

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Prostate Cancer Immunotherapy – Strategy with a Synthetic GnRH Based Vaccine Candidate

J.A. Junco¹ et al.*

¹Department of Cancer,
Center for Genetic Engineering and Biotechnology of Camaguey,
Ave. Finlay y Circunvalación Norte, Camaguey,
Cuba

1. Introduction

Recent clinical trials have shown therapeutic vaccines to be promising treatment modalities for prostate cancer. Additional strategies are being investigated that combine vaccines and standard therapeutics as anti-hormone treatment to optimize the vaccines' effects.

Previous studies with Gonadotropin Releasing Hormone (GnRH/LHRH) vaccines have demonstrated the usefulness of immunization against this hormone in prostate cancer. To this purpose, we generated the completely synthetic GnRHm1-TT peptide which has been validated in a proof of concept formulated together with Montanide ISA 51 adjuvant. Such vaccine preparation induced a significant anti-GnRH immune response and correspondingly reduced testosterone to castration levels and thus produce a biological response in hormone-dependent tumors. As a novel strategy the GnRHm1-TT peptide has been also emulsified with the adjuvant combination Montanide ISA 51 and VSSP. The use of this candidate in healthy animal models showed a significant increase in the anti GnRH immune response in comparison with the previous candidates, including their advantages regarding prostate and its testicle atrophy. Moreover, the use of the GnRHm1-TT/Montanide ISA 51/VSSP vaccine candidate produced a significant inhibition of tumor growth in mice transplanted with hormone-sensitive murine tumor Shionogi SC-115. The development of a Phase I clinical trial in patients with advanced prostate cancer using GnRHm1-TT/Montanide/VSSP vaccine, demonstrated the safety of using this candidate in humans as well as the therapeutic elements which must be demonstrated more widely in future clinical trials.

*F. Fuentes¹, R. Basulto¹, E. Bover¹, M.D. Castro¹, E. Pimentel¹, O. Reyes², R. Bringas², L. Calzada¹, Y. López¹, N. Arteaga¹, A. Rodríguez³, H. Garay², R. Rodríguez⁴, L. González-Quiza⁴, L. Fong⁴ and G.E. Guillén²

¹Department of Cancer, Center for Genetic Engineering and Biotechnology of Camaguey, Ave. Finlay y Circunvalación Norte, Camaguey, Cuba

²Center for Genetic Engineering and Biotechnology, Havana, Cuba

³University of Medical Sciences of Camaguey, Carretera Central SN, Camaguey, Cuba

⁴Marie Curie Oncologic Hospital of Camaguey, Carretera Central SN, Camaguey, Cuba

2. Epidemiology of prostate cancer

Prostate cancer is the most commonly diagnosed malignancy in men in the Western Hemisphere, with 33% incidence rate and the second leading cause of cancer death in men, exceeded only by lung cancer despite the efforts to achieve early diagnosis of disease through the use of the serological marker "prostate specific antigen" (PSA). Worldwide, there are about 650 000 reported new cases of prostate cancer each year and a mortality of about 200 000 cases. Autopsy studies in men show that 70% of men develop prostate cancer sometime in their lives, although many of them are clinically irrelevant (Russel et al, 1994; Cancer Statistics, 2008).

Prostate cancer is diagnosed in a clinically relevant stage in one out of six men around the world and is usually diagnosed by elevated levels of prostate specific antigen (PSA) or the presence of an abnormal digital rectal examination.

The Prostate Specific Antigen (PSA), is a protein produced by normal and pathological prostate cells. This protein is found in relatively small levels in the bloodstream of men with normal prostate, however, is considerably increased in most individuals suffering from a malignant disease of the prostate, but also tends to increase in benign diseases of the gland such as prostatitis and benign prostatic hyperplasia (BPH).

While assessing the levels of PSA takes into account the individual's age, it is generally considered that these values may reach up to 4 ng/mL (Sonpavde et al, 2010). Unfortunately, however, the PSA does not distinguish between the stages of the disease.

3. Prostatic tumorigenesis

Prostate cancer seems to develop over a period ranging from 20 to 30 years (Kabalin et al, 1989; Sacker et al, 1996). In most cases, the tumorigenesis begins as a prostatic inflammatory atrophy which progresses to prostatic intraepithelial neoplasia (PIN), which in some cases leads to carcinoma (De Marzo et al 2003, Nelson et al, 2007). In addition to genetic changes occurred, androgens act as promoters of proliferation and prostate growth. Thus, testosterone passes into the prostate cell where the action of the enzyme 5 alpha reductase is converted to its metabolically active form, dihydrotestosterone (DHT). Once DHT binds to the receptor in the cytoplasm, this favors the formation of dimers crossing the nuclear membrane where the complex binds to genes with androgen-responsive elements, a process modulated by co-activators and co-repressors (You and Tindall, 2004).

Thus, if normal prostatic epithelial cells are deprived of testosterone, this progresses to death by apoptosis. Similarly, the majority of prostatic adenocarcinomas are androgen-dependent, which means they are responders to testosterone hormone ablation. This behavior of prostate cells has influenced therapeutic concepts in prostate cancer for decades (Culig et al, 2005). Thus even in cases of metastatic disease, the first therapeutic step is represented by androgen suppression, which causes death by apoptosis in most prostate cells and leads to remission in about 85% to 90% of individuals.

4. Current therapies in prostate cancer

Prostate cancer patients at initial stages of the disease are treated successfully with radical prostatectomy or radiation therapy, however, approximately 30–40% of them will ultimately develop recurrent diseases (Roehl et al, 2004).

Once prostate cancer reach the prostatic capsule and seminal glands, the androgenic ablation represents the most useful therapeutic procedure since the decade of the 40's of the last century (Culig et al, 2005; Pienta et al, 2006). The normal intervention of the prostate cancer includes: the surgical castration, the use of estrogens, anti-androgenic therapy to inhibit the testosterone actions and, the use of GnRH analogues which prevent the production of androgens in the testicles.

The anti-hormones therapies, although very much in use, have various inconveniences. Thus, the surgical castration is not ethically accepted by most patients. The estrogens, such as the diethyl-stilbestrol (DES) are highly toxic to the cardiovascular system and the anti-androgens frequently produce severe gastro-intestinal toxicity (Finstad et al, 2004) while pituitary adenomas and hot flush during the first weeks of treatment are reported for GnRH analogs treatment. However, the most important drawback of anti-hormonal therapy in prostate cancer consists in that the benefit of this therapy last for an average of between 18 and 36 months (Casper et al, 1991). At that stage those clones of cells that escaped to the requirements of the absence of testosterone or lower levels, begin to grow or proliferate as castration resistant prostate cancer (CRPC) and emerge as the predominant cell phenotype. When CRPC appears, chemotherapy and steroids represent the alternative palliative treatment left for patients who no longer respond to hormone therapy. The half-life for these patients ranges between 18 and 24 months (Tannock et al, 2004). Of the many treatment approaches for recurrent prostate cancer that no longer responds to hormonal agents, immunotherapy is particularly promising, due to several unique characteristics of both the disease and the treatment.

5. Prostate cancer immunotherapy

Prostate cancer is a relatively indolent disease, allowing time for the immune system to generate an immunologic response. Furthermore, since the prostate is a nonessential organ, targeting prostate cancer-associated antigens are unlikely to have significant negative side effects. Finally, therapeutic cancer vaccines have been shown to be much less toxic than chemotherapy, hormonal therapy or targeted therapies, thus significantly improving a patient's quality of life.

Studies in animals and humans developed over decades using non-specific immune therapies, suggest the usefulness of these therapies in prostate cancer. However, these therapies have been used mainly in advanced stages of cancer. Immunotherapies are classified as passive and active. The former include the treatment with immunomodulatory substances, the infusion of cytokines and immune effector agents such as antibodies or lymphocytes.

In medical practice the most widely used cytokine-stimulating factor has been the Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF). This cytokine is known to act at different levels of the immune response which includes the stimulation to arachidonic acid release in neutrophils and active cellular response mediated by antibodies (Weisbart et al, 1985).

An active immunotherapy vaccine includes strategies in which the goal is to produce an immune response against the tumor/host factors that aid the maintenance and growth of metastatic tumor (Rini et al, 2004).

Ideally, therapeutic prostate cancer vaccines should induce a focused antitumor immune response by targeting defined tumor-associated antigens (TAAs) through TH1 cell stimulation. The ideal TAA should be specific to, or overexpressed on the surface of prostate cancer cells. Several prostate-associated TAAs have been identified and that include: the prostate specific antigen (PSA), a 34-kD kallikrein-like serine protease expressed almost exclusively by prostate epithelial cells and is the most widely used serum marker for diagnosis and monitoring of prostate cancer (Freedland et al, 2008; Madan et al, 2009). The prostate specific membrane antigen or PSMA which is a 100-kD transmembrane glycoprotein commonly found on the surface of late stages, undifferentiated metastatic prostate cancer and is an imaging biomarker for staging and monitoring of therapy. It also represents an attractive antigen for antibody-based diagnostic and therapeutic intervention in prostate cancer, since it is highly restricted to the prostate and overexpressed in all tumor stages (Fishman et al, 2009). The prostatic acid phosphatase or PAP is a secreted glycoprotein (50 kDa) that serve as a well-known tumor marker of differentiated prostate epithelial cells (Becker et al, 2010) whose primary biologic function is still unclear. Another potential target, TARP, is a protein expressed in patients with prostate and breast cancer and is present in both normal and malignant prostate cancer tissue. It is found in about 95% of prostate cancer specimens, making TARP a promising target antigen for cancer vaccines (Maeda et al 2004; Epel et al, 2008). Recently, a prostate-specific gene encoding a protein named NGEF has been discovered. The full length protein (NGEF-L) is expressed in normal, hyperplastic and cancer prostate tissue (Cereda et al, 2010).

Despite the long list of tumor markers, PSA has been the most useful TAA as a diagnostic tool and for immunotherapy. In this sense, PSA has been used as part of the PSA-TRICOM vaccine design, which showed a beneficial impact on metastatic CRPC patients and is currently in Phase III clinical trial (Bavarian Nordic, 2011).

As part of the immune response modulation, Cytotoxic Lymphocyte Antigen 4 (CTLA-4), represents an important immune checkpoint molecule expressed after activation by an APC. CTLA-4 blocking could disrupt the transmission of the regulatory signal and may increase the immune response of CTLs against tumor cells (Fong et al, 2008). In this sense, Ipilimumab is a fully humanized monoclonal antibody against CTLA-4 that demonstrated PSA decline in a Phase I clinical trial (Small et al, 2007) and is currently in Phase III trial. (<http://www.clinicaltrials.gov>).

The most successful prostate cancer immunotherapy intervention is represented however, by the ex vivo vaccine called "Provenge" or "Sipuleucel T", which is generated from each patient's own Peripheral Mononuclear Blood Cells (PMBCs), that are later "charged" with the fusion protein PAP/GM-CSF and the GM-CSF. (Burch et al, 2000). These immunotherapy demonstrated a significant improvement in the overall survival for Sipuleucel-T (25.8 months) vs. placebo (21.7 months) (Kantoff et al, 2010) and has been registered by the FDA in the United States and is the only therapeutic vaccine of its kind in the world for this condition.

Combining therapeutic cancer vaccines with hormonal therapies is a potential approach for hormone-sensitive tumors, such as breast and prostate cancer. Preclinical data indicate that testosterone suppression affects not only prostate tumors, but also the immune system (Aragon et al, 2007). Increasingly data suggest that androgen deprivation therapy (ADT) in prostate cancer can augment the immune response by increasing T-cell infiltration into the

prostate (Mercader et al, 2001). Furthermore, ADT has been shown to decrease immune tolerance of TAAs, increase the size of the thymus (where CTLs are produced), and enhance the T-cell repertoire (Drake et al, 2005; Sutherland et al, 2005; Aragon et al, 2007; Goldeberg et al, 2007). It may also stimulate CTLs by reducing the number of regulatory T cells (Tregs), improving immune-mediated tumor-specific response (Wang et al, 2009).

6. Vaccines design with autologous molecules

Immunization against endogenous molecules requires a sufficient level of neutralizing antibodies during the treatment period to obtain the desired effect. To produce an immune response against these molecules, several of which are not immunogenic themselves, many strategies, such as the coupling to a carrier protein and the use of powerful adjuvants are required (McKee et al, 2010).

6.1 Prostate cancer immunotherapy based on GnRH. State of the art

GnRH-based vaccines represent a promising anti-hormonal treatment alternative in prostate cancer, because these can reduce serum testosterone to castration levels, avoid the "hot flushes" produced by GnRH analogues and can be administered in acute and complicated forms of prostate cancer. In turn, the ability to generate a memory immune response in vaccinated patients allows them to do without medications for relatively long periods of time which also results in lower medication costs and marketing. This aspect gives vaccines high added-value and very competitiveness in the market.

6.1.1 Carriers molecules to GnRH vaccine delivery

The most often used approach to make a peptide immunogenic, is to couple it to a protein molecule. Commonly used carrier proteins are KLH, TT, DT, OVA, BSA and HSA. The origin of the carrier protein could be of importance for the level of immunogenicity of the conjugate. The use of "foreign" proteins is expected to result in conjugates with a stronger immune response. In general, most exogenous proteins can be used as carriers, although non-mammalian proteins are expected to be more immunogenic. Additionally, the site of conjugation may determine the efficacy of the immunization. Conjugation via glutamine at position 1 induced a higher GnRH specific antibody response and reduced testosterone levels in rabbits more effectively than conjugation via positions 6 or 10. Similarly, no difference in GnRH antibody response has been observed in male sheep when GnRH was conjugated to KLH via a substituted cysteine either at position 1, 6 or 10 (Goubau et al, 1989). These results have been confirmed in mice. At the same time, specificity of the antisera depend on the site of conjugation. So, experiment carried out by conjugation via cysteine on position 1 resulted in C-terminal directed antibodies, conjugation via cysteine on position 10 generated N-terminal directed antibodies, while conjugation via cysteine at position 6 generated both N- and C-terminal antibodies (Silversides et al, 1988). In contrast, Ferro et al. (1998) showed that the N-terminal conjugation via a cysteine substitution at position 1 resulted in effective immunization of rats, while conjugation via cysteine substitution at position 10 was not effective. Other groups used native GnRH extended with glycine and cysteine, conjugated to a carrier protein (Ferro et al., 1996; Miller et al., 2000) or longer spacer peptides (Simms et al., 2000; Parkinson et al., 2004). Thus, it seems that immunization with GnRH peptides, conjugated to a carrier protein via the N-terminus

results in more effective antibody titers than conjugation via the C-terminus. However, this was not confirmed in all studies and may depend on the chemical approach used and substitution of amino acids required for coupling.

One of the most promising vaccine candidate based on GnRH have been developed by United Biomedical (UBI, Hauppauge, USA), which is a complete synthetic vaccine comprising the GnRH decapeptide, several promiscuous T-cell epitopes and a domain from Yersinia invasus protein to improve the immunogenicity of the GnRH-T cell epitope constructs (Finstad et al., 2004). Although the single constructs were not completely effective in rats, mixtures of constructs caused serum testosterone to drop to very low levels, whereas testes weights were less than 25% of the controls. The antigens in a water-in-oil formulation and oil-in-water formulation were effective in baboons and dogs, respectively. Phase I clinical trials have been planned recently using this vaccine candidate, however, no results have been published so far.

In the hope of developing a better anti GnRH vaccine apart from the mentioned carrier molecules, some alternatives have been used like the *Mycobacterium tuberculosis* hsp 70, linked to GnRH-6-DLys by elves and Roitt's group employing either Ribi adjuvant or incomplete Freund's adjuvant. With either adjuvant, all mice produced sufficient antibodies to cause atrophy of the uro-genital complex. LHRH-6-DLys was also employed after conjugation to albumin and mixed with Specol as adjuvant. These vaccine candidate have been used in pigs as an alternative to castration (Zeng et al, 2002).

Previous studies of the Population Council group (Ladd et al,1990) found that the conjugation of TT at the N-terminal was better than at the C-terminal. Desirability of keeping the LHRH C-terminal free has also been advocated by Ferro et al. (2002). Linkage of TT at the N-terminal in Des 1-GnRH, where glutamic acid at position 1 is replaced by cysteine, gives better conjugate that induces antibodies specific to the classical GnRH-1. Dimerization enhances antibody titers but the monomer conjugate was found to be more effective (Ferro et al., 2002).

6.1.2 Recombinant vaccines against LHRH/GnRH

Enough data have accumulated to conclude that the vaccines against GnRH-I can be employed in humans and in animals without side-effects. These vaccines are beneficial in the treatment of prostate carcinoma patients to reduce fertility of wild animals and sex steroid hormone production thus regulating estrus and libido of animals raised for meat production.

