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review

Prostate cancer immunotherapy, particularly in combination with androgen deprivation or radiation treatment. Customized pharmacogenomic approaches to overcome immunotherapy cancer resistance

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SUMMARY: **Prostate cancer immunotherapy, particularly in combination with androgen deprivation or radiation treatment. Customized pharmacogenomic approaches to overcome immunotherapy cancer resistance.**

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Conventional therapeutic approaches for advanced prostate cancer - such as androgen deprivation, chemotherapy, radiation - come up often against lack of effectiveness because of possible arising of correlative cancer cell resistance and/or inadequate anti-tumor immune conditions. Whence the timeliness of resorting to immune-based treatment strategies including either therapeutic vaccination-based active immunotherapy or anti-tumor monoclonal antibody-mediated passive immunotherapy. Particularly attractive, as for research studies and clinical applications, results to be the cytotoxic T-lymphocyte check point blockade by the use of anti-CTLA-4 and PD-1 monoclonal antibodies, particularly when combined with androgen deprivation therapy or radiation. Unlike afore said immune check point inhibitors, both cell-based (by the use of prostate specific antigen carriers autologous dendritic cells or even whole cancer cells) and recombinant viral vector vaccines are able to induce immune-mediated focused killing of specific antigen-presenting prostate cancer cells. Such vaccines, either used alone or concurrently/sequentially combined with above-mentioned conventional therapies, led to generally reach, in the field of various clinical trials, reasonable results particularly as regards the patient's overall survival. Adoptive trasferred T-cells, as adoptive T-cell passive immunotherapy, and monoclonal antibodies against specific antigen-endowed prostate cancer cells can improve immune micro-environmental conditions. On the basis of a preliminary survey about various immunotherapy strategies, are here also outlined their effects when combined with androgen deprivation therapy or radiation. What's more, as regard the immune-based treatment effectiveness, it has to be pointed out that suitable personalized epigenetic/gene profile-achieved pharmacogenomic approaches to target identified gene aberrations, may lead to overcome – as well as for conventional therapies – possible prostate cancer resistance to immunotherapy.

KEY WORDS: Prostate cancer - Immunotherapy - Androgen deprivation - Radiation - Immunotherapy cancer resistance - Pharmacogenomic approaches.

Introduction

Prostate cancer (PCa) is the most common cancer in men, except for skin tumors, it foreseeably consisting, this year (2016), of 180,890 new cases diagnosed in USA, where, moreover, the PCa incidence mainly concers local/regional stages with over 99% of 5-year survival rate that, instead, falls to 28% for the advanced-metastatized stages particularly found in USA/Caribbean countries inhabitant African men (1). Similarly, PCa is the leading tumor – among the most common noncutaneous malignancies – in European male population, nevertheless its mortality ranking 3th after lung- and colorectal carcinoma. Particularly, in Italy, the PCa diagnosis occurrence arises at about 20% of all cancers in men, even though, in the last decade, it year after year decreasing – as well as more considerably in USA, Australia and Nordic European countries – *versus* 2005, correlatively with decline in PSA testing use era (2, 3).

Prostate cancer therapy approaches include, as dependent on different stage assessment, either localized procedures – as radical prostatectomy, external beam radiation therapy (EBRT)/brachytherapy, focal laser ablation, cryo-ablation, high intensity focused ultrasound – or systemic treatments, such as androgen deprivation therapy (ADT), chemotherapy, radiopharmaceuticals among which particularly ^{223}Ra and ^{153}Sm, although possible anti-tumor immunosuppressive conditions might make timely the resort to immunotherapy measures.

Among the first significant immunotherapy approaches in oncology clinical trials, the use of anti-tu-

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mor antibody *rituximab* was approved by the FDA, in 1997, for the therapy of follicular lymphoma. *Sipuleucel-T*, the first dentritic cell-based cancer vaccine, was granted in 2010 for the prostate cancer treatment. In 2011, the FDA issued the approval to immune check point inhibitor *ipilimumab* for metastatic melanoma patients with encouraging results on overall survival (OS). The further considerable development of various immunotherapy strategies has led to make feasible several preclinical/clinical trials also in the field of advanced prostate cancer (4-6).

Immune-based treatment strategies

Current immune-based treatment strategies for both advanced and recurrent PCa forms mainly fall within two modalities, such as, on the one hand, the *passive immunotherapy* including either the immune check point blockade or specific monoclonal antibodies (mAbs) against tumor associated antigens (TAAs) and, on the other hand, the *active immunotherapy* by tumor specific antigens vaccination (Table 1). Among passive immunotherapy ways, also the adoptive T-cell transfer (adoptively transferred T-cells) and adjuvant immunomodulatory drugs may help reach suitable immune responses.

