How is Cancer Immune Response Formed?

When the cancer cells first start to multiply, macrophages phagocyte the cancer cells while other cancer cells occupy the nearby tissues and cells. Macrophages digest the cancer cells and demonstrate antigenic parts of cancer cells on the surface. After that by connecting to the microphages (dendritic cells), T helper cells recognize the presented antigens and this connection causes the release of many cytokines from both cells. Thus, antigen presentation as an immune response to cancer takes place. The released cytokines induce the formation of more cytokines and antibodies by inducing especially the IL-2, T helper, cytotoxic T and B cells. Induced cytotoxic T cells headed towards the cancer cells which are carrying the same antigen start to form holes on these. Thus, a cytotoxic effect towards cancer is demonstrated. Finally, antibodies released from B cells connect to free floating cancer cells, and thus, a target to destroy is shown to the microphages, and complement system is activated. When the cancer cells are under control, B and T cells are passivated by suppressor T cells. Stored T and B Cells stay ready in order to provide quick response in case antigens for cancer cells are observed (1,2).

Vaccine Platforms

Used vaccine treatments can be divided into main titles as the following (3);
- Tumour cells (autologous and allogenic)
- Dendritic cell
- DNA
- Viral vector
- Protein/peptide
- Immune regulators

**Immunotherapy in Prostate Cancer**

**GVAX: Tumour Cell Vaccine (Intradermal)**

Allogenic prostate cancer (PCa) cells are used as immunotherapy vectors in this tumour vaccine. It is formed by two PCa cell series called PC3 and LNCaP.

**LNCaP:** Cancer cells with lymph node metastasis secrete many prostate surface antigens including prostate-specific antigen/prostate-specific membrane antigen (PSA/PSMA).

**PC-3:** These are androgen-resistant cells obtained from bone metastasis (4).

Normally, cancer cells are not immunogenic. The cells are genetically modified by applying radiation and adenoviral transfer to PC-3 and LNCaP cell series. These cells take effect inhibiting the division of cancer cells with cytokines secreted by becoming immune active (5,6).

Simons et al. (phase 2 study) followed up 24 patients with castration-resistant prostate cancer (CRPC) for two years giving high-dose and low-dose GVAX treatment and found that the high dose group survival was 70% compared to the 41% survival rate in low-dose survival group (7). Average survival was 26.2 months in a phase 2 study on 55 metastatic CRPC patients, 23.1 months in low-dose group in phase 2 study on 80 metastatic CRPC patients and 35 months in high-dose group and, it was detected that GVAX treatment was safe and tolerated. Autoimmune toxicity was not present in the patients and it was observed that high-dose treatment was more effective on survival. The most common treatment-based side effects were fatigue, myalgia, arthralgia, and infection-site reaction (8,9). After that, phase 3 studies for GVAX were started. In a phase 3 VITAL 1 study, 626 asymptomatic CRPC patients not having chemotherapy were randomized in sipuleucel-T and placebo branches. In this study, no advantage was observed in sipuleucel-T branch in 3-year survival (15). In a similar phase 2 study, 98 patients with metastatic PCa were randomized in sipuleucel-T and placebo branches. In this study, no advantage was found in 3-year survival in sipuleucel-T branch (16). In post-hoc analysis of these two studies (225 patients), average survival period was reported to be 23.2 months in sipuleucel-T branch while it was 18.9 months in placebo branch (17).

In FDA controlled phase 3 IMPACT study in which 75 centres were included, 512 asymptomatic or minimal symptomatic metastatic CRPC patients were separated into placebo (171 patient) and Sipuleucel-T (341 patients) branches and patients were followed for 34.1 months in average. Decrease in relative death risk was determined as 22% in medicine branch. Average survival was observed as 25.8 months in medicine branch and 21.7 months in placebo branch. 4.1 months of survival advantage was detected in medicine branch. Although the 3 year total survival was 38% more than placebo, it was reported that the medicine had no effect on the time passing up to progression. After this study Sipuleucel-T FDA consent was taken (16,18).

**Disadvantages of Sipuleucel-T treatment:** They can be listed as having no effect on the time passing up to progression, the possibility of late regression in secondary PSA to immune response time, inability to effectively follow the patients with PSA and progression response for these reasons and the cost of 3 leukopheresis sessions being 93000 USD and not being docetaxel compared study.

**Advantages of Sipuleucel-T treatment:** They were observed as general survival advantage, good tolerability, short treatment duration (30 days), not prohibiting treatment which may be applied after as in chemotherapy, mild side effect profile (4).

In 2015 National Institute for Health and Care Excellence (NICE) stated that asymptomatic or minimal symptomatic CRPC usage instead of Sipuleucel-Ts usage in metastatic patients is more appropriate (19).

**Prostvac VF-Tricom: Viral Vector Vaccine**

The aim of this vaccine is activating a stronger immune system activation by synthesizing high amount of prostate cancer cell or antigen by viral vectors. The vaccine called Prostvac was formed by synthesizing from the combination of flower viruses and recombinant PSA with heterologous prime-boost strategy (16). A more immugenic vaccine called TRICOM was formed by using viral DNA plasmids with the combination of three stimulator proteins CD80, intracellular adhesion molecule-3 (IAM3) and leukocyte function antigen-3 (LFA3) (20).

No toxicity was observed in 4 weeks in the phase 1 study made and 8 week PSA stabilization was observed in 40% of the cases (21). In phase 2 study, 82 (42/40) metastatic CRPC patients were randomized into placebo and prostvac branches and survival was observed as 25.1 months in survival medicine branch and 16.6 months in placebo
A two-stage treatment was applied to PCA patients without apparent metastasis in the current phase 2 study made and Prostvac+GM-CSF treatment in the first stage and androgen ablation treatment in the second stage was given. While pre-treatment median PSA velocity was 0.13log(PSA) per month and PSA doubling time was 5.3 months, PSA velocity was measured 0.03log(PSA) per month and PSA doubling time was measured 7.7 months. Complete response was reported in 20 of 27 patients after two staged treatment. As the number of patients is limited in this study and there is no control group, it is limited to evaluate the success of the treatment (27).

**DNA and RNA Based Vaccines**

**DNA–Prastatic Acid Phosphatase and DNA–Prostate Specific Antigen: DNA-Based Vaccines**

They contain genetic structure coded specifically for prostate specific proteins. Plasmid DNA vaccine for PAP is called DNA-PAP (pTVG-HP/PAP) and PSA producing DNA plasmid vaccine is called DNA-PSA (pVAX/ PSA) vaccine.

In the phase 1/2a study made, human PAP coding plasmic vaccine (DNA-PAP) + GM-CSF was given to 22 non-metastasic PCA patients with biochemical progression and PSA doubling time prolongation in PAP specific T lymphocyte response was determined (28).

In phase 1 study which was made by giving DNA-PSA + GM-CSF+IL-2 to nine CRPC patients, PSA specific significantly increased T cell response and PSA doubling period prolongation in two out of five patients given high dose were reported (29). In spite of these results, use of DNA based vaccines are limited with potential low efficiency.

**CV9103 and CV9104: mRNA vaccine (RNActive®)**

It is a nucleotide based vaccine. Sufficient antigen expression formation, autologous immune stimulation and flexibility in production and application are positive characteristics of these vaccines. In phase 1/2 studies, there are studies showing that CV9103 is well tolerated, makes immune-activation and CV9104 has positive effect as neoadjuvant in high risk prostate cancer and CRPC patient (30).

**AdV-tk: Cytotoxic Immunotherapy with Gene Agent**

It includes the inner tumour application of adenovirus coded with thymidine kinase which is a herpes simplex virus enzyme. Tumour cells with transduction become over sensitive with valaciclovir (VCV) and ganciclovir (GCV). These medicines especially effect the neighbour cells which reproduce quickly afterwards (local bystander effect). They immunologically attack systematic metastasises and protect against tumour recurrence (systemic bystander effect) (31).

In Phase 1-2 study made with 23 local advanced PCA patients, safety and treating potential of AdV-tk was tested before the prostatectomy and AdV-tk was injected intraprostatically and prostatectomy was made 2-4 weeks after the 2 week GCV treatment. As a result, significant CD8+T lymphocyte increase in blood and resected prostate tissue was detected and no change in CD4+T lymphocyte, natural killer level and no significant recovery in biochemical PSA recurrence and prognosis was observed. AdV-tk-based and chemotherapy combination therapies have not been completely researched clinically (32).