Recombinant vaccines would be substantially cheaper to produce at industrial scale than synthetic vaccines conjugating multiple copies of GnRH with receptor-binding domain of *Pseudomonas* exotoxin (Hsu et al, 2000). This recombinant protein containing 12 repeats of LHRH along with this carrier generated high antibody titres in rabbit and is recommended for the treatment of hormone-sensitive cancer. At the same time, genes for three repeats of GnRH linked through an eight amino acid hinge fragment of human IgG1 to a helper T-cells peptide of measles virus have been constructed in order to increase immunogenicity. The DNA coding for a dimer of this complex assembly was fused to the C-terminal (199-326)-encoding sequence of asparaginase (Jinshu et al.(2006). This protein was expressed in *E. coli* and generated an anti LHRH response .

Talwar *et al.* (2004) reported the ability of a multimer recombinant anti-LHRH vaccine to cause decline of testosterone to castration level and atrophy of rats prostate. In the design of this vaccine, DT/TT used as carriers in the previous semisynthetic vaccines were replaced by four or five T non-B-cell peptides interspersed in four or five LHRH units. This was done to avoid carrier-induced epitope suppression brought by DT/TT carrier conjugates (Sad *et al.*, 1991), and also to communicate through an array of these T-cell determinants with MHC across the spectrum in a polygenetic population. The genes were assembled, cloned and expressed at high level (15% of total cellular protein) in *E. coli* (Gupta *et al.*, 2004). Employing a buffer at pH 3, it was possible to extract the protein from inclusion bodies employing low concentrations of chaotropic reagents (2 mol/l urea instead of 8 mol/L). The protein was purified and refolded to native immunoconformation (Raina *et al.*, 2004).

The company Biostar, (Saskatoon, Canada), developed a GnRH vaccine comprising a recombinant fusion protein produced in *E. Coli* bacteria. Several copies of a GnRH-tandem molecule were fused to the terminal ends of leukotoxin. This vaccine has shown full efficacy in young pigs and cats (Manns and Robbins, 1997; Robbins *et al.*, 2004), while antibody responses were variable in heifers (Cook *et al.*, 2001). For application in prostate cancer patients, the vaccine called Norelin™, was out-licensed to York Medical BioSciences (Mississauga, Canada). In 2001 clinical studies indicated that the vaccine with an aluminium salt-based adjuvant was safe to be used in humans, however it was not immunogenic enough to raise a sufficiently strong immune response. In 2003, a second clinical trial was initiated. This vaccine was well tolerated with no major adverse events (www.ymbiosciences.com). In a recent press release it has been announced a trial in prostate cancer patients in China (www.unitedbiomedical.com). On the other hand Proterics developed a GnRH vaccine containing the GnRH decapeptide with an additional glycine and cysteine 'Prolog', which was out-licensed to ML Laboratories. They completed phase II clinical studies in 2000, but at present no results have been published (www.mllabs.co.uk). Most recently, the chimeric peptide called GnRH3-hinge-MVP which contains three linear repeats of GnRH (GnRH3), a fragment of the human IgG1 hinge region, and a T-cell epitope of measles virus protein (MVP). The expression plasmid contained the GnRH3-hinge-MVP construct ligated to its fusion partner (AnsB-C) via a unique acid labile Asp-Pro linker. The recombinant fusion protein was expressed in an inclusion body in *Escherichia coli* under IPTG or lactose induction and the target peptide was easily purified using washing with urea and ethanol precipitation. The target chimeric peptide was isolated from the fusion partner following acid hydrolysis and purified using DEAE-Sephacel chromatography. Further, immunization of female mice with the recombinant chimeric peptide resulted in generation of high-titer antibodies specific for GnRH. The results showed that GnRH3-hinge-MVP could be considered as a candidate anti-GnRH vaccine, however the reports just include their use as immunocastration vaccine (Jinshu X, 2006). In 2008, the group of Li Yu and colleagues at Department of Biochemistry, Medical College, Jinan University in China developed GnRH-PE40, one of the recombinant single-chain fusion proteins consisting of GnRH fused to a binding defective form of *pseudomonas aeruginosa* exotoxin A (PE40), which has been developed as a preparation with potential functions of immune castration in male reproductive system (Li *et al.*, 2008).

6.1.3 Adjuvants and other strategies employed for enhancement of immune response

The adjuvants most commonly used in human and veterinary vaccines are oil-based adjuvants and aluminum hydroxide (Alum). Responses to Alum are often low and of short

duration. Oil-based adjuvants are effective in generating a high immune response, but may cause inflammatory reactions. Complete Freund's adjuvant (CFA) is a mineral oil, which forms a water-in-oil emulsion, and contains killed and dried bacteria to stimulate the immune response. This combination induces high antibody responses; because of these characteristics and CFA being one of the oldest adjuvants used, it is 'the gold standard' among adjuvants. However, due to the inflammatory side effects, which may occur at the site of injection, its use is limited to experimentation in laboratory animals.

Instead of whole bacteria, bacterial compounds such as muramyl dipeptide (MDP), lipopolysaccharide (LPS) or monophosphoryl lipid A (MPL) can be used to stimulate the immune system. Alternative immune stimulating compounds are saponins, i.e. Quil A and the purified QS21 fraction, bacterial DNA, microparticles, Iscoms, liposomes, virus-like particles, block polymers and dimethyldioctadecylammonium bromide (DDA).

Among the new adjuvant in development we count Titermax, which contains non-mineral oil and a block polymer that forms a water-in-oil emulsion, and RIBI adjuvant, which contains non-mineral oil with microbial components that forms an oil-in-water emulsion (Bennett *et al.*, 1992; Kiyama *et al.*, 2000). The comparison of CFA to Montanide ISA 51, which forms a water-in-mineral oil emulsion, showed that CFA was superior to ISA 51 with respect to antibody titers and subsequent effects on testosterone levels when tested in sheep. In contrast, others found effective GnRH antibody responses using ISA 51 combined with DDA in baboons (Finstad *et al.*, 2004). In conclusion, effective antibody titers can be generated with adjuvants other than CFA, however, responses may differ among studies due to differences in target species, number of immunizations, antigen type and dose.

A novel retro-inverso GnRH composed of D-amino acids assembled in reverse order (C to N terminus) was found to induce high titers of antibodies reactive with native GnRH without conjugation to a carrier or use as an adjuvant (Fromme *et al.*, 2003). On the other hand non-ionic surfactant vesicles, aluminium hydroxide, Quil A, polylactide co glycolide acid (PLGA) and Quil A/PLGA combination, with their cysteine-modified LHRH linked to TT have been the best adjuvant used by Ferro *et al.* (2004) and interestingly, there exist reports from 1998 that the encapsulation of GnRH-6-DLys-TT in PLGA microspheres induces a bio-effective antibody response within 15 days after a single administration, obliterating the necessity of repeated injections (Diwan *et al.*, 1998)

7. Clinical trials in prostate carcinoma patients

Several GnRH vaccines have been developed for the treatment of prostate cancer. Clinical trials in patients with advanced prostate cancer revealed that in contrast to rodents and monkeys, high antibody titers were obtained in some, but not all treated patients. A reduction in prostatic size was observed in 3 out of 6 patients treated with 400 µg conjugate in Alum and in 1 out of 6 patients treated with 200 µg conjugate (Talwar *et al.*, 1995). The same group used a vaccine comprising a modified GnRH decapeptide with a D-Lysine at position 6 linked to DT in the development of a Phase I/ II clinical trials in 28 patients of advanced stage carcinoma of prostate (12 patients at the All India Institute of Medical Sciences, New Delhi, India, 12 at the Post Graduate Institute of Medical Education and Research, Chandigarh, India, and 4 at the Urologizche Klinikum, Salzburg, Austria). The vaccine employed alhydrogel, an adjuvant permissible for human use. It was used at 200 µg

and 400 µg dose, and three injections were given at monthly intervals. The vaccine was well tolerated by all patients with no side-effects attributable to immunization. A 400 µg dose produced antibody titres >200 pg dose. Patients generating >200 pg of antibodies/ml benefited clinically and testosterone declined to castration levels. The prostate-specific antigen (PSA) and acid phosphatase declined to low levels. Ultrasonography and serial nephrostograms showed the regression of prostatic mass (Talwar *et al.*, 1998). A preclinical study was previously developed by Fuerst in rats bearing androgen-dependent prostatic tumors R3327-PAP. As a result of this study carried out in Copenhagen rats implanted SC with the tumor fragments, after three immunizations tumor growth was suppressed compared to untreated controls. Surprisingly, tumor growth was also suppressed in rats implanted with androgen-independent Dunning tumor cells R3327-AT2.1. This Phenomenon is suggested to be related with the presence of a local GnRH-loop in the prostate, which is affected by GnRH neutralizing antibodies and produce a tumor growth reduction even in testosterone-independent tumors.

A very promising approach using the GnRH antigen was developed by the Apton company in USA. That comprises the GnRH molecule extended with a linker peptide of 6 amino acids conjugated to DT. Two clinical trials with a GnRH-DT vaccine have been carried out at Nottingham, UK by Bishop's group. In the first study (Simms *et al.*, 2000), the vaccine was used at two doses 30 µg and 100 µg, administered three times over 6 weeks in 12 patients with advanced prostate cancer. It was well tolerated and in five patients a significant reduction in serum testosterone and PSA levels was seen. Testosterone declined to castration level in four patients for 9 months. Since the modest results obtained in this trial, 3 and 15 µg doses were evaluated in order to determine how the doses reduction can work (Parkinson *et al.*, 2004). As result of this approach, suppression of testosterone to castrate levels was detected in 2 out of 6 patients treated with 15 µg antigen, whereas none of the patients treated with 3 µg responded. The above-mentioned clinical studies in India, Austria and UK confirmed the safety of LHRH or GnRH linked to DT vaccine in prostate carcinoma patients. These studies further showed that in patients generating adequate antibodies, testosterone declined to castration levels with concomitant decline of PSA, and there was clinical benefit to the patients.

8. GnRHm1-TT, a new strategy to prostate cancer immunotherapy

The development of the vaccine candidate GnRHm1-TT has as its main invention the substitution of L-glycine in position 6 of the GnRH molecule by the amino acid L-proline and the addition of a T helper epitope of TT (Bringas *et al.*, 2000). With this modification we expected to guarantee a "change" in the "U" native conformation of the natural GnRH peptide structure, which is known to play a pivotal role in binding to the receptor (Millar *et al.*, 1977; Millar *et al.*, 2008) and on the other hand, to generate a more rigid molecule that makes it more available to the immune system in the hope to break the B cells tolerance (Goodnow *et al.*, 1991; Bizzini and Achour, 1995) while the incorporation of the TT 830-844 promiscuous epitope give an additional "immunologic target" to recognize this molecule. (Hoskinson *et al.*, 1990, Ferro and Stimson, 1998, Finstad *et al.*, 2004).

The high production of natural antibodies against GnRH, induced by the GnRHm1-TT peptide in AF and in the two types of Montanide, demonstrated the immunogenicity of the

peptide used and its potential to “fool” the physiological mechanisms of immunologic tolerance together with the fact that the Montanide ISA 51 showed some superiority over the FA in terms of antibody titers.

An important fact in the development of a GnRH based vaccine has been the decision about the peptide doses to employ. In this sense, experiments carried out with doses ranging from 125 to 750 μg in rats addressed the usefulness of the 750 μg doses in comparison with lower doses according to the time to develop the anti immune response and the anti GnRH titres. That demonstrate the desirability of using high peptide doses of GnRHm1-TT to achieve the breaking of B cell tolerance and at the same time the utility of using fortnightly and monthly immunization schedules. That is in correspondence with previous works using similar candidates (Talwar et al, 1997, Finstad et al, 2004)

In the hope to demonstrate the feasibility of generating an effective immune response with the vaccine candidate GnRHm1-TT/ Montanide ISA 51 in other species of mammals, given that although GnRH is a hormone that is 100% homologous in all of them (Talwar et al, 1997, Millar et al, 2008), HLA system among species produces a different behavior of the immune response (Friederike et al, 2008), experiments were carried out in two animal models that share different genetic homology; the New Zealand rabbits and Macacus monkeys.

The development of an immunization schedule in both species showed that 100% of the animals generated anti-GnRH antibody titres, regardless of the dose used (750 μg or 1mg). However, the increase in dose up to 1mg, produced a significant rise in antibody titres generated, although this increase did not result in different levels of testosterone.

In this sense, the results suggest that, once antibodies against GnRH hormone reach "critical" levels, they achieve the formation of nearly 100% of circulating immune complexes with GnRH and they will result in the fall of testosterone levels. That observation is in accordance with some reports that suggest that rather than their isotype or antibody affinity, the biological effect of anti-GnRH antibodies depends on the speed of its appearance and its maintenance in sufficient concentrations (Talwar et al 2004, Miller et al, 2006). The use of doses over 1 mg did not improve significantly the characteristics of the immune response produced, neither seemed to generate a phenomenon of immunological tolerance.

These results have great relevance for making decisions regarding the selection of the peptide dose in the following of experiments in other animal models and in humans. In turn, the increased robustness of the *Macacus irus* model, allowed to explore how the immune response would behave when using the intramuscular route (IM). The results found with GnRHm1-TT formulated in Montanide ISA 51, corresponded to those obtained by other authors that report similar immunogenicity between IM and SC routes (Talwar et al, 1997). These similar behavior can be related to the ability of the adjuvant Montanide ISA 51, similar to the AF, to produce a reservoir of antigen and its slow release to the immune system, which allows a better efficiency of antigen presentation by macrophages and Dendritic cells (DC) to T cells (Guerrero et al, 1982; Forsbucher et al, 1996). That approach demonstrates the feasibility of using IM route in human trials where both, the Montanide ISA 51 and Montanide ISA 51 VG, produce a marked local toxic effect when administered subcutaneously (manufacturer's data SEPPIC, France).

In a step forward, the vaccine candidate GnRHm1-TT/Montanide ISA 51 was evaluated in the Dunning R3327-H tumor model, which shares similar characteristics to cancer in humans (Isaacs et al, 1994). The results of the high anti-GnRH seroconversion (88%) in the model demonstrated the feasibility of the vaccine in generating a consistent humoral immune response, despite the presence of established tumor. So, despite the variability in the anti-GnRH titres it dropped testosterone levels until castration in all the animals that seroconverted and in consequence a significant tumor growth inhibition was observed (Table 1).

Animal number	Time to seroconversion (in days)	Anti GnRH titres (day 90)	Testosterone (nmol/L) (day 90)	Survival (in days)
32-	60	1/ 200 ^c	1,70±0,04 ^a	150 ^b
36-	45	1/ 400 ^c	0,07±0,02 ^a	150 ^b
43	45	1/400 ^c	0,01±0,00 ^a	265 ^a
46-	45	1/800 ^b	0,07±0,02 ^a	195 ^b
49-	45	1/ 800 ^b	0,00±0,00 ^a	265 ^a
51-	45	1/ 1600 ^a	0,00±0,00 ^a	95 ^c
55-	45	1/200 ^d	0,84±0,28 ^a	60 ^c
56-	60	1/100 ^d	0,18±0,01 ^a	265 ^a
60-	-	0/00	1,9 0±0,3 ^b	85 ^c

Table 1. Effects of the immunization with vaccine candidate GnRHm1-TT/Montanide in Copenhagen rats implanted with the Dunning R3327-H tumor.

Different letters denote significant differences calculated according to the U Mann Whitney test. The differences in the anti-GnRH antibody titres and Testosterone were calculated using a simple ANOVA and for the survival a Log Rank test was carried out.

As an important fact it was noted that despite the uncontrolled tumor growth observed in cases beyond the hormone-dependence, Dunning R3327-H tumor does not generate distant metastases (Isaacs et al, 1978, 1986; Canena-Adams, 2007, Cho et al, 2009; Peschke et al, 2011).

In order to determine the direct role of the anti GnRH antibodies in the described immunocastration effects in healthy and tumor implanted animals, purified serum obtained from rabbits immunized with the GnRHm1-TT/Montanide ISA 51 vaccine candidate was used and tested in the mammalian COS-7 cells model (Millar et al, 2003). As a result, a gradual decrease in the Inositol Phosphate (IP) concentration was detected once the anti GnRH antibodies concentration was increased from 0,65 µg to 12.5 mg, showing the neutralizing capacity of the anti GnRH antibodies generated with the vaccine candidate.

Despite the satisfactory results obtained with the use of the vaccine candidate GnRHm1-TT/Montanide ISA 51 in the different tested models, the slow appearance of anti-GnRH antibodies (after 3 immunizations) and hence; the consequently slow fall in testosterone levels and the heterogeneity in the atrophy effects in prostate and testes in the vaccinated individuals, led us to a new strategy to include the use of a combination of adjuvants in order to enhance the immune-response attributes induced and the biological effects.

The use of combinations of potent adjuvants to promote the inflammatory cells to escape from the regulatory circuit, is an attractive idea, well addressed by the scientific literature that has been recently practiced in the development of preventive vaccines (Ambrosino et al, 1992; Udono et al, 1993; Nestle et al, 1998). This strategy, however, has not been used so far in the development of therapeutic vaccines based on poorly immunogenic self-molecules as GnRH. In the current vaccine design, a Montanide ISA 51/VSSP adjuvant combination was explored.

The VSSP belongs to the range of pathogen-derived adjuvants which have the ability to stimulate dendritic cells (DC) through receptors similar to those described in *Drosophila* Tolls (TLR). This adjuvant has within its active molecules, those classified as "dangerous", according to the so-called danger theory (Andersen et al, 1989, Lowell et al, 1990; Zollinger, 1990, 1994, Jeannin et al, 2000; 2003; Matzinger et al, 2001).

As a result of the immunization of rats with the GnRHm1-TT peptide emulsified with the adjuvant combination Montanide ISA 51/VSSP, a strong humoral response manifested as three-fold increase in anti-GnRH antibody titres and a significant improvement in the speed of the anti-GnRH was produced (Fig.1).

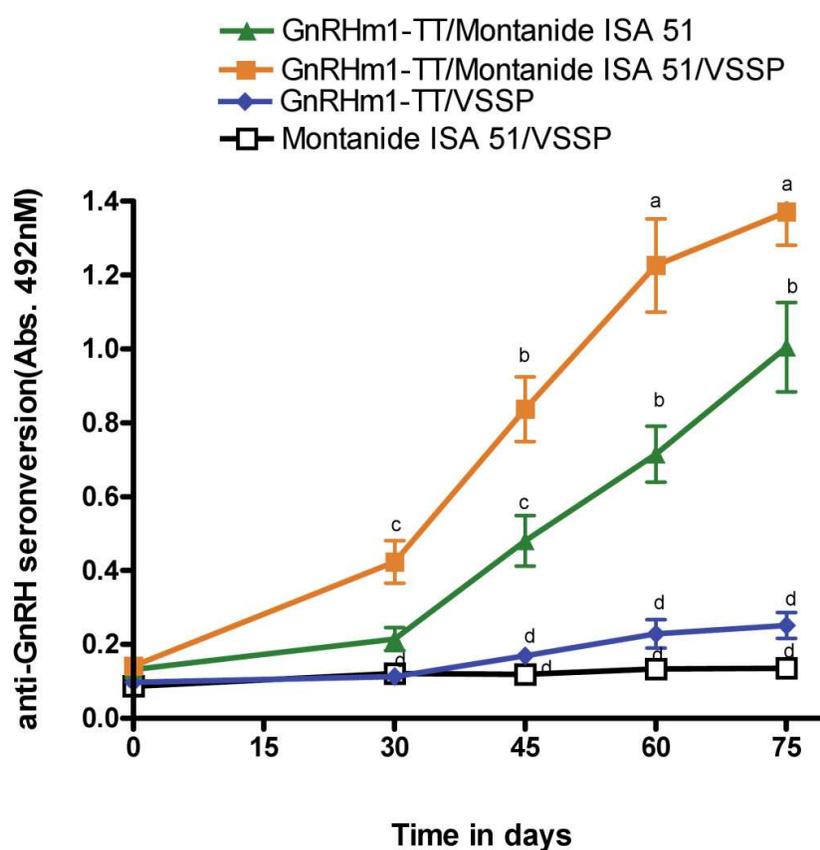


Fig. 1. Graphic representation of anti GnRH seroconversion generated in male rats immunized with the vaccine composition GnRHm1-TT, with or without the presence of VSSP. Serums from different experimental groups were obtained at days 0, 30, 45, 60, and 75. These were diluted in a blocking buffer 1:50. The T bars for each point convey the absorbance mean \pm DE. Different letters denote statistical differences between the points according to the non-parametrical Kruskal Wallis analysis.

It also highlights the fact that this formulation, is the first to allow to obtain mean levels of castration at day 60 after the beginning of the immunization. In addition the adjuvant combination generated a reduction of over 60% of the size of the prostate and testes which was significantly higher than that achieved with the traditional vaccine candidate GnRHm1-TT/Montanide and represents an unprecedented result in the development of such vaccines.

The VSSP classifies as a type 2 adjuvant, acting by stimulating antigen presenting cells through TLR 2 and 6 by a mechanism independent of LPS, producing increased maturation of the humoral immune response in a Th1 pattern (personal communication) and together with the adjuvant Montanide ISA 51, favors the direct stimulation of the innate immune response (Aguiar et al, 2009). To evaluate the antitumoral potentiality of the GnRHm1-TT/Montanide ISA 51 VG /VSSP vaccine candidate, the hormone-sensitive Shionogi SC-115 murine prostate tumor model was used.

Similar to the pattern described in healthy rats, immunization of DD/S mice bearing the Shionogi tumor generated a fast seroconversion and high antibody titers against GnRH after 2 administrations. These results, although expected, are further evidence of the immunogenicity of the GnRHm1-TT peptide in a new species of rodents. In accordance with those anti GnRH antibodies, testosterone ablation was observed in all the immunized mice and a controlled tumor growth was seen in most cases (Fig. 2).

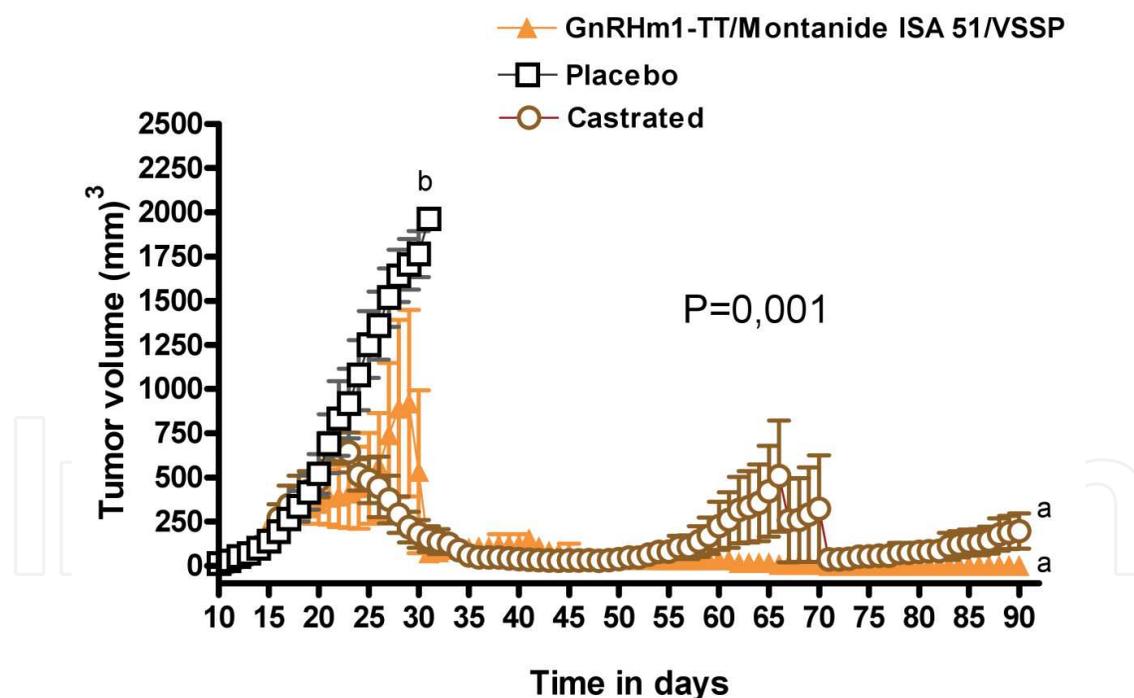


Fig. 2. Tumor growth behaviour of the Shionogi tumor in DD/S mice immunized with the vaccine candidate GnRHm1-TT/Montanide/VSSP. The mice were immunized with the vaccine candidate GnRHm1-TT/Montanide/VSSP at days 0, 15, 30, 45, and 60. (n=5). The castrated group was orchietomized at day 15 after the tumor cells were inoculated. The placebo group was immunized with the same frequency as the immunized one. The former group received a mixture of Montanide ISA 51 VG/VSSP. The curve comparison was made using the Kruskal Wallis test. Different letters denote significant differences ($p < 0,05$).