Check point blockade immunotherapy

Antitumor immunity restored by immune check point CTLA-4 receptor blockade

CTLA-4 (cytotoxic T-lymphocyte associated antigen-4), is an immune check point T-cell receptor that normally allows the immune system to mantain an adequate immunological homeostasis by particularly preventing possible autoimmunity events. Indeed, such receptor, expressed on the surface of both TCD8+ and TCD4+ lymphocytes (T-cells) as well as of regulatory immunosuppressive T cells (Tregs), competitively acts towards T cell CD28 co-receptor that, instead, mainly works for T-cell immune function activation. Both CTLA-4 and CD28 receptors can bind two ligand proteins, B7-1 and B7-2 – made from antigen-presenting cells (APCs) – towards which CTLA-4 receptor shows higher affinity than the CD28 one (5, 7). It results that CTLA-4 is one of the most powerful immunosuppressive molecular factor on T-cell surface. In addition, Tregs can make easier the drop of immune responses also by release of both interleukin-10 (IL-10) and transforming growth factor-β (TGF-β) immunosuppressive agents (4-10). Since the CTLA-4 link with tumor cell B7-1/B7-2 ligands can suppress T-cell immune responsiveness, it follows that the disruption of CTLA-4 activity by anti-CTLA-4 mAbs (so-called immune T-cell check point modulators) can

TABLE 1 - GENERALLY CLASSIFIED PROSTATE CANCER IMMUNOTHERAPY APPROACHES.

Active immunotherapy, triggering the host immune system to focusly target cancer cells.

Prostate cancer *therapeutic vaccines:*

- cell-based vaccines, by using tumor pulsed-dendritic cells or autologous cancer cells, quite as personalized immunotherapy approaches to target patient's mutation-derived specific antigens.
- vector-based vaccines, by using engineered virus (recombinant viral vector-based vaccine) or other vectors to carry:
  - DNA/mRNA, encoding tumor-associated antigens.
  - peptides, as tumor specific antigens.

Cancer vaccines may be also provided with *adjuvants* (e.g., interleukin-2) to enhance the immune response.

Passive immunotherapy, including the use of monoclonal antibodies or adoptively transferred T-cells.

Immune check point blockers, as monoclonal antibodies targeting immune check point receptors such as CTLA-4 and PD-1. Novel monoclonal antibodies against T-cell check point inhibitory receptors are able to prolong their anti-immunosuppressive effects, thus substantially enhancing the response to combined other immunotherapeutic approaches.

Specific monoclonal antibodies targeting tumor-associated antigens, just as a main option of passive immunotherapy even though repeated antibody infusions are required to mantain anti-tumor immune conditions, given the lack of real immune system memory deriving from such immunotherapy measure.

Adoptive T-cell transfer (ACT), by use of adoptively transferred T-cells (tumor specific autologous cytotoxic lymphocyte transfer).

results in enhancing anti-tumor immune response with consequent effective challenges against tumor progression/recurrence.

Ipilimumab, as a fully human IgG1k-based anti-CTLA-4 mAb – the first immune check point blocker taking role in oncology clinical trials – has been used either alone or in combination with conventional therapies (ADT, radiation, chemotherapy) in several clinical trials for metastatic castration-resistant prostate cancer (mCRPC) patients, it resulting a significant decrease in PSA serum levels (4, 5, 11, 12). Moreover, from a trial concerning combination therapy of T-cell check point modulator ipilimumab with EBRT it was followed that the radiation exposure, besides not inducing immunosuppressive effects, can, instead, synergically act with ipilimumab to effectively increase the antitumor immune response (4, 5, 11-13).

Also ***tremelimumab***, as a wholly human IgG2-mediated specific anti-CTLA-4 mAb, has allowed to obtain, either alone or in combination with bicalutamide-mediated ADT, in patients suffering from recurrent PCa, a prolonged decrease in serum PSA levels together with a delay in metastatic PCa progression (9, 14).

Antitumor immunity restored by immune checkpoint PD-1 receptor blockade

PD-1 (programmed cell death-1), as T-cell transmembrane glycoprotein-based immune check point receptor, performs its immune inhibitory function on the T-cell activation effector phase, so differently from CTLA-4 that inhibits T-cell activation triggering phase. The interaction between PD-1 and PCa cell surface-expressed PD-L1/ PD-L2 ligands can induce a decrease in antitumor immune response, with further PCa progression and metastatic spread (4, 5, 9, 11, 15).

PD-L1 is over-expressed on both PCa cells and tumor microenvironmental stromal cells, that's why it follows that, because of high PD-1/PD-L1 interaction, the immune response goes down with increase in cancer cell proliferation (16).

It follows that the resort to anti-PD-1/PD-L1 pathway mAb-mediated immunotherapy may lead to restore the immune response against the tumor with following decrease in its load and metastatic spread. In this regard, the use of single specific mAb-based agents (***nivolumab, atezolizumab, pembrolizumab***) to disrupt PD-1/PD-L1 interaction has allowed to reach, at first, encouraging effects in patients with melanoma as well as subsequently in subjects suffering from some solid tumors (renal cell carcinoma, non-small cell lung carcinoma, colorectal cancer) (17-19).

Unfortunately, as regards advanced PCa treatment, among seventeen CRPC patients enrolled in the human IgG4-mediated anti-PD-1 mAb ***nivolumab*** trial, only one achieved just a 28% tumor load reduction, without significant other immune responses (5, 11, 17-20). In this regard, highly increased levels of PD-L1 on PC cells are, indeed, a poor prognostic marker given the easy onset of PCa adaptive immune resistance. What's more, an elevated number of PD-L1 positive dendritic cells (DC), often found in enzalutamide-resistant PCa patients, may explain the ineffectiveness of nivolumab treatment (21).

However, a recent intriguing trial has allowed, instead, to find out unexpected antitumor effects, including PSA significant decrease, in enzalutamide-resistant mCRPC patients undergone immune-based treatment with ***pembrolizumab*** – as another anti-PD-1 mAb agent – therefore the enzalutamide enhancing such check point blockade-mediated antitumor immune response (9, 21, 22).

The resort to combined treatment of both anti-CTLA-4 and anti-PD-1 mAbs (even, in addition, with ***durvalumab***, as a monoclonal antibody anti-PD-L1), may lead to reach better immune responses rather than when they are used separately (4, 5).

Moreover, quite recently it has been detected that tumor cells result more attackable by a subset of human *natural killer cells* (Nk), expressing PD-1 higher levels rather than by T-cells, whence it follows the timeliness of referring to such intriguing detection to perform novel anti-tumor immunotherapy methods (23).

Prostate cancer therapeutic vaccination

Unlike aforesaid immune check point modulators, therapeutic vaccines are able to induce – as active immunotherapy measures triggering the host immune system to target cancer cells – specific immune responses to tumor associated antigens (TAAs), showing to be either *generic* when engineered to deliver certain immunogenic TAAs or quite *personalized* as resulting generated from the PCa patient's own tumor-responsive immune cells (4, 24).

About PCa vaccines, those *peptide-based* consist of clearcut molecular antigen targets such as PSA (prostate specific antigen), PSMA (prostate-specific membrane antigen), PAP (prostate acid phosphatase), PSCA (prostate stem cell antigen), STEAP-1 (six-transmembrane epithelial antigen of prostate) and Muc-1(mucin-1 glycoprotein) while the *nucleic acid carriers* ones are endowed with DNA/RNA which, by encoding above-mentioned peptides, can induce analogous immune responses (4, 25).

As far as the PSA antigen is concerned, it should be mentioned that though PSA gene transcription, under normal androgen receptor modulation, is drawing to come down – with PSA serum levels decline – as response to ADT, nevertheless, in mCRPC patients, high PSA levels are dependent on PCa cell DNA bent to make per-

sistent, in this way, the androgen receptor activity despite environmental androgen ablation (26).

Apart from *cell-based* vaccines – by resorting to either autologous dendritic cells (DC), as peptide antigen carriers, or even to autologous whole cancer cells – various *recombinant viral vector-based* vaccines, by using viral vectors of DNA-exones encoding specific peptide antigens, have been properly developed.

Leukapheresis-harvested autologous dendritic cells (DC), then *ex vivo* prepared to be efficient specific antigen presenting cells,are generally used to reach an effective *in vivo* T-cell immune activation (4, 5,26-28).

Among the dendritic cell-based vaccines, ***sipuleucel-T*** – that has been approved by FDA in 2010 to treat minimally symptomatic mCRPC patients – consists, indeed, of dendritic cells generated from autologous peripheral blood mononuclear cells loated with fusion immunostimulatory protein (adjuvant GM-CSF) containing the antigen PAP, as appropriate PCa target given that its expression increases proportionally to tumor progression and metastatic spread (4, 12, 23, 26-28).

In mCRPC patients, sipuleucel-T has been put to various trials, either alone or concurrently/sequentially combined with other therapeutic measures – such as ADT (abiraterone, enzalutamide) or EBRT or even chemotherapy (particularly taxane derivatives as docetaxel and cabazitaxel)) – without their negative interference on the immune cell function (5, 12, 29-32).

Moreover, the trial-use, in mCRPC patients, of ***DC-VAC/PCa*** – another autologous therapeutic vaccine composed of dendritic cells pulsed with killed PSA-positive LNCaP cells (androgen-sensitive cells derived from lymph node metastasis) – led to significantly reach a delayed PSA doubling time without remarkable side effects (4, 5).

Differently from above autologous vaccines, ***Prostvac-VF*** (TRICOM or PSA-TRICOM) is a recombinant virus-based vaccine resulting, indeed, from two recombinant viruses (just pox viruses appearing as extensively potential vaccine vectors) engineered to be provided with DNA encoding PSA and, in addition, three T-cell costimulating molecules. Its research trials in mCRPC patients, by inducing immune responses against PSA antigen presenting tumor cells, have led to achieve satisfactory OS positive results (particularly when combined with T-cell check point modulator ipilimumab), nevertheless without improvements in progression free survival (PFS) (33-35).

Also the plasmid DNA-based vaccine ***pTVG-HP*** , consisting of plasmid DNA encoding human PAP, can stimulate the patient immune system to trigger-off a T-cell proper immune response against PAP expressing PCa cells, it implying possible benefits for mCRPC patients, particularly when associated, as a additive booster vaccine, with sipuleucel-T (36).

Apart from DNA, nucleic acid-based vaccines may also consist of mRNA-transfected DCs, by using the mRNAs – encoding peptide antigens – derived from various PCa cell lines (DU145, LNCaP, PC3) (37).

Quite as whole tumor cell-made vaccine, ***GVAX*** – granulocyte macrophage colony stimulating factor (GM-CSF) endowed cancer cell vaccine – consists of allogenic whole PCa cells (LNCaP and PC3) genetically engineered with such recombinant stimulating factor adeno-based viral vector, which can enhance the immune response against antigens expressed by PCa cells, even more significantly when GVAX is combined with immune check point modulator ipilimumab (38-42).

Moreover, multiepitope-DCs pulsed vaccinations (consisting of PSA/PCA/PSMA/PSCA cocktail) have been performed in CRPC patients, with significant immune responses against any involved antigen (43).

Generally, about any anti-tumor vaccination approach, although the vaccine combination with immune check point blockade (mAbs against T-cell inhibitory CTLA-4 and PD-1 receptors) can foreseeably overcome possible individual immunosuppressive conditions, nevertheless such combined treatment may sometimes expose to the risk of immune response excess-dependent adverse events (most commonly, rash and pruritus, colitis, hepatitis, thyroiditis and/or other immune-based severe inflammatory lesions (44).

Adoptive T-cell transfer

Another way leading to reach immune responses against PCa is the adoptive T-cell transfer therapy (ACT). As regards this passive immunotherapy approach, tumor infiltrating autologous lymphocytes (TILs), removed from PCa patients, are genetically engineered to recognize specific cancer antigens and, after their expansion *ex vivo* are reinfused into the same patient in order to kill cancer cells on the basis of specifically targeted immune attack.

Such approach, besides the immune activation of T-cell, implies, instead, the suppression of Tregs, so allowing to improve the microenvironmental immune conditions.

CAR T cells, chimeric antigen receptor T-cells (CAR-modified "designer" T-cells, dTc), may represent, indeed, an immunotherapy strategy clearly availing of engineered patient's T-cells – as cells reprogrammed to express chimeric antigen receptors – that allow to target specific tumor antigens (5, 45-47).

Therefore, as far as PCa immunotherapy is concerned, CAR T cells, engineered to target PSMA and PSCA antigen markers, have been effectively used in clinical trials with mild enough immune adverse events nevertheless including sometimes cytokine-release syndromes with the consequent resort to steroid therapy. CAR T cells

against PSMA (otherwise known as glutamate-carboxypeptidase) can specifically target *in vitro* such antigen-expressing cells and *in vivo* kill the tumor cells. In concurrent addition to CAR T cell adoptive immunotherapy,an administration of interleukin-2 (IL-2) may further increase immune responses (29,48-52).

Monoclonal antibodies and immune adjuvant cytokines

Among the monoclonal antibodies (Abs) to target – in the field of passive immunotherapies for advanced PCa stages – specific tumor own antigens, are here reported both ***tisotumab*** (antibody-drug conjugate targeting tissue factors involved in tumor neoangiogenesis) and ***sacituzumab*** (against TROMP-2, as a cell surface glycoprotein highly expressed in various tumours) besides ***vandortuzumab***, directed against STEAP antigen gene mainly upregulated in several PCa cell lines.

Recently, it has been developed, to treat mCRPC patients, a new humanized bispecific antibody – the ***Mor209/ES414 –*** to direct T-cell cytotoxicity against PCa cells by PSMA specific targeting (53).

Moreover, anti-PSCA mAbs seem to significantly inhibit tumor cell proliferation together with prolonging survival of mices carrying human prostate cancer xenografts (54).

About the immune adjuvant cytokines – as proteins regulating the immune system behaviour, especially showing the ability to passively enhance antitumor immune effects – may be sometimes useful, in mCRPC patients, either concurrently with immunotherapy active measures or sequentially, some interleukins, among which ***IL-10, IL-7, IL-12.*** On this subject, it should be mentioned that ***IL-2*** and ***TGF-β*** are attractive targets for PCa recognition/therapy, given that respectively the enhanced *IL-2* cytokine and, instead, the decreased *TGFβ* can lead to reach a proper immunologic identification of prostate cancer cells with consequent their suitable targeting (55).

Some new adjuvant immunomodulatory drugs

Apart from novel pharmacogenomic approaches to increase the tumor immunotherapy sensitivity – here subsequently subject of an outlined report (56, 57) – some adjuvant immunomodulatory drugs may be used in addition to above outlined immunotherapy strategies.

Lenalidomide, as thalidomide analog, besides its anti-inflammatory and antiangiogenic properties, can act as boosting the immune response, that's why its administration, either alone or in combination with chemotherapeutics (particularly,docetaxel and paclitaxel), has been trialled in mCRPC patients, although up till now with modest antitumor results and, unfortunately, serious adverse effects (4, 56, 58).

Indoximod, as methylated tryptophan-based blocking agent of the well-known enzymatic indoleamine 2,3-dioxygenase (IDO) pathway – that, by the tryptophan depletion, induces immunosuppressive effects due to both T-cell function arrest and increase, instead, in number of Tregs – can enhance, in mCRPC patients, immune responses to sipuleucel-T vaccine (59).

Tasquinimod (quinolone-3-carboxamide analog), counteracts tumor development by inhibiting regulatory myeloid cell-mediated immunosuppressive effects and,moreover,by blocking the hypoxic HIF-α1 signaling-mediated tumor growth. Such drug has been mainly trial-used in in mCRPC patients, it following somet particularly when in combination with cabazitaxel and prednisone, an improvement in clinical outcomes consisting of PFS with moderate adverse events, such as skeletal muscle pain, gastrointestinal disorders and fatigue. As regards such drug, other trials are in pipeline within current year (5, 60).

Immune-based treatments combined with other therapies

Combination with androgen deprivation therapy

On the basis of a recent report, it would seem that androgen receptor antagonist-based ADT might likely compromise T-cell immune responses against PCa, it leading to early tumor relapse. What, moreover, could occur owing to radiation and chemotherapy, such treatments quickly reducing cancer burden but, at the same time, leading to immunesuppression. Whence the timeliness of carrying out suitable measures that might enhance the immune response to prevent PCa relapse (61).

In enzalutamide-resistant mCRPC patients it has often shown an enhanced number of PD-L1 positive dendritic cells, it leading to think that the PCa antiandrogen-resistance is dependent on intrinsic tumor cell PD-L1 increased expression rather than on androgen receptor refractory sensitivity (4, 5, 9, 18).

However, other studies show that ADT compounds – from traditional both GnRH agonists/antagonists and antiandrogens to second generation particularly including enzalutamide and abiraterone – can, instead, intensify the anti-cancer immune response. The ADT, indeed, by lowering the human cancer cells expression of antiapoptotic genes, may enhance cancer sensitivity to immune-mediated lysis, so that making clear the effectiveness of ADT/immunotherapy combination (22, 62).

Immunomodulatory properties of enzalutamide and

abiraterone can induce PCa cells to be more sensitive to T-cell killing effects quite through androgen receptor-due apoptotic pathway modulation, whence it resulting that such antiandrogen immunomodulation activity might justify their combination with immunotherapy measures in CRPC patients (22, 63).

CTLA-4 immune check point inhibitor ***ipilimumab***, combined with ADT in mCRPC patients, has allowed to obtain, indeed, *versus* ADT alone, a significant improvement in biomarker tumor response, particularly with decrease in serum PSA levels. In this regard, also the trial combination of ipilimumab with GnRH antagonist degarelix seems to reach encouraging results against advanced PCa (5, 9, 14, 64, 65).

If ***nivolumab***, as PD-1 immune check point blocker, shows lack in efficacy for mCRPC patients therapy, the use, on the other hand, of ***pembrolizumab***, as PD-1 inhibitor too, can elicit surprising effective anti-tumor immune responses in enzalutamide-resistant mCRPC patients (14, 22, 64, 65).

The use, in advanced stage PCa patients, of autologous cell-based vaccine ***sipuleucel-T*** , either concurrently or sequentially combined with enzalutamide, has elicited, in a variety of trials, a considerable PSA decrease. Moreover, sipuleucel-T immunotherapy together with abiraterone administration has led, given correlative satisfactory results, to stick to this therapeutic integration (27, 31, 32, 66-68).

With reference to ADT sinergically combined with ***adoptive T-cell transfer*** (ACT), the ADT effects appear to be improved by TILs including specific tumor antigens.That's why,as regards the prostate cancer, chimeric antigen receptor T-cells (CAR T cells) are engineered to properly recognize and target PSMA and/or PSCA specific antigens (5, 51, 52). Such immunotherapy strategy can reasonably enhance the ADT-related anticancer effects (69).

Combination with radiation therapy

If the exposure of blood pool/bone marrow significant volume to large ionizing radiation fields may cause a considerable decrease in white blood cell number so it inducing to consider the radiation as an inductor of immunosuppressive effects, the current therapeutic resort, instead, to linear accelerator-mediated highly focused radiation delivery – properly named stereotactic radiotherapy – allows to avoid, by really restricting blood pool/bone marrow volume irradiation, potential correlated immunosuppression conditions (70, 71).

Quite from various trials it results that radiation focused on the tumor can convert it into *in situ tumor vaccine* by implying antigen release at tumor cell death, so synergically acting with immunotherapy to enhance anticancer immune responses through additively triggering the activation of prostate cancer specific T-cells (13, 72).

Indeed, radiation can activate the major histocompatibility complex (MHC) upregulation together with the cancer cell surface antigen expression and, moreover, radiation-induced both DNA damage and ROS (reactive oxygen species) development may lead to tumor cell death as well as to significant proliferation of tumor specific antigen-correlated T-cells. It has been proved that radiation can, in addition, influence the output of various interleukins and growth factors (among which, particularly, IL-1/-2/-6, transforming growth factor-β, tumor necrosis factor-α) that act as immune response regulators (5, 70, 73-79).

Besides the *photon* radiation (external beam radiation therapy, EBRT), also the *proton* one can induce a tumor cell surface antigens up-regulation involved in immune recognition/therapeutic targeting, and, in addition, may enhance the cancer cell sensitivity to cytotoxic T-cell killing effects (80).

Radiation combined with ***check point blockade immunotherapy*** can increase, in mCRPC patients, im systemic effects, the antigen specific-related immune responses that result positively influenced, besides by the stereotactic radiation alone, particularly by such treatment combination. Indeed, the ***ipilimumab*** administration can potentiate anticancer immunity deriving from radiation-induced conversion of the tum into *in situ cancer vaccine* provided with uncovered cryptic cancer antigens (70, 79, 81).

Given that experimental trials in an animal model – transgenic mice spontaneously developing a prostate cancer – have shown that radiation combined with ***vaccine immunotherapy*** can support antitumor T-cell activation, so removing possible immune system tolerance, it has also been noted that combination of EBRT, in mCRPC patients, with autologous cell-based therapeutic vaccine ***Sipuleucel-T*** , has led to elicit satisfactory immune responses (30, 31, 70, 76, 81).

Moreover, also EBRT, combined with ***PSA-TRICOM*** vaccine, has allowed to obtain a remarkable increase in immune responses to tumor-linked antigens, if compared with immune-based treatment alone (70, 82, 83).

Recently, β-*emitting Samarium-153* (^{153}Sm), as radiopharmaceutical binding osteoblastic bone metastases, has been used in combination with PSA-TRICOM vaccine,in mCRPC patients, making attainable a more significant decrease in PSA serum levels rather than that reached by ^{153}Sm alone administration (84). Also the α-*emitter Radium-223* (^{223}Ra) use is for ages involved in the treatment of bone mCRPC, sometimes togheter with immunotherapy approaches (85).

From integrative trial including stereotactic radiation treatment – in TRAMP (transgenic adenocarcinoma of mouse prostate) male mouse model – with ***T-GVAX***, it followed, besides a significant enhancement of animal

overall survival, also the into-tumor histological detection of radiation-mediated increase in immune T-cells infiltration (70, 81, 86).

On the basis of what above-mentioned about combined radiation/immunotherapy, it appears to be clear that immune-based various treatments can enhance the radiation-driven local tumor responses and, reciprocally, the radiation may potentially increases the immunotherapy-mediated systemic effects (79, 81, 86).

Immunotherapy supported by customized pharmacogenomic approaches

Though the immunotherapy strategies might be frequently useful, particularly when combined with other above-mentioned treatments, to improve the state of health in advanced PCa patients, it has to be pointed out that suitable personalized therapeutic measures – as individual epigenetic/gene profile recognition-based pharmacogenomic approaches targeting correlative molecular PCa cell aberrations – may lead to overcome possible immunotherapy tumor resistance conditions as well as to conventional therapies (ADT, radiation, chemotherapy).

In this regard, the *epigenetic silencing* of prostate specific antigens encoding genes, can lead, indeed, to the extintion of PCa cell immune recognition with consequent lack of immunotherapy effectiveness (57, 87). The epigenetic deregulation particularly involves DNA methylation and/or histone deacetylation, that's why a timely gene function restoration may results from resort to either DNA methyltransferase- or histone deacetylase (HDAC) inhibitors among which particularly efficacious prove to be vorinostat and entinostat whose administration can induce, indeed, a substantial enhancement of PCa cell sensitivity to immune cytotoxc T-cell mediated lysis (57, 88).

On the basis of individual gene profile recognition, customized pharmacogenomic approaches allow to target *individual cancer cell gene aberrations* – regarding oncogenes and/or tumor suppressor genes – that elicit specific tumor resistance to immune-based therapeutic measures as well as to above- mentioned conventional therapies even when these are combined with immune-based measures.

Among gene mutation-derived worse PCa conditions, particularly significant appear to be – besides aberrant gene-due tumor cell growth/apoptosis pathways alterations together with sometimes cancer stem cell gene-dependent overactivation of tumor progression/recurrence (Table 2) – the prostate cancer cell specific antigen impaired expression with consequent decrease in anti-tumor immune responses (89-91).

In this regard, a glycosylphosphatidylinositol-anchored prostate stem cell antigen (PSCA) protein overexpression in PCa cells is strongly correlated – by leading to PCa cell both vimentin and β-catenin abnormal accumulation and drop, instead, in E-cadherin (E-calcium dependent adhesion glycoprotein) – with fast tumor growth/metastatic spread, refractory to various therapies. An experimental small harpin *RNA-mediated knockdown* of such antigen in DU145 PCa cell line has induced an appreciable reduction of cancer cells proliferation, this suggesting the hypothesis of possible recovered therapy-sensitivity (92).

What's more, the implication of prostate cancer stem cells (PCSCs) in tumor resistance to various therapies make quite advantageous to identify the correlative aberrant genes, given that PCSC1- and PCSC2-RAN (Ras-associated nuclear protein) genetic influences are remarkably involved in DNA synthesis overactivation and cell-cycle redundant induction (93, 94).

However, it is acquired knowledge that antigen-specific immune response to prostate cancer therapeutic gene-based vaccination (e.g.,DNA vaccine encoding PAP) shows to be specifically relevant to host-encoded antigens even if deriving from DNA single nucleotide polymorphisms, therefore without impaired immune effects due to epitope differences between such DNA-vaccine and individual somatic encoding sequence (95).

Nevertheless, that isn't exempting from the necessity of suitably choosing personalized epigenetic/gene profile-related antitumor immunotherapeutic strategies to avoid the lack of proper immune responses in mCRPC patients undergone immunotherapy (96-99). Indeed, given that individual cancer cell genome mutated exome sequencing gene identification and consequent targeting pharmacogenomic approaches result to be advantageous to improve conventional treatments outcomes, such personalized diagnostic and therapeutic measures – even by *genome editing* supply, possibly resorting to CRISPR-CAS9 technique – should be taken into consideration also in the field of current immunotherapy developments to particularly support the vaccination-induced anti-tumor immune response (97, 100, 101).

Conclusion

Given the current novel developments of above-mentioned anti-tumor immunotherapeutic strategies, particularly in combination with conventional treatments (ADT, radiation, chemotherapy), to reach more and more satisfactory results, it is to be properly hoped that the immune-based measures might play, in the next future, a more important role to manage the advanced PCa, whose reliably preventing both progression and recurrence.

A foreseeable methodical resort to individual epigenetic/gene profile recognition-based customized phar-

TABLE 2 - SOME CUSTOMIZED PHARMACOGENOMIC WAYS AGAINST INDIVIDUAL PCA RESISTANCE TO VARIOUS THERAPIES (mod. from Alberti C. G Chir. 2015;36:133-136).

Aberrant gene-due resistance to therapy	Personalized sensitizers to therapy
Cancer cell growth pathway hyperactivation:	
- Overexpression of HER2 (Human epidermal growth factor receptor type 2 of tyrosine kinase).	- Trastuzumab (Herceptin) that, by blocking HER2, inhibits PCa cell proliferation.
- Phosphatidyl inositol 3-kinase(PI3K)-Akt/mammalian target of rapamicin (mTOR) pathway.	- NVP-BEZ 235 or NU7 441,as dual ATP-competitive PI3K and mTOR blockers. Zotarolimus, as analog of rapamicin, blocks mTOR.
- Janus tyrosine kinase - Signal transducer activator of transcription (Jak-STAT) pathway.	- AG 490,as a suitable specific blocker of Jak-STAT pathway, can sensitize the PCa cells to therapy. Ruxolitinib and fludarabine are respectively selective inhibitors of Jak 1/2 and STAT3.
- Interactions between overexpressed MDM2 (mouse double minute 2) and p53 with subsequent lack of p53 normal function,whence enhancement of cancer cell growth.	- Nutlins, as cis-imidazoline analogs, to prevent p53-MDM2 interactions, so inhibiting cancer cells growth meanwhile restoring PCa therapy-sensitivity. MDM2 antagonist Nutlin-3 also facilitates apoptosis.
Cancer cell apoptotic pathway evasion:	
- Suppression of apoptosis machinery by overexpression of antiapoptotic Bcl-2 gene.	- HA14-1 and ABT-263 (Navitoclax), as inhibitors of Bcl-2, facilitate the apoptotic process.
- Suppression of proteolytic cleavage of poly(ADP-ribose) polymerase-1 (PARP-1), so preventing apoptosis-proper DNA fragmentation.	- Olaparib, veliparib, niraparib, as blockers of PARP-1, allow the cancer cell death, so it reaching the prostate cancer cell therapy sensitization.
- Survivin gene overexpression, by interfering with caspase activity, supports cancer cell survival.	- YM155, as survivin inhibitor, can restore sensitivity of prostate cancer cells to therapy.
- Clusterin, as inhibitor of Bax proapoptotic activity, protects cancer cells from TGFβ-induced apoptotic mechanisms.	- OGX-011 antisense nucleotide, by promoting a down regulation of clusterin expression, can restore cancer cell apoptosis and therapy-sensitivity.
Cancer stem cell-related therapy-resistance:	
- Particular gene mutation-dependent over-activation of stem cell specific pathways – such Wnt/βcatenin-, Hedgehog-and Notch signaling pathways – plays an important role in inducing both self-renewal process and PCa therapy resistance.	- Perifosine,besides blocking the Akt pathway,can also inhibit the Wnt signaling, with following restoration of tumor therapy-sensitivity.
- CXCR4 (chemochine CXC of receptor 4), by interacting with its ligand CXCL12, can cause both cancer stem cell chemo- and radioresistance.	- Foreseeable block of CXCR4-CXCL12 interactions should represent a promising opportunity to refine the prostate cancer sensitivity to such therapies.
- Also PSCA (prostate stem cell antigen) gene overexpression can promote, because of vimentin/β-catenin increase compared with E-cadherin decrease, prostate carcinogenesis with further PCa progression and resistance to therapies.	- PSCA knock-down decreases PCa progression and metastatic potentials meanwhile restoring the PCa cells sensitivity to therapies.

macogenomic approaches could lead to avoid/overcome, by targeting PCa specific gene aberrations, the tumor immunotherapy resistance, what's already feasible for conventional therapies.

Conflict of interest statement

The author declares that this work was conducted without any potential conflict of interest.

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