Overexpressed oncogenic tumor-self antigens
New vaccine targets

Robert K Bright1,*, Jennifer D Bright1, and Jennifer A Byrne2,3

1Department of Immunology and Molecular Microbiology and the TTUHSC Cancer Center; Texas Tech University Health Sciences Center; Lubbock, TX USA; 2Molecular Oncology Laboratory, Children’s Cancer Research Unit; Kids Research Institute; The Children’s Hospital at Westmead; Westmead, NSW Australia; 3The University of Sydney Discipline of Paediatrics and Child Health; The Children’s Hospital at Westmead; Westmead, NSW Australia

Keywords: TPD52, mD52, hD52, Overexpressed tumor-self antigen, oncogenic, shared, universal, vaccine

Abbreviations: TPD52; tumor protein D52; mD52, murine TPD52; hD52, human TPD52; TAA, tumor associated antigen; Treg, T regulatory cell; ODN, oligodeoxynucleotide; TRAMP, Transgenic adenocarcinoma of the mouse prostate; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; WT-1, Wilms tumor-1; VEGFR2, vascular endothelial growth factor receptor 2; ALK, Anaplastic lymphoma kinase; Her-2/neu, human epithelial growth factor receptor 2; AR, androgen receptor; CTL, cytotoxic T lymphocyte; HLA, human leukocyte antigen.

Overexpressed tumor-self antigens represent the largest group of candidate vaccine targets. Those exhibiting a role in oncogenesis may be some of the least studied but perhaps most promising. This review considers this subset of self antigens by highlighting vaccine efforts for some of the better known members and focusing on TPD52, a new promising vaccine target. We shed light on the importance of both preclinical and clinical vaccine studies demonstrating that tolerance and autoimmunity (presumed to preclude this class of antigens from vaccine development) can be overcome and do not present the obstacle that might have been expected. The potential of this class of antigens for broad application is considered, possibly in the context of low tumor burden or adjuvant therapy, as is the need to understand mechanisms of tolerance that are relatively understudied.

Introduction

Current statistics reveal an increase in cancer incidence with very little decrease in mortality. It is estimated that about 1 660 290 new cancer cases were diagnosed in the USA in 2013, with 580 350 (35%) deaths.1 Improvement of treatments to decrease mortality may be met through immune-based therapies. The employment of the immune system to treat malignant tumors, commonly referred to as tumor immunotherapy, encompasses two general categories: passive and active.2 Passive immunotherapy largely involves the administration of specific antibodies, cytokines, or cells (most commonly tumor antigen-specific T cells generated ex vivo, known as adoptive cell therapy). Passive administration of T cells or of monoclonal antibodies against the T cells inhibitory receptor CTLA-4, which is referred to as immune checkpoint blockade, has recently gained international acclaim as the breakthrough of the year (2013),3 though not without immune related adverse effects.4 Active immunotherapy can be most easily defined by vaccination. While passive immunotherapies often engage the immune system independent of the knowledge of defined tumor antigens (an exception being some forms of adoptive cell therapy5), vaccines elicit specific immune responses by targeting defined tumor antigens.6

With the exception of vaccines to prevent cervical cancer by targeting select serotypes of human papillomaviruses,7 most if not all vaccine clinical trials have focused on therapy. In this approach the vaccine is administered when tumors are present in the patient, with the primary goal of shrinking the tumor mass. However, the majority of clinical trials to treat established solid tumors by vaccination have yielded disappointing results.8 This lack of success is underscored by the existence of only one FDA approved cell-based therapeutic vaccine Sipuleucel T (Provenge®), approved in 2010.8 However this is not to say that cancer vaccination research is without merit. On the contrary, much work is still being done to advance and improve cancer vaccines.

The idea of preventive cancer vaccination has recently gained new attention,9 and may represent the next significant advance in the field of immune-based cancer treatments. The logic behind preventive vaccination is sound and supported by centuries of success against infectious agents. In this case, why hasn’t vaccination been applied more broadly to cancer prevention? It is reasonable to speculate that cancer vaccines have been applied as an option for compassionate administration as a last effort for patients who have failed conventional therapy. This is essentially the approved application for Sipuleucel T, which is not curative but extends life for some late stage prostate cancer patients.10 Advances in early cancer detection have opened the door to develop vaccines to prevent primary and recurrent tumors rather than shrink existing tumor masses.

Whether the vaccine is therapeutic or preventive, it is clear that future success will depend on the character of the tumor.
antigen targeted by the vaccine. The current collective of tumor antigens ranges from specific to associated, non-self to self, and comprises hundreds of vaccine candidates (far too many to discuss in a single review article). In this light, an emerging group of cancer vaccine target antigens, defined primarily by their overexpression in tumor cells compared with low but detectable levels in normal cells, and a role in oncogenesis, represents the focus of this review. Other related topics such as comparisons of vaccine strategies, or the use of adjuvants from different clinical studies, have been recently reviewed elsewhere.11

**Tumor Antigen Classifications**

Tumor antigens recognized by the immune system have been most often defined based on the nature of the antigen (protein, mucin) and its expression pattern, a practice that gave rise to numerous categories and complexities. Examples include, viral proteins specifically associated with virus-induced malignancies (approximately 12% of all cancers),12 and oncogene and tumor suppressor gene products or their mutant variants.6 Oncofetal antigens and melanoma associated antigens represent those with restricted expression in non-malignant tissues,13,14 whereas cancer testis antigens are only expressed, as the name suggests, in the tumor and in testes, with testes being protected by mechanisms of immune privilege.15 Overexpressed antigens are a large and diverse group that includes any protein found at increased levels in tumors compared with normal healthy cells and tissues.

Recently, Coulie and colleagues classified tumor antigens as either high tumor specificity or low tumor specificity, each with two sub-categories; mutation (most tumors) or tumor-specific expression (many tumors) being associated with high tumor specificity, and tissue-specific expression (melanomas) or overexpression (some tumors) being associated with low tumor specificity.6 The overall premise of this classification is whether or not the immune system (T cells in particular) recognizes the tumor antigen.

**Figure 1.** Immunologic character of tumor antigens. Simplified tumor antigen classification based on the immunologic character of the tumor antigen relative to its potential immunogenicity. Depicted are two main classes of antigens represented by tumor-specific and -associated antigens defined by normal cell expression, and four sub-classes ranging from stronger to weaker immunity. Non-self (e.g., viral proteins) and altered-self (e.g., mutant protein or restricted expression) antigens are proposed to elicit strong immune responses, and self antigens, whether involved in oncogenesis (tumor-dependent) or not (tumor-independent), would elicit weaker immune responses.

Figure 1 illustrates a simplified classification based on the immunologic character of the tumor antigen relative to its potential immunogenicity. Arguably tumor-specific antigens represent the obvious choice for vaccine targets, with no or limited expression in normal cells, inferring the potential for a stronger immune response without tolerance. Some examples of tumor-specific antigens include viral antigens (non-self), cancer testis antigens, and melanoma associated antigens (altered-self, with respect to limited expression in normal cells). Antigens from each of these groups are in the advanced stages of vaccine development. Tumor associated antigens (variable expression in normal cells) may represent a more obscure class of antigens, but ironically also represent the largest class of candidate vaccine target antigens. It is presumed that tumor associated antigens will induce a weaker immune response insufficient for tumor rejection, which may be why many have been understudied as vaccine targets. A subclass of overexpressed tumor associated antigens involved in the generation of oncogenesis and critical for maintaining the oncogenic phenotype may represent some of the most promising, widely applicable vaccine targets.

**Overexpressed Oncogenic Tumor-Self Antigens**

A National Cancer Institute sponsored project to prioritize cancer vaccine target antigens for translational research revealed that aberrantly expressed self-proteins represent the largest number of candidate antigens for vaccine development (nearly half of those categorized).17 The study used preweighted objective criteria for prioritizing candidate vaccine target antigens and selected 75 antigens for comparison and ranking (from hundreds considered). Nine criteria were used to rank the antigens. Therapeutic function was considered as the most important followed by immunogenicity, specificity, and oncogenicity. The relative weights of the remaining five criteria were orders of magnitude less than those applied to the top four.17 Cellular localization of expression (internal or surface) carried the least weight of importance, but circulating antigen was determined to be not preferable. Therapeutic function data resulting from clinical trials were heavily considered in selecting the 75 antigens that were ranked.17 Because the goal of the study was to accelerate translational (clinical) research, this bias was logical and acknowledged. However, some newer and potentially promising antigens were not ranked, largely because relevant studies were still in the preclinical phase. To summarize the conclusions, an ideal vaccine target antigen would be immunogenic, eliciting a response that eliminates tumor cells leaving normal cells unharmed (immunogenicity, therapeutic function, and specificity) and play a role in inducing or maintaining the oncogenic phenotype making the antigen indispensable to the tumor.

When considering the specificity criterion and focusing only on those antigens exhibiting aberrant expression (varying degrees of normal cell expression) 87% (65/75) of the ranked antigens can be classified as having some normal cell expression.17 If post-translational modifications (e.g., the mucin, MUC1), and tissue specific (e.g., gp100), mixed (e.g., ALK), stromal (e.g.,...
VEGFR2), and oncofetal (e.g., WT1) expression are excluded from the aberrant expression class, only overexpressed antigens remain, representing nearly 40% of the ranked antigens (28/75). If overexpressed antigens that do not play a role in oncogenesis are excluded from the list of 75, there are 9 overexpressed tumor-self antigens that are immunogenic and oncogenic (two criteria considered to be heavily weighted and important for an ideal tumor vaccine antigen). In the following sections, we will briefly highlight several of the ranked overexpressed oncogenic tumor-self antigens, and end by focusing on perhaps the newest, widely shared overexpressed oncogenic tumor-self antigen, tumor protein D52 (TPD52).

Limited tumor expression
Androgen Receptor

The androgen receptor (AR) is a steroid receptor involved in prostate development, and a target for hormone deprivation therapy for advanced metastatic prostate cancer. Its function ascribes the AR with a role in prostate oncogenesis. AR is expressed in normal tissues with the prostate being a major site, and is widely overexpressed in prostate cancer, and in a subset of breast cancers. Pre-existing AR-specific antibodies and T cells have been detected in prostate cancer patients, demonstrating immunogenicity and the potential for targeting the AR by vaccination. The fine specificity of the AR-specific CTL responses in prostate cancer patients was defined by recognition of two HLA-A2-restricted peptides, AR805 and AR811, with the latter capable of eliciting specific CTLs following immunization of A2/DR1 transgenic mice. An AR-based DNA vaccine targeting the AR ligand-binding domain (LBD) effectively induced CTLs in HHDII-DR1 (HLA-A2+ and HLA-DR1+) transgenic mice which were capable of lysing HLA-A2+ human prostate cancer cell lines. In vivo prostate tumor regression was also observed in rats immunized with the AR-LBD DNA vaccine, supporting the potential for targeting the AR with vaccination. These studies demonstrate that AR is immunogenic in patients and AR-based vaccines are capable of in vivo tumor rejection in pre-clinical animal models.

Shared tumor expression

We defined shared tumor expression as overexpression in multiple different cancers, but not as widely shared or potentially universal in tumor overexpression as that reported for human telomerase reverse transcriptase (hTERT) and survivin (discussed in the following section). The following are three examples of shared overexpressed oncogenic tumor-self antigens that are currently being evaluated in clinical trials as vaccine targets. Her-2/neu and p53 are perhaps the most commonly recognized and extensively studied TAAs of this category.

Her-2/neu

The transmembrane tyrosine kinase Her-2/neu is one of the most studied cancer proteins and therapeutic targets (nearly 1000 reviews). Her-2/neu is overexpressed in multiple cancers including lung, prostate and most notably breast cancer. To date the majority of clinical vaccine trials have been conducted in patients with breast cancer. Early evidence supporting Her-2/neu as a vaccine target came from the demonstration that specific CTLs exist in the peripheral blood of breast cancer patients. Clinical trials evaluating vaccines targeting the intracellular and extracellular domains of Her-2/neu demonstrated the generation of specific T cells without the induction of autoimmunity and no significant toxicity, supporting the use of vaccines in the adjuvant setting.

p53

The important role played by p53 in cancers of multiple types has been recognized for decades. CTLs have been generated against mutated and non-mutated epitopes of p53 without inducing autoimmunity and pre-existing p53-specific CTLs have been demonstrated in cancer patients. Clinical trials are underway evaluating therapeutic vaccines targeting p53 in ovarian cancer, colorectal cancer, and non-small cell lung cancer. Overall, p53-specific immune responses have been observed in patients, but significant reductions in tumor burdens have not been demonstrated. It has been suggested that multiple epitope vaccines, Treg elimination, and/or CTLA-4 blockade should be assessed in combination with p53 vaccines to increase their clinical efficacy.

EphA2

EphA2 is a cell surface-expressed receptor tyrosine kinase and is one of 14 members of the Eph family (named for erythropoietin-producing hepatocellular carcinoma cell lines). This family and its ligands play key roles in normal development and in tumorigenesis, where EphA2 is overexpressed in multiple cancers including brain malignancies, which have been the focus of most vaccine trials. The vaccine potential of targeting EphA2 in gliomas was initially demonstrated by generating HLA-A2-restricted CTLs from the peripheral blood of HLA-A2+ normal donors and glioma patients using a single synthetic peptide, and by vaccination of HHD mice with the same EphA2 peptide, demonstrating that tolerance could be broken by vaccination without inducing autoimmunity. Subsequent studies in murine models of colon, liver and brain cancer further support the potential of EphA2 as a vaccine target. Initial clinical trials testing EphA2 vaccines against adult and pediatric brain cancers have shown them to be safe, immunogenic and promising.

Widely shared (universal) tumor expression

There are but a few tumor associated antigens that appear to be universal for most if not all cancers, and these have shown promise as candidates for vaccine development. The two most studied universal tumor associated antigens are hTERT and the anti-apoptotic protein survivin. Neither of these antigens are specific for tumor cells, but both are over-active and/or aberrantly expressed in tumor cells compared with normal or non-malignant cells, and play a role in prolonging survival of tumor cells by preventing natural death mechanisms associated with proliferation such as telomere shortening and apoptosis. These attributes
classify hTERT and survivin as shared overexpressed oncogenic tumor-self antigens.

hTERT
The activity of hTERT is a rate limiting step in the proliferative capacity of advanced cancers and represents a prototypical and universal cancer antