement, even though the antibodies bind with the cells. We linked Curcumin (diferuloyl methane), the active principle of Curcuma longa with a humanized monoclonal against hCG, cPiPP. This was achieved by creating an amino linker on terminal hydroxyl group of Curcumin, which was condensed with carboxylic acid of acidic amino acids of the antibody. It may be stated that Curcumin has anti-inflammatory and anti-cancerous properties [7]. It is totally non-toxic and fully safe. Phase I clinical trials showed that amounts taken upto 8 gms per day orally were well tolerated and caused no side effects of any type in humans [8]. While cPiPP, the anti-hCG antibody did not kill any MOLT 4 cell (Figure 8a), the same cells incubated with c PiPP-curcumin conjugate were killed in proportion to the dose of the conjugate, reaching 98.3% at 100 µg concentration of the conjugate (Figure 8b and 8c). The antibodycurcumin conjugate itself was not cytotoxic. It did not exercise any cytotoxicity on peripheral blood mononuclear cells (PBMCs) of normal healthy donor (Figure 8d) [9].



**Figure 8:** Cytotoxic effect of cPiPP-curcumin on MOLT- 4 cells. (a) 0.1 million cells were cultured with RPMI 1640 supplemented with (i) 0  $\mu$ g, (ii) 10  $\mu$ g, (iii) 50  $\mu$ g, and (iv) 100  $\mu$ g/ml of the antibody equivalent, for 48 h. FACS analysis of cells was carried out after staining with Propidium Iodide. (b) Cytotoxic effect of cPiPP curcumin conjugate on MOLT-4 cells by FACS analysis of PI-stained cells at (i) 0  $\mu$ g, (ii) 10  $\mu$ g, (iii) 50  $\mu$ g, and (iv) 100  $\mu$ g/ml. Percentages of dead cells appearing in right lower quadrant were 0.9, 68, 96 and 98.3%, respectively. (c) The cytotoxic effect of the immunoconjugate was confirmed by trypan blue exclusion assay. Curcumin conjugated to an irrelevant antibody (MoAb 730) was devoid of cytotoxicity on MOLT-4 cells. (d) Lack of cytotoxic action of cPiPP-curcumin conjugate on PBMCs bearing CD13 marker of an AML patient (R.D.) (9).

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The cytotoxic action of cPiPP-curcumin was not only exercised on MOLT-4 cells, but also on U-937 lymphoma cells killing at saturating dose of the conjugate the entire lot of cells expressing hCG ectopically. Thus employing "homing" antibodies to deliver curcumin acted as a magic bullet killing the target cancer cells. It may be stated that Curcumin is a potent inhibitor of Stat 3, which plays a pivotal role in tumor growth, invasion, and metastasis of many cancers [10].

# Expression of hCG/subunits by Advanced Stage Cancers

Human Chorionic Gonadotropin (hCG) is normally made by the early embryo soon after fertilization of the egg [11]. It plays a vital role in implantation of embryo and in sustenance of pregnancy. Neither non pregnant females, nor healthy males make this hormone. Since late, however several reports have appeared on ectopic or unexpected expression of hCG or its subunit by a variety of cancers: lung cancer [12], bladder carcinoma [13,14], pancreatic carcinoma [15,16], breast cancer [17], cervical carcinoma [18,19], oral cancers [20,21], head and neck cancers [22], prostate cancer [23], renal carcinoma [24], colon adenocarcinoma [25], gastric carcinoma [26,27], vulva/vaginal cancers [28,29]. Invariably the expression of hCG/subunits takes place at an advanced stage of cancer. The prognosis of such cancers is poor and survival adverse of the patients carrying the  $\beta$ -hCG expressing cancers

than the patients suffering from the same type of cancers but not expressing hCG or its subunits [30]. It appears that the dedifferentiation of cells goes to a stage that they become like embryonic cells, thereby expressing proteins such as hCG and Carcinoembryonic antigen (CEA). hCG is a promoter of invasiveness and angiogenesis [31]. Anti-hCG antibodies exercise a cytotoxic effect on A549 lung cancer cells *in vitro* [32]. In nude mice, the growth of Chago lung cancer is blocked in proportion to the antibodies injected (Figure 9) [33]. Similar observations have been made on colorectal cancer cells (CCL-253). These cells express hCG, and anti-hCG antibodies kill these cells in presence of Complement *in vitro* [34]. Also in nude mice implanted with CCL-253 colorectal cancer cells, administration of anti-hCG antibodies caused a significant reduction in tumor uptake & all treated animals with anti-hCG antibodies survived in contrast to the mortality of control animals [34].