Although routine prostate cancer immunotherapy refer to those interventions related to the use of specific TAA or cell based vaccines, and GnRH vaccines are considered just as hormonal ablation therapy; the introduction of powerful adjuvant as part of the GnRH vaccines open the possibility to works at the same time as enhancers of antigen spreading and DC stimulation and suppression of T regs cell population. Additionally there are published results (Nesslinger et al, 2007), that argue that castration alone is capable of inducing antibodies against tumor in both, animal models and in humans as a result of efficient presentation of tumor antigens obtained from apoptotic bodies.

In the case of the vaccine candidate under study, which has the powerful components, Montanide ISA 51 and VSSP, we hypothesized that the apoptotic bodies resulting from testosterone ablation of prostate cancer cells in the presence of the “danger” signals produced by VSSP through TLR 2 and 6 in a context of the inflammatory environment, produce an immunological spreading of specific CTL that recognizes the most representative TAA. Similarly, a specific humoral response against tumor antigens can be reached contributing to a better antitumoral effect (Nesslinger et al, 2007). These aspects must be studied in depth in new preclinical and clinical studies to characterize a more efficient vaccine candidate GnRHm1-TT/Montanide ISA 51/VSSP as an advantageous alternative for the treatment of prostate cancer. In Clinical setting an important milestone of the trial was to demonstrate the safety of this candidate. As evidence of vaccine efficacy, recently the GnRHm1-TT/Montanide ISA 51/VSSP candidate (Heberprovac), have been employed in a Phase I clinical trial in advanced prostate cancer patients. The vaccine was well tolerated and no important side effects were detected. As results of immunization, all the 6 patients that concluded the treatment developed anti GnRH antibodies and had depleted the testosterone until castration levels and, in concordance normalized their PSA values. After 4 year of clinical and haematological follow up of the clinical trial, 5/6 patients are alive and keep a favourable clinical picture and normal PSA behaviour.

9. Concluding remarks

Therapeutic cancer vaccines have been in use for several years now. At the beginning, with disappointing results, but after many attempts our understanding growth in the sense of figuring out how the immune system works. This knowledge permitted the successful development of more potent vaccines and other immunotherapeutic agents that are currently in advanced clinical trial or registered as Sipuleucel-T.

GnRH-based vaccines represent a promising anti-hormonal treatment alternative in prostate cancer, because these can reduce serum testosterone to castrate levels, avoid the “hot flushes” produced by GnRH analogues and can be administered in acute and complicated forms of prostate cancer.

Although regularly prostate cancer immunotherapy refer to those interventions related with the use of specific TAA or cell based vaccines, the introduction of powerful adjuvants as part of the GnRH vaccines enables them to work similarly as enhancers of antigen spreading and DC stimulation and immune response modulation.

The development of the vaccine candidate GnRHm1-TT have as main invention the substitution of L-glycine in position 6 of the GnRH molecule by the amino acid L-proline and the addition of a T helper epitope of TT (Bringas et al, 2000) and the use of the

Montanide ISA 51/VSSP adjuvant combination in order to improve the immunogenicity and antitumoral effects of such vaccine. Additionally, it is supposed that the vaccine candidate GnRHm1-TT/Montanide/VSSP take advantage of the tumor apoptosis produced by the testosterone ablation and the special conditions available with the use of the VSSP adjuvant to stimulate a successful antigen presentation to DC and a prominent immunological spreading of effector T cell directed to prostate TAA. Additionally as Nesslinger states, castration alone is capable of inducing antibodies against tumor in both, animal models and in humans as a result of efficient presentation of tumor antigens obtained from apoptotic bodies. (Nesslinger et al, 2010).

In this context, we consider GnRH vaccine like GnRHm1-TT/Montanide/VSSP to represent a powerful weapon that could be employed by uro-oncologist to control the course of prostate cancer toward the CRPC.

10. Acknowledgments

The authors acknowledge Dr. Peter Peschke, at DKFZ, Heidelberg, Germany, Dr. Brad Nelson at DRC BCCRC at Vancouver island, Canada and Dr. Robert Millar MRC Human Reproductive Sciences Unit, Edinburgh, UK for their support of this study.

We specially thanks the Union for International Cancer Control (UICC) and Deutscher Akademischer Austausch Dienst (DAAD), Germany, for the fellowships and support to carry out this work. We also are thankful to Lic. Orestes Padrón Yordi for the manuscript revision.

11. References

- [1] Ambrosino DM, Bolon D, Collard H, Van Etten R, Kanchana MV, Finberg RW. Effect of Haemophilus influenzae polysaccharide outer membrane protein complex conjugate vaccine on macrophages. *J Immunol* 1992; 149:3978-83.
- [2] Andersen BM. Endotoxin releases from Neisseria Meningitides. Relationship between key bacterial characteristics and meningococcal disease. *Scand J Infect Dis Suppl* 1989; 64:1-43.
- [3] Aragon-Ching JB, Williams KM, Gulley JL. Impact of androgen-deprivation therapy on the immune system: implications for combination therapy of prostate cancer. *Front Biosci* 2007;12:4957-71.
- [4] Bavarian Nordic Receives Special Protocol Assessment Agreement from the FDA for Phase 3 Trial of PROSTVAC®. Accessed: April 2011; Available from: <http://www.bavariannordic.com/investor/announcements/2010-40.aspx>
- [5] Becker JT, Olson BM, Johnson LE, Davies JG, Dunphy EJ, McNeel DG. DNA vaccine encoding prostatic acid phosphatase (PAP) elicits long-term T-cell responses in patients with recurrent prostate cancer. *J Immunother* 2010;33(6):639-47.
- [6] Bennett, B., Check, I.J., Olsen, M.R., Hunter, R.L., 1992. A comparison of commercially available adjuvants for use in research. *J. Immunol. Methods*, 153: 31-40.
- [7] Bizzini B y Achour A. "Kinoids": the basis for anticytokine immunization and their use in HIV infection. *Cell. Mol. Biol* 1995; 41: 351-356.
- [8] Bringas R, Basulto R, Reyes O. De la Fuente J. Vaccine preparation for reversible mammals immunocastration 2000. PCT patent N0. A61K37/38, C07K 7/00.

- [9] Burch PA, Breen JK, Buckner JC, Gastineau DA, Kaur JA, Laus RL, Padley DJ, Peshwa MV, Pilot HC, Richardson RL, Smits BJ, Sopapapan P, Strang G, Valone FH, Vuk-Pavlovic S. Priming tissue specific cellular immunity in a phase I trial of autologous dendritic cells for prostate cancer. *Clin Cancer Res* 2000; 6:2175-82.
- [10] Cancer statistics. *CA Cancer J Clin* 2008; 58: 71-96.
- [11] Canene-Adams K, Lindshield BL, Wang S, Jeffery EH, Clinton SK, Erdman JW. Combinations of tomato and broccoli enhance antitumor activity in dunning r3327-h prostate adenocarcinomas. *Jr. Cancer Res.* 2007; 7(2):836-43.
- [12] Casper RF. Clinical uses of gonadotropin-releasing hormone analogues. *Can Med Assoc J* 1991; 144:53-158.
- [13] Cereda V, Poole DJ, Palena C, Das S, Bera TK, Remondo C, et al. New gene expressed in prostate: a potential target for T cell-mediated prostate cancer immunotherapy. *Cancer Immunol Immunother* 2010;59(1):63-71.
- [14] Cho H, Ackerstaff E, Carlin S, Lupu ME, Wang Y, Rizwan A, O'Donoghue J, Ling CC, Humm JL, Zanzonico PB, Koutcher JA. *Neoplasia* 2009; 11(3):247-59.
- [15] Cook T, Sheridan WP. Development of GnRH antagonists for prostate cancer: New approaches to treatment. *Oncologist* 2001; 5:162-168.
- [16] Culig Z, Steiner H, Bartsch G and Hobisch A. Mechanisms of endocrine therapy-responsive and-unresponsive prostate tumors. *Endocrine-Related Cancer* 2005; 12:229-244.
- [17] De Marzo A.M, Nelson W.G, Isaacs W.B, and Eptein J.I. Pathologic and molecular aspects of prostate cancer. *Lancet* 2003; 361:955-964.
- [18] Diwan M, Dawar H and Talwar GP (1998) Induction of early and bioeffective antibody response in rodents with the anti LHRH vaccine given as a single dose in biodegradable microspheres along with alum. *Prostate* 35,279-284.
- [19] Drake CG, Doody AD, Mihalyo MA, Huang CT, Kelleher E, Ravi S, et al. Androgen ablation mitigates tolerance to a prostate/prostate cancer-restricted antigen. *Cancer Cell* 2005;7(3):239-49.
- [20] Epel M, Carmi I, Soueid-Baumgarten S, Oh SK, Bera T, Pastan I, et al. Targeting TARP, a novel breast and prostate tumor-associated antigen, with T cell receptor-like human recombinant antibodies. *Eur J Immunol* 2008;38(6):1706-20.
- [21] Ferro VA, Costa R, Carter KC, Harvey MJ, Waterston MM, Mullen AB, Matschke C, Mann JF, Colston A and Stimson WH (2004) Immune responses to a GnRH-based anti-fertility immunogen, induced by different adjuvants and subsequent effect on vaccine efficacy. *Vaccine* 22,1024-1031.
- [22] Ferro, V.A, O'Grady, J.E., Notman, J, Stimson, W.H. An investigation into the immunogenicity of a GnRH analogue in male rats: a comparison of the toxicity of various adjuvants used in conjunction with GnRH glycols. *Vaccine* 1996; 14: 451-457.
- [23] Ferro, V.A., Stimson, W.H., Investigation into suitable carrier molecules for use in an anti-gonadotrophin releasing hormone vaccine. *Vaccine* 1998; 16: 1095-1102.
- [24] Finstad C.L, Wang C.Y, Kowalsky J, Zhang M, Li M, Li X, Xia W, Bosland M, Murthy k.k, Walfield A, Koff W.C, Zamb T.J. Synthetic luteinizing hormone releasing hormone (LHRH) vaccine for effective androgen deprivation and its application to prostate cancer immunotherapy. *Vaccine* 2004; 22:1300-1313.