In Phase I/II study made AdV-tk and combined radiotherapy (RT) was applied to patients with prostate cancer, the patients were separated in three categories with 4 patients in each as 29 patients with low risk (stage T1-T2a, Gleason score <7), 26 patients with high risk (stage T2b-T3, Gleason score >6) and 44 patients with D1 disease. Average follow-up period in the study was more than 13 months. It was observed that PSA levels were under control in all low risk and high risk patients. But in three of D1 patients, biochemical failure was detected (33). Phase 3 study *ProstAtak™* including medium-high risk localized prostate cancer patients with placebo controlled, AdV-tk+radiotherapy combinations application still goes on.

Main studies with vaccine treatment in prostate cancer and continuing studies are summarized in Table 1 and Table 2.

**Immune Regulators**

**Anti-CTLA-4 Antibody "Ipilimumab" and "Tremelimumab": Checkpoint Inhibitor (Checkpoint Blocking Antibodies)**

As cytotoxic T-lymphocyte antigen-4 (CTLA-4) is the T cell activation negative regulator, Anti-CTLA-4 antibody "Ipilimumab" is started to be used as target treatment in cancer immunotherapy. It shows effect by providing tumour regression by T cell activation and proliferation (34,35).

In Phase 1-2 study on 50 patients in which monotherapy and radiotherapy are applied together, average survival period was measured as 17.4 months, complete response from 3.6% of the patients and partial response from 7.1% of the patients were taken and stable disease in 21.4% and PSA decrease over 50% in 16% of the patients were observed (36). Significant PSA decrease and objective clinical response were observed in phase 1 studies made by combining GM-CSF GVAX and Prostvac (37). Ipilimumab and placebo was applied (399/400) to metastatic CRPC patients who had progressed radiotherapy after docetaxel chemotherapy in phase 3 study made with 799 patients and average survival was detected as 11.2 months in medicine group and 10 months in placebo group.

Survival period without progression was found statistically significant when compared to the placebo branch (4/3.1 months, p<0.001) (38). Common side effects were weakness, rash, itching, vomiting, constipation and weight loss and it was stated that adrenal deficiency, hepatitis and autoimmune colitis were observed when evaluated immunologically (37,38).
<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment agent</th>
<th>Patient selection</th>
<th>Study design</th>
<th>Phase</th>
<th>Number of patients</th>
<th>Survival/Progression time</th>
<th>PSA response</th>
<th>Immunological response</th>
<th>Trustability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higano et al. 2009 (10)</td>
<td>GVAX VITAL-1</td>
<td>Asymptomatic, CT (-), CRPC</td>
<td>GVAX and docetaxel and prednisone</td>
<td>III</td>
<td>626</td>
<td>MS: 20.7 vs 21.7 months (p=0.78)</td>
<td>N/E</td>
<td>N/E</td>
<td>Study was stopped due to safety</td>
</tr>
<tr>
<td>Small et al. 2006 (15)</td>
<td>Sipuleucel-T</td>
<td>Asymptomatic, CT (-), mCRPC</td>
<td>Control and Sipuleucel-T</td>
<td>III</td>
<td>127</td>
<td>MS: 25.9 vs 21.4 months (p=0.01) survival advantage: 4.5 months 3 year survival: 34% vs 11%</td>
<td>N/E</td>
<td>Increased T cell response and stimulation</td>
<td>Safe, well tolerated</td>
</tr>
<tr>
<td>Higano et al. 2009 (17)</td>
<td>Sipuleucel-T</td>
<td>Asymptomatic, CT (-), mCRPC</td>
<td>Control and Sipuleucel-T</td>
<td>III</td>
<td>225</td>
<td>MS: 23.2 vs 18.9 months (p=0.01) survival advantage: 4.3 months</td>
<td>N/E</td>
<td>Safe, well tolerated grade 3-4 SE: 24.4% vs 24.4%</td>
<td></td>
</tr>
<tr>
<td>Kantoff et al. 2010 (18)</td>
<td>Sipuleucel-T IMPACT</td>
<td>Asymptomatic or minimal symptomatic, CT (-), mCRPC</td>
<td>Control and Sipuleucel-T</td>
<td>III</td>
<td>512</td>
<td>MS: 25.8 vs 21.7 months (p=0.032) survival advantage: 4.1 months 3 year survival: 31.7% vs 11% PSA decrease ≥50%: 2.6%/vs 3.3% T cell proliferation increase, Significant increase in antibody titer</td>
<td>Safe, well tolerated grade 3 SE: 6.8% vs 1.8% grade 4 SE: 2.8% vs 1.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kantoff et al. 2010 (22)</td>
<td>Prostvac-VF</td>
<td>minimal symptomatic, mCRPC</td>
<td>Prostvac-VF and Control</td>
<td>II</td>
<td>125</td>
<td>MS: 25.1 vs 16.8 months 3 year survival: 30% vs 17% PSA response rare T cell response was not evaluated, no increase in antibody titer</td>
<td>Grade 3-4 SE: 2 patients (1 TTP, 1 MI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gulley et al. 2010 (23)</td>
<td>Prostvac-VF</td>
<td>CT (-), mCRPC</td>
<td>Prostvac-VF</td>
<td>II</td>
<td>32</td>
<td>MS: 26.6 vs 17.4 months PSA decrease 12/32 (37.5%) PSA specific T cell decrease</td>
<td>N/E</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Another Anti-CTLA-4 antibody "Tremelimumab" was evaluated in phase 1 study in 11 patients with biochemical recurrence and although the safety profile was good no significant oncological data was obtained (39).

**Anti-PD-1 (Programmed Death)/PD-L1: Checkpoint Inhibitor (Checkpoint Blocking Antibodies)**

As they are also effective on B lymphocyte and Natural killer cells together with Anti-PD-1 T lymphocytes, they have a wider effect compared to Anti-CTLA-4 antibody. PD1 is an immune system blocking antibody. For this reason Anti-PD-1 shows antitumour effect. PD-L1 is the ligand of PD-1. Anti-PD-1 (programmed death) "Nivolumab" was given to 17 CRPC patients in phase 1 study made and no objective response related to medicine was observed in patients (38,40). It was observed that PD-L1 was expressed low in CRPC patients (40). In study made using PD-1/PD-L1, it was observed that objective response was received in different cancer types. Phase 1b/2 studies (NCT01420965, NCT00730639) in prostate cancer are still continued (38).

Studies made on check point inhibitors and continuing studies are summarized in Table 3 and Table 4.

Anti-OX40, anti-Her-2/neu (MDXH210), anti-TAG (mAb CC49), anti-PSMA (trastuzumab, rituximab) and similar medicines show antitumoural effect by the passive immunization provided by monoclonal antibodies (40).

**Result**

Together with cytoreductive treatments, vaccine treatments have an effective potential lengthening general survival, decreasing tumour load in long term and increasing cancer development (38,41,42). As the results in the studies made so far are positive, combining radiotherapy, chemotherapy or new antiandrogens and immunotherapy in order to be used for metastatic CRPC patients especially and the early stage in prostate cancer will be a more commonly applied modality in the future. But other clinical data and studies to support this are still needed.

**Concept:** Mehmet Giray Sönmez, Cengiz Kara

**Design:** Mehmet Giray Sönmez, Cengiz Kara

**Data Collection or Processing:** Data collection or Processing wasn’t done.

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**References**

### Table 2. Continuing studies on the prostate cancer vaccinations (except immune regulatory agents)

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment Agent</th>
<th>Phase</th>
<th>Aim of the Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT01057810</td>
<td>Prostvac-VF</td>
<td>III</td>
<td>GM-CSF+ vs GM-CSF- survival comparison</td>
</tr>
<tr>
<td>NCT00450463</td>
<td>Prostvac-VF</td>
<td>II</td>
<td>Disease progression comparison of Flutamide+Prostvac vs Flutamide branches</td>
</tr>
<tr>
<td>NCT01688492</td>
<td>DNA-PAP</td>
<td>II</td>
<td>GM-CSF ± DNA-PAP comparison</td>
</tr>
<tr>
<td>NCT00849121</td>
<td>DNA-PAP</td>
<td>II</td>
<td>Medicine safety and immunogenicity research</td>
</tr>
<tr>
<td>NCT01436968</td>
<td>Adv-tk</td>
<td>III</td>
<td>Adv-tk+ RT vs. Placebo branches healthy survival comparison</td>
</tr>
<tr>
<td>NCT00715078</td>
<td>Sipuleucel-T</td>
<td>II</td>
<td>Evaluation of CD54 increase with variable fusion protein (PAP2024) concentrations</td>
</tr>
<tr>
<td>NCT00715104</td>
<td>Sipuleucel-T</td>
<td>II</td>
<td>Evaluation of immune response in prostate tissue after neo-adjuvant application</td>
</tr>
<tr>
<td>NCT00901342</td>
<td>Sipuleucel-T</td>
<td>II</td>
<td>Immunity response evaluation of patients with metastatic prostate cancer</td>
</tr>
<tr>
<td>NCT0079402</td>
<td>Sipuleucel-T</td>
<td>III</td>
<td>Efficiency evaluation in early stage, nonmetastatic prostate cancer patients</td>
</tr>
<tr>
<td>NCT01306980</td>
<td>Sipuleucel-T</td>
<td>II</td>
<td>Cerebro vascular occurrence risk evaluation after sipuleucel-T treatment CRPC patients</td>
</tr>
<tr>
<td>NCT01487863</td>
<td>Sipuleucel-T</td>
<td>II</td>
<td>Evaluation of consecutive and simultaneous application of abiraterone acetate + prednisone treatment and Sipuleucel-T</td>
</tr>
<tr>
<td>NCT01431391</td>
<td>Sipuleucel-T</td>
<td>III</td>
<td>Evaluation of the effects of ADT + Sipuleucel-T application in non-metastatic prostate cancer on immune response</td>
</tr>
</tbody>
</table>

GM-CSF: granulocyte macrophage colony stimulating factor, CRPC: castration resistant prostate cancer, RT: radiotherapy, ADT: androgen deprivation treatment

### Table 3. Studies made on check point inhibitors (37)

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment Agent</th>
<th>Phase</th>
<th>Number of patients</th>
<th>Patient Population</th>
<th>Average survival</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small et al. 2007</td>
<td>Ipilimumab</td>
<td>I</td>
<td>14</td>
<td>mCRPC</td>
<td>N/A</td>
<td>Safe, PSA decrease &gt;50%; 14.3%</td>
</tr>
<tr>
<td>Fong et al. 2009</td>
<td>Ipilimumab + GM-CSF</td>
<td>I</td>
<td>6</td>
<td>mCRPC</td>
<td>N/A</td>
<td>Safe, PSA decrease &gt;50%; 50% RECIST criteria: Partial response: 16.7%</td>
</tr>
<tr>
<td>Madan et al. 2012</td>
<td>Ipilimumab + PROSTVAC</td>
<td>I</td>
<td>30</td>
<td>mCRPC</td>
<td>34.4 months</td>
<td>Safe, PSA decrease &gt;50%; 50%</td>
</tr>
<tr>
<td>Van den Eertwegh et al. 2012</td>
<td>Ipilimumab + GVAX</td>
<td>I</td>
<td>16</td>
<td>mCRPC</td>
<td>29.2 months</td>
<td>Safe, PSA decrease 50%; 50%</td>
</tr>
<tr>
<td>Slovin et al. 2013</td>
<td>Ipilimumab</td>
<td>I-II</td>
<td>50</td>
<td>mCRPC</td>
<td>17.4 months</td>
<td>Safe, PSA decrease &gt;50%; 16%; RECIST criteria: 3.6% complete, 7.1% partial response, 21.4% stable disease</td>
</tr>
<tr>
<td>Kwon et al. 2014</td>
<td>Ipilimumab</td>
<td>III</td>
<td>799</td>
<td>CT-mCRPC</td>
<td>11.2 months</td>
<td>Average survival period according to placebo is statistically insignificant. (11.2/10 months, p=0.053) Survival period without progression is statistically significant (4/3.1 months, p&lt;0.001)</td>
</tr>
<tr>
<td>McNeel et al. 2012</td>
<td>Tremelimumab</td>
<td>I</td>
<td>11</td>
<td>PC with PSA recurrence after local treatment</td>
<td>N/A</td>
<td>Safe, Psa doubling time no significant increase</td>
</tr>
<tr>
<td>Topalian et al. 2012</td>
<td>Nivolumab</td>
<td>I</td>
<td>17</td>
<td>CRPC</td>
<td>N/A</td>
<td>Safe, No objective response</td>
</tr>
</tbody>
</table>

N/A: not applicable, RECIST: response evaluation criteria, CT-mCRPC: post chemotherapy metastatic castration resistant prostate cancer, mCRPC: metastatic castration resistant prostate cancer, PC: prostate cancer, PSA: prostate specific antigen, GM-CSF: granulocyte macrophage colony stimulating factor


34. Walunas TL, Lenschow DJ, Bakker CY, Linsley PS, Freeman GJ, Green JM, Thompson CB, Bluestone JA. CTLA-4 can function as a negative regulator of T cell activation. Immunity 1994;1:405-413.