Susana Rulli has developed transgenic mice expressing hCG $\beta$ . The female transgenic mice develop pituitary hypertrophy, mammary tumors over & above ovarian dysfunction [35]. Immunization of these transgenic hCG $\beta$  mice with a recombinant anti-hCG vaccine developed by us [36] prevents them becoming obese, develop insulin resistance and various other abnormalities [32]. Their life span was longer.



**Figure 9:** Inhibition of tumour induction by anti- $\alpha$ -human chorionic gonadotropin (hCG) antibody. Human lung cancer Chago cells (expressing hCG $\alpha$ ), 1×10<sup>6</sup> in 0.5 mL of PBS buffer along with different concentrations of anti-hCG $\alpha$  antibody, were transplanted under the dorsal skin of athymic mice (three animals in each group). The control group was given transplants of the same number of cells and an equivalent amount of normal serum (designated as 0 ng of anti-hCG $\alpha$  antibody [ $\alpha$ -HCG-ab]. Series of panels under A, B, C, D, and E show tumour sizes photographed after 2, 4, 6, 8, and 10 weeks, respectively, after transplantation of cells with indicated concentrations of antibody [33].

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## Clinical evaluation of vaccines against hcg in patients with advanced epithelial malignancies

A vaccine CDX-1307 was developed in which hCG beta subunit was fused to mannose receptor specific monoclonal antibody [37]. It was given intradermally and intravenously in patients with advanced epithelial malignancies. To improve its immunogenicity, GM-CSF and Toll-like receptor (TLR)-3 agonist poly-ICLC and TLR7/8 agonist Resiquimod (which activate the APCs), were given as adjuvants. While no significant anti-hCG response was seen in patients with CDX-1307 alone but those delivered in combination with TLR agonists elicited some response. The response in patients varied with the degree of immune response induced and was the greatest in one patient where the circulating hCG could be decreased by immunological intervention. Only two patients had a stable disease for 8.8 and 18.2 months. Both had evidence of humoral and cellular immune responses generated by the vaccine [37].

These studies point out to the possible benefit that a potent antihCG vaccine can bring in patients with advanced stage cancers. The recombinant hCG-LTB vaccine developed by us is highly immunogenic [36] and evokes hundred percent positivity of response in mice. It employs human use permissible adjuvant, *Mycobacterium indicus pranii* (MIP) which is potent invigorator of immune responses. The vaccine has received approval of the National Committee on Genetically Modified Recombinant Products (RCGM) and has completed toxicity on an International protocol in rodents and marmosets. It is due to go for human trials for control of fertility in the coming months under the aegis of the Indian Council of Medical Research (ICMR). These trials would provide further data on its immunogenicity in humans. There is every hope that this vaccine would be available in the near future for therapeutic intervention in patients with advanced stage cancers refractory to available drugs.

## Anti-tumour Properties of a Vaccine Invigorating Immune Responses

We developed many years back, an immuno-therapeutic vaccine for multi-bacillary lepromatous leprosy [38]. It was based on nonpathogenic mycobacteria, coded in our investigations as M.w. It has since been sequenced. As no such Bacillus existed previously in World Data Bank, it has been named as Mycobacterium indicus pranii (MiP), Pran being first name of Talwar [39,40]. MiP renders nearly 70% of lepromin negative multibacillary patients to lepromin positivity status, who otherwise continue to be lepromin negative even after they are cured by persistant multidrug (MDT) regime. Lepromin is a Delayed hypersensitivity test to M. leprae antigens. Lepromin negativity is one of the criteria for diagnosis & classification of leprosy patients to the lepromatous category. The immunological deficit in these patients is their inability to recognize and react to some key M. leprae antigens. MiP used as adjunct to MDT, expediated bacterial clearance and shortened the period of recovery [38]. Mycobacterium indicus pranii is also observed to be a potent adjuvant for enhancing antibody titres to a vaccine against human chorionic gonadotropin (hCG). It induces both Th1 and Th2 response, which is reflected in the production of not only IgG1, but also IgG2a and IgG2b antibodies [41].

*Mycobacterium indicus pranii* has received the approval of the Drugs Controller General of India (DCGI) and also of the USFDA. It is licensed to a company in India. It is in the market and available to all for human use. Figure 10 is an electron micrograph of this Bacillus. It is active in autoclaved killed form, as well as in a live form, where it

manifests a more pronounced protective effect against tuberculosis [42]. It is also highly effective as adjunct to chemotherapy for tuberculosis.

What is amazing is the ability of *Mycobacterium indicus pranii* to both prevent & treat tumours, such as Myeloma in mice. This work has been done by Dipankar Nandi at the Indian Institute of Science Bangalore. SP2O Myeloma cells develop into tumour in mice. Immunization with *Mycobacterium indicus pranii* before implantation of the tumour, as well as given after SP<sub>2</sub>O cells were given, prevented the growth of the tumour to variable extent. Figure 11 is a summary of their observations, reported by them elsewhere [43].



## Immunological Approaches against Carcinoembryonic Antigen (CEA)

Carcinoembryonic antigen (CEA), a tumor-associated marker is expressed by a large number of human cancers particularly at the advanced stage of the disease. CEA is reported to be expressed by 90% pancreatic cancers, 90% colorectal cancers, 90% gastrointestinal cancers, 70% Non-small cell lung cancer, 50% breast cancer [44,45], and also by cervical and ovarian cancers [46]. Furthermore, its role in cellular adhesion, invasion and cancer metastasis has also been well established. In the past decade a number of groups have been engaged in developing immunological approaches against CEA. It is observed that the vaccines against CEA, differ in either the vector employed and/or the epitopes which induce variable degree of protective response. A group led by Dr. Mohebtash [47] investigated the efficacy of PANVAC vaccine, which is a recombinant poxvirus vaccine that contains transgene for two tumor associated antigens CEA and MUC1 (cell surface associated glycoprotein) and three T-cell costimulatory molecules (B7.1, ICAM-1 and LFA-3). The vaccine was tested in a clinical trial in 14 ovarian and 12 breast cancer patients, all of whom were previously treated by chemotherapy. In the breast cancer patients, median survival time was 13.7 months but one patient remained on study for 37 months and 4 patients had stable disease. In Citation: Talwar GP, Gupta JC, Kumar Y, Nand KN, Ahlawat N, et al. (2014) Immunological Approaches for Treatment of Advanced Stage Cancers Invariably Refractory to Drugs. J Clin Cell Immunol 5: 247. doi:10.4172/2155-9899.1000247

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the ovarian cancer patients, median survival was 15 months, one ovarian cancer patient was stable for 38 months before her disease progressed. Staff et al. [48] have reported that a DNA vaccine against CEA in combination with GM-CSF was well tolerated and did not show any sign of autoimmunity. 10 patients were enrolled in this trial, all of whom had undergone surgical resection of colorectal cancers. 8 patients did not show any sign of disease after a median follow-up of 72 weeks. One patient had disease recurrence at week 52 but was still alive at the end of 72 weeks while one died of bladder cancer which was detected later. Kaufman et al. [49] reported that a canary pox based vaccine (ALVAC-CEA/B7.1) induced T-cell immunity in patients with metastatic colorectal cancer. Increase in CEA-specific T cells was detected in 50%, 37%, and 30% of patients in 3 different groups comprising a total of 118 patients studied. Wahid et al. [50] have reported that a vaccine against CEA N-domain blocks the formation of tumor in CEA-expressing transgenic mice. Zheng et al. [45] have reported a novel monoclonal antibody, CC4, against CEA which suppresses colorectal tumor growth and enhances NK cells-mediated tumor immunity. A group led by Sarkar et al. [51] has reported that a Dendritic cell vaccine against CEA in combination with neem-leaf glycoprotein induces anti- tumor immunity, which protected mice from tumor development and these mice remained tumor free following second tumor inoculation, indicating generation of effector memory response.



**Figure 11: MIP treatment suppresses tumor growth and induces a Th1 cytokine response. (a)** General outline of the *in vivo* experiment protocol. **(b)** Comparison of the anti-tumor effects of MIP administered at different time points. Cohorts of ten mice were inoculated s.c. with ~10<sup>7</sup> Sp2/0 cells. Mice were injected i.d. with a single dose of MIP (~5×10<sup>8</sup>) either one day (-1D) before or 3 (+3D) or 6 (+6D) days after tumor inoculation. Mice injected i.d. with PBS on day 3 were included as controls. The growth of tumors (mean  $\pm$  SD mm<sup>3</sup>) at indicated days post implantation. **(c)** Representative photographs of solid tumors from different treatment groups dissected on day 14 [43].

## Antibodies for Negating Immune Inhibitorycheckpoints

It is increasingly being realized that cancers are recognized by the immune system, and under normal circumstances, the immune system may control and even eliminate tumors at the nascent stage. Tumors can avoid immune surveillance by stimulating immuno-inhibitory receptors that function to turn off established immune responses. By blocking the ability of tumors to stimulate inhibitory receptors on T cells, sustained, anti-tumor immune responses can be generated. Thus, therapeutic blockade of immune inhibitory checkpoints provides a potential method to boost anti-tumor immunity. This approach has been exploited successfully for the generation of a new class of anticancer therapies, "checkpoint-blocking" antibodies, exemplified by the recently FDA-approved agent, Ipilimumab, an antibody that blocks the co-inhibitory receptor CTLA-4 (cytotoxic T lymphocyte antigen-4). Taking advantage of the success of Ipilimumab, agents that

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target a second co-inhibitory receptor, PD-1, or its ligand, PD-L1, are in clinical development [52].

### CTLA-4 (Cytotoxic T Lymphocyte Antigen-4)

The T-cells are regulated at multiple levels to prevent inappropriate activation (i.e. autoimmunity) and the inhibitory activity exerted by CTLA-4 represents an important checkpoint at the periphery. CD4+ and CD8<sup>+</sup> T-cells require at least two signals between T-cells and antigen presenting cells (APCs) to get activated. The first signal consists of the presentation of an antigen to T cell Receptor (TCR) by a major histocompatibility complex molecule on an APC. The second co-stimulatory signal is generated by binding of the CD28 receptor on T-cells to B7 molecules on APCs. The activated CD28 receptor engages the same B7 molecules as the inhibitory CTLA-4 receptor (though with reduced affinity). CD28 and CTLA-4 display a different pattern of expression on T-cells: CD28 is constitutively expressed on the surface of T-cells; CTLA-4 is slightly detectable in naïve T-cells and appears upon the activation of T-cell. Binding of CTLA-4 to B7 molecules negatively regulates activated T-cells. In addition to this competition with CD28, CTLA-4 can directly inhibit TCR signals, reduce IL-2 production and IL-2 receptor expression, and regulate cell cycle progression. The final result of CTLA-4 activation is the induction of peripheral tolerance in antigen specific T-cells [53].

Ipilimumab, an anti-CTLA-4 antibody, was approved by US Food and Drug Administration in March 2011 to treat patients with late stage melanoma (a type of skin cancer) that had spread and could not be removed by surgery. It is a new generation immunotherapeutic agent that has shown activity in terms of disease free and overall survival in metastatic melanoma patients [53]. In addition to melanoma, Ipilimumab is undergoing clinical trials for the treatment of non-small cell lung carcinoma (NSCLC), metastatic hormonerefractory prostate cancer and other advanced solid tumors [54].

### PD-1 (Programmed Cell Death-1 protein)

PD-1 is one of the most important inhibitory checkpoint responsible for mediating tumor-induced immune suppression, normally involved in promoting tolerance. PD-1 is a cell surface coinhibitory receptor expressed on T cells, B cells, monocytes, and natural killer cells, following activation. If another molecule, called Programmed Cell Death 1 ligand 1 (PD-L1), binds to PD-1, the activated lymphocytes die. PD-1 expression by tumor-infiltrating lymphocytes (TILs) is associated with impaired effector function (cytokine production and cytotoxic efficacy against tumor cells) and/or poor outcome in several tumor types [55]. Moreover, a variety of tumors, including renal cell carcinoma (RCC), melanoma (MEL), stomach, breast, ovarian, pancreatic, and lung cancers, have been shown to express PD-L1, potentially contributing to immune suppression and evasion. PD-L1 expression on tumor cells has been shown to correlate with poor prognosis in patients with RCC, MEL, breast, pancreatic, stomach, bladder, lung, liver, and ovarian cancers [55].

Three monoclonal antibodies against PD-1, and one against PD-L1, have undergone Phase 1 clinical trial. All four antibodies (Nivolumab, Lambrolizumab, Pidilizumab, mAb BMS-936559) have shown encouraging preliminary activity, and those that have been evaluated in large number of patients have shown encouraging safety profiles. The fully human anti–PD-1 mAb Nivolumab, tested in renal cell cancer (RCC), MEL, castration resistant prostate cancer (CRPC), non– small cell lung cancer (NSCLC), and colorectal cancer (CRC), has demonstrated antitumor activity in Phase 1 trials [55,56]. The humanized anti-PD-1 antibody Lambrolizumab has also demonstrated antitumor activity in patients with solid cancers in a Phase 1 study [55]. Pidilizumab, a humanized anti-PD-1 antibody, has been evaluated in advanced hematologic malignancies, and demonstrated potential clinical activity in patients with non-Hodgkin's lymphoma, chronic lymphocytic leukemia, Hodgkin's lymphoma, multiple myeloma, and acute myeloid leukemia [55,57]. The anti-PD ligand 1 (PD-L1) mAb BMS-936559 has shown preliminary antitumor activity (tumor regression and prolonged stabilization of disease) against various solid cancers: non-small-cell lung cancer, melanoma and renal cell carcinoma [55,58].

## **Concluding Comments**

People die of cancer, even though surgery, radiations and a plethora of drugs are available. These take care of the patient till the stage, when neither of these is functional. It is at this advanced terminal stage, immunological approaches in form of vaccines and monoclonal therapeutic antibodies offer the last solace.

Reviewed is the work of the author and his coworkers on 2 vaccines: against LHRH and hCG, and on monoclonal antibodies developed against androgen-independent carcinoma of prostate. Both vaccines are highly immunogenic. LHRH vaccine is usable for prostate carcinoma as well as for hormone dependent breast cancers, being given that the decapeptide is common to both males and females.

The recombinant hCG vaccine is highly immunogenic in all genetic strains of mice tested. Adsorbed on Alhydrogel, and given along with autoclaved suspension of *Mycobacterium indicus pranii* (MiP) as adjuvant, it induces both  $Th_1$  and  $Th_2$  response in 100% of animals. MiP by itself is a strong invigorator of immune response and has demonstrated the capability of preventing and treating  $SP_2O$  myelomas in mice.

A combination of 2 monoclonal antibodies developed by us, are competent to kill near to 98-100% of DU145 and PC3 cells derived from patients dying of androgen-independent carcinoma of prostate.

Antibodies may by themselves kill the target cancer cells by inactivating a growth promoting molecule, or these may lyse the cells in presence of Complement. An alternate but highly effective use of an antibody recognizing an epitope on the cancer cell membrane, is to employ these for 'homing' a safe, anti-cancerous compound such as Curcumin to the cancer cells. The efficacy of such 'targeted magic bullets' is demonstrated by the ability of an anti-hCG monoclonal antibody linked to Curcumin to kill 100% of Molt-4 lymphoblastic leukemia cells.

A number of vaccines and antibodies against Carcinoembryonic antigen (CEA) are in clinical trials. There are also vaccines and monoclonal antibodies directed against a variety of other target molecules impacting the growth of cancer cells. Table 2 shows a number of monoclonal antibodies and vaccines which are either approved for clinical use or at different stages of development. The entire field is abuzz with activity around the World. Some of these, but not all, are reviewed in this chapter. Their success in controlling advanced stage cancers varies with the degree of their immunogenicity, and indeed a number of adjuvants and immunostimulating agents have been employed to improve the efficacy of intervention. Citation: Talwar GP, Gupta JC, Kumar Y, Nand KN, Ahlawat N, et al. (2014) Immunological Approaches for Treatment of Advanced Stage Cancers Invariably Refractory to Drugs. J Clin Cell Immunol 5: 247. doi:10.4172/2155-9899.1000247

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Antibodies	Target Antigen	Developed by	Indication	Status		
lpilimumab	CTLA-4	Bristol-Myers Squibb	Metastatic Melanoma	FDA approval		
Bevacizumab	VEGF-A	Genentech/Roche	Metastatic colon cancer	FDA approval		
Panorex 17-1A	EpCAM	GlaxoSmithKline/Centocor	Colorectal cancer	German approval		
Rituxan	CD20	IDEC Pharm	Non-Hodgkin's Lymphoma	FDA approval		
Herceptin	Her2/neu	Genentech	Metastatic Breast cancer	FDA approval		
MoAb730	Tumor antigen on DU145 cell line	Talwar Research Foundation	Castration-resistant prostate cancer cells DU145 and PC3	Preclinical development		
MoAb7B2G10	Tumor antigen on PC3 cell line	Talwar Research Foundation	Castration-resistant prostate cancer cells DU145 and PC3	Preclinical development		
MoAb3C8D4	Tumor antigen on PC3 cell line	Talwar Research Foundation	Castration-resistant prostate cancer cells DU145 and PC3	Preclinical development		
PiPP	β subunit of human chorionic gonadotropin (hCGβ)	Talwar Research Foundation	Advanced stage cancers ectopically expressing hCG	Preclinical development		
PiPP-curcumin conjugate	β subunit of human chorionic gonadotropin (hCGβ)	Talwar Research Foundation	Kills MOLT-4 cells (human T- lymphoblastic leukemia cells) and U-937 cells (histolytic lymphoma cells)	Preclinical development		
MoAbCC4	Carcinoembryonic antigen (CEA)	National laboratory of Biomacromolecules, Chinese Academy of Sciences, Beijing	Colorectal cancer	Preclinical development		
Nivolumab	PD-1	Bristol-Myers Squibb	Renal cell cancer, melanoma, castration resistant prostate cancer, non-small cell lung cancer, colorectal cancer	Undergone Phase-I trial		
Lambrolizumab	PD-1	Merck	Solid cancers	Undergone Phase-I trial		
Pidilizumab	PD-1	CureTech Ltd.	Advanced hematologic Malignancies	Undergone Phase-I trial		
MoAb BMS-936559	PD-L1	Bristol-Myers Squibb	Non-small-cell lung cancer, melanoma, renal cell carcinoma	Undergone Phase-I trial		
Vaccines						
Sipuleucel-T	Prostatic acid phosthatase	Dendreon	Castration-resistant prostate cancer	FDA approval		
Racotumomab	NeuGcGM3	Recombio	Advanced stage lung cancer	Argentina and Cuba approval		
Oncophage	HSPPC-96	Antigenics.Inc.	Kidney cancer	Russian approval		
PANVAC	Carcinoembryonic antigen (CEA)	National Institute of Health (NIH), USA	Ovarian and breast cancers	Undergone Phase-I trial		
CEA-LTB	Carcinoembryonic antigen (CEA)	Talwar Research Foundation	Advanced stage cancers ectopically expressing CEA	Preclinical development		
ProstVac-VF	Prostate specific antigen	National Institute of Health (NIH), USA	Castration-resistant prostate cancer	Undergone Phase-I trial		
CDX-1307	$\beta$ subunit of human chorionic gonadotropin (hCG $\beta$ )	Celldex	Advanced epithelial cancers expressing hCGβ	Undergone Phase-I trial		
hCGβ-LTB	$\beta$ subunit of human chorionic gonadotropin (hCG $\beta)$	Talwar Research Foundation	Advanced stage cancers ectopically expressing hCGβ	Ready for clinical trials		
LHRH-D-Lys-R-N=CH- (CH2)3-CH=N-DT	Luteinizing-Hormone-Releasing Hormone (LHRH)	Talwar Research Foundation	Carcinoma of prostate	Undergone successfully Phase II trials in 3 centers in India and Austria		

**Table 2:** Antibodies and vaccines against cancers and their status.

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