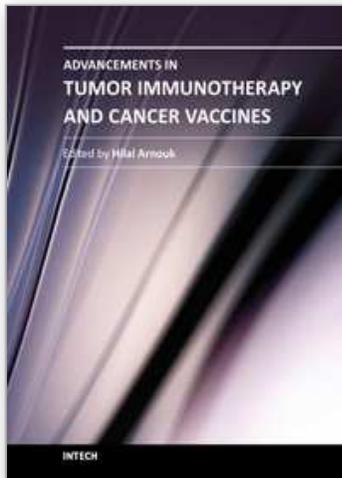
- [25] Fishman M. A changing world for DCvax: a PSMA loaded autologous dendritic cell estrogen by anastrozole enhances the severity of experimental polyarthritis. *Exp Gerontol* 2009;44(6-7):398-405
- [26] Fong L, Small EJ. Anti-cytotoxic T-lymphocyte antigen-4 antibody: the first in an Matzinger P. Essay 1: the danger model in its historical context. *Scand J Immunol* 2001; 54:4-9.
- [27] Forsthuber T, Yip HC, Lehmann PV. Induction of TH1 and TH2 immunity in neonatal mice. *Science* 1996; 271:1728-30.
- [28] Freedland SJ, Hotaling JM, Fitzsimons NJ, Presti JC, Jr., Kane CJ, Terris MK, et al. PSA in the new millennium: a powerful predictor of prostate cancer prognosis and radical prostatectomy outcomes--results from the SEARCH database. *Eur Urol* 2008;53(4):758-64;discussion 65-6.
- [29] Friederike F, Heinen T, Wunderlich FT, Yogev N, Buch T, Roers A, Betelli E, Muller W, Anderton S, Waisman A. Tolerance without clonal expansion: A self -antigen-expressing B cell program self reactive T cells for future deletion. *The journal of immunology* 2008; 181:5748-5769.
- [30] Goodnow C. C., Brink R., Adams E. Breakdown of self tolerance in anergic B Lymphocytes. *Nature* 1991, 532-536.
- [31] Goubau S, Silversides D.W, Gonzalez A, Laarveld B, Mapletoft, R.J, Murphy B.D. Immunisation of sheep against modified peptides of gonadotropin releasing hormone conjugated to carriers. *Domest. Anim. Endocrinol* 1989; 6: 339-347.
- [32] Gupta JC, Raina K, Talwar GP, Verma R and Khanna N (2004) Engineering, cloning and expression of genes encoding the ultrimeric luteinising- hormone-releasing-hormone linked to T-cell determinants in *Escherichia coli*. *Protein Express Purif* 37,1-7.
- [33] Hoskinson R.M, Rigby R.D, Mattner P.E, Huynh V.L, D'Occhio M, Neish A, Trigg T.E, Moss B.A, Lindsey M.J, Coleman G.D, Schwartzkoff C.L. Vaxtrate®: an anti-reproductive vaccine for cattle. *Aust. J. Biotechnol* 1990; 4: 166-170.
- [34] Hsu CT, Ting CY, Ting CJ, Chen TY, Lin CP, Whang-Peng J and Hwang J (2000) Vaccination against gonadotropin-releasing hormone (GnRH) using toxin receptor-binding domain-conjugated GnRH repeats. *Cancer Res* 60,3701-3705.
- [35] Isaacs J .T, Lundmo P.I, Berges R, Martikainen P, Kyprianou N, English H .F. Androgen regulation of programmed death of normal and malignant prostatic cells. 1992. *Journal of andrology* 1992; 13(6):457-64.
- [36] Isaacs J.T, Isaacs W.B, Feitz W.F, Scheres J. Establishment and characterization of seven Dunning rat prostatic cancer cell lines and their use in developing methods for predicting metastatic abilities of prostatic cancer. *Prostate* 1986; 9:261-81.
- [37] Isaacs JT, Heston WD, Weisman RM, Coffey DS. Animal models of the hormone-sensitive prostatic adenocarcinomas, Dunning R-3327-H, R-3327-HI, and R-3327-AT. *Cancer Res* 1978; 38:4353-4359.
- [38] Jeannin P, Renno T, Goetsch L, Miconnet I, Aubry J.P, Deineste Y. et al. OmpA targets dendritic cells, induces their maturation and deliver antigen into the MHC class I presentation pathway. *Nat Immunol* 2000; 6:502-9.
- [39] Jinshu Xu, Zheng Zhu, Peng Duan, Wenjia Li, Yin Zhang, Jie Wu, Zhuoyi Hu, Rouel Roque , Jingjing L. Cloning, expression, and purification of a highly immunogenic

- recombinant gonadotropin-releasing hormone (GnRH) chimeric peptide. *Protein Expression and Purification* 50 (2006) 163-170.
- [40] Kabalin, J.M., et al. Undiscovered adenocarcinoma of the prostate in patients undergoing cystoprostatectomy for other causes: Incidence, histology and morphometric observations. *J. Urol* 1989; 141:1091-1094.
- [41] Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med* 2010;363(5):411-22.
- [42] Kiyama, Z., Adams, T.E., Hess, B.W., Riley, M.L., Murdoch, W.J., Moss, G.E, 2000. Gonadal function, sexual behavior, feedlot performance, and carcass traits of ram lambs actively immunized against GnRH. *Journal of Animal Science* 78: 2237-2243.
- [43] Ladd A, Tsong YY, Lok J and Thau RB (1990) Active immunization against LHRH: Effects of conjugation site and dose. *Am J eprod Immunol Microbiol* 22,56-63.
- [44] Li Yu, Zhong-Fang Zhang, Chun-Xia Jing, Feng-Lin Wu. Intraperitoneal administration of gonadotropin-releasing hormone-PE40 induces castration in male rats. *World J Gastroenterol* 2008; 14(13): 2106-2109.
- [45] Madan RA, Arlen PM, Mohebtash M, Hodge JW, Gulley JL. Prostatevac-VF: a vectorbased vaccine targeting PSA in prostate cancer. *Expert Opin Investig Drugs* 2009;18(7):1001-11.
- [46] Maeda H, Nagata S, Wolfgang CD, Bratthauer GL, Bera TK, Pastan I. The T cell Receptor gamma chain alternate reading frame protein (TARP), a prostate-specific protein localized in mitochondria. *J Biol Chem* 2004;279(23):24561-8.
- [47] Manns J.G, Robbins S.R. Prevention of boar taint with a recombinant based GnRH vaccine. In: Bonneau, M., Lundström, K. and Malmfors, B. (Eds.) *Boar taint in entire male pigs*. Wageningen Pers, Wageningen 1997; 92:137-140.
- [48] McKee AS, MackLeod KL, Kappler JW, Marrack P. Immune mechanism of protection: Can adjuvants rise to the challenge? *BMC Biology* 2010; 8:37, 1-10.
- [49] Mercader M, Bodner BK, Moser MT, Kwon PS, Park ES, Manecke RG, et al. T cell infiltration of the prostate induced by androgen withdrawal in patients with prostate cancer. *Proc Natl Acad Sci U S A* 2001;98(25):14565-70.
- [50] Millar R.P, Pawson A.J, Morgan K, Emilie F, Rissman E.F, Zi-Liang Lu. Diversity of actions of GnRHs mediated by ligand-induced selective signaling. *Frontiers in Neuroendocrinology* 2008; 29:17-35.
- [51] Miller, L.A., Johns, B.E., Killian, G.J., 2000. Immunocontraception of white-tailed deer with GnRH vaccine. *Am. J. Reprod. Immunol.* 44: 266-274.
- [52] Nesslinger N.J, Sahota R.A, Stone B, Johnson K, Chima N, King C, Rasmusen D, Bishop D, Rennie P.S, Gleave M, Blood P, Pai H, et al. Standard treatments induce antigen specific immune responses in prostate cancer. *Clin Cancer Res* 2007; 13: 1493-502.
- [53] Nesslinger NJ, Ng A, Tsang KY, Ferrara T, Schlom J, Gulley JL, et al. A viral vaccine encoding prostate-specific antigen induces antigen spreading to a common set of self-proteins in prostate cancer patients. *Clin Cancer Res* 2010;16(15):4046-56.
- [54] Nestle FO, Aljagic S, Gilliet M, Sun Y, Grabbe S, Dummer R, Burg G, Schadendorf D. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nat Med* 1998; 4:328-32.

- [55] Parkinson R.J, Simms M.S, Broome P, Humphreys J.E, Bishop M.C. A vaccination strategy for the long-term suppression of androgens in advanced prostate cancer. *Eur. Urol* 2004; 45: 171-175.
- [56] Patients: long-term results. *J Urol* 2004;172(3):910-4. 2008;26(32):5275-83.
- [57] Peschke P, Karger CP, Scholz M, Debus J, Huber PE. Relative biological effectiveness of carbon ions for local tumor control of a radioresistant prostate carcinoma in the rat.. *Int J Radiat Oncol Biol Phys* 2011; 79(1):239-46.
- [58] Phase 3 Study of Immunotherapy to Treat Advanced Prostate Cancer. Accessed: April 2011; Available from: <http://www.clinicaltrials.gov/ct2/show/NCT01057810?term=NCT01057810&rank=1>.
- [59] Pienta K.J, and Brandley D. Mechanisms underlying the androgen-independent prostate cancer. *Clin. Can. Res* 2006; 12:1665-1671.
- [60] Raina K, Panda AK, Ali MM and Talwar GP (2004) Purification, refolding, and characterization of recombinant LHRH-T multimer. *Protein Express Purif* 37,8-17.
- [61] Rini B. Recent clinical development of dendritic cell-based immunotherapy for prostate cancer. *Expert Opin Biol Ther* 2004; (11):1729-1734.
- [62] Robbins S.C, Jelinski M.D, Stotish R.L. Assessment of the immunological and biological efficacy of two different doses of a recombinant GnRH vaccine in domestic male and female cats (*Felis catus*). *J. Reprod. Immunol* 2004; 64: 107-119.
- [63] Roehl KA, Han M, Ramos CG, Antenor JA, Catalona WJ. Cancer progression and
- [64] Russel DW, Wilson J.D. 5 alpha reductase: Two genes/to enzymes. *Annu Rev Biochem* 1994; 63:25-61.
- [65] Sacker W.A, et al. Age and racial distribution of prostatic intraepithelial neoplasia. *Eur. Urol* 1996; 30:138-144.
- [66] Sad S, Gupta H.M, Talwar G.P, Raghupathy R. Carrier induced suppression of the antibody response to self hapten. *Immunology* 1991; 74:223-7.
- [67] Silversides D.W, Allen A.F, Misra V, Qualtiere L, Mapletoft R.J, Murphy B.D. A synthetic luteinizing hormone releasing hormone vaccine I. Conjugation and specificity trials in BALB/c mice. *J. Reprod. Immunol* 1988; 13: 249-261.
- [68] Simms M.S, Scholfield D.P, Jacobs E, Michaeli D, Broome P, Humphreys J.E, Bishop M.C. Anti-GnRH antibodies can induce castrate levels of testosterone in patients with advanced prostate cancer. *Br. J. Cancer* 2000; 83: 443-446.
- [69] Small EJ, Tchekmedyan NS, Rini BI, Fong L, Lowy I, Allison JP. A pilot trial of CTLA-4 blockade with human anti-CTLA-4 in patients with hormone-refractory prostate cancer. *ClinCancer Res* 2007;13(6):1810-5.
- [70] Sutherland JS, Goldberg GL, Hammett MV, Uldrich AP, Berzins SP, Heng TS, et al. Activation of thymic regeneration in mice and humans following androgen blockade. *J Immunol* 2005;175(4):2741-53.
- [71] Talwar G.P, Diwan M, Dawar H, Frick J, Sharma S.K, Wadhwa S.N.. Counter GnRH vaccine. In: M. Rajalakshmi and P.D. Griffin (Editors). *Proceedings of the Symposium on 'Male Contraception: present and future*. New Delhi 1995: 309-318.
- [72] Talwar G.P, Raina K, Gupta J.C, Ray R, Wadhwa S, Ali M.M. A recombinant luteinising-hormone-releasing hormone immunogen bioeffective in causing prostatic atrophy. *Vaccine* 2004; 22:3713-3721.
- [73] Talwar G.P. Vaccines for control of fertility and hormone-dependent cancers. *Immunology and Cell Biology* 1997; 75:184-189.

- [74] Tannock I.F, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N. Engl. J. Med* 2004; 351:1502-1512.
- [75] Udono H, Srivastava P.K. Heat shock protein 70-associated peptides elicit specific cancer immunity. *J Exp Med* 1993; 178:1391-6.
- [76] Vaccine for prostate cancer. *Expert Opin Biol Ther* 2009;9(12):1565-75.
- [77] W.G. Prostate cancer prevention. *Curr. Opin. Urol* 2007; 17:157-167.
- [78] Wang J, Zhang Q, Jin S, Feng M, Kang X, Zhao S, et al. Immoderate inhibition of
- [79] Weisbart R.H, Golde D.W, Clarck S.C, Wong G.G, Gasson J.C. Human GMCSG is a neutrofil activator. *Nature* 1985; 314:361-3.
- [80] Yu L, Wang L, Wang H and JW Xuan. Application of Gleason analogous grading system and flow cytometry DNA analysis in a novel knock-in mouse prostate cancer model. *Postgrad. Med. J* 2006; 82: 40-45.
- [81] Zeng W, Ghosh S, Lau YF, Brown LE and Jackson DC (2002) Highly immunogenic and totally synthetic lipopeptides as self-adjuvanting immunocontraceptive vaccines. *J Immunol* 169,4905-4912.
- [82] Zollinger W.D. New and improved vaccines against meningococcal disease. In: Woodrow GC, Levine MM, editors. *New generation vaccines*. Marcel Dekker: NY 1990; 325-48.

IntechOpen



Advancements in Tumor Immunotherapy and Cancer Vaccines

Edited by Dr. Hilal Arnouk

ISBN 978-953-307-998-1

Hard cover, 218 pages

Publisher InTech

Published online 03, February, 2012

Published in print edition February, 2012

Harnessing the potential of the human body's own immune system to attack malignant tumor cells has been the goal of many scientific investigators in recent years, with advances in cancer biology and immunology enabling cancer immunotherapy to become a reality. World-class bench and clinical researchers have joined forces to collaborate and review current developments and trends in cancer immunology for the purposes of this book, and the result is a promising review of contemporary clinical treatments. In each chapter the authors present the scientific basis behind such therapeutic approaches, including cancer vaccines with special focus on prostate cancer, melanoma and novel approaches utilizing both innate and adaptive immune responses.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

J.A. Junco, F. Fuentes, R. Basulto, E. Bover, M.D. Castro, E. Pimentel, O. Reyes, R. Bringas, L. Calzada, Y. López, N. Arteaga, A. Rodríguez, H. Garay, R. Rodríguez, L. González-Quiza, L. Fong and G.E. Guillén (2012). Prostate Cancer Immunotherapy – Strategy with a Synthetic GnRH Based Vaccine Candidate, *Advancements in Tumor Immunotherapy and Cancer Vaccines*, Dr. Hilal Arnouk (Ed.), ISBN: 978-953-307-998-1, InTech, Available from: <http://www.intechopen.com/books/advancements-in-tumor-immunotherapy-and-cancer-vaccines/synthetic-gnrh-based-vaccine-to-prostate-cancer-immunotherapy>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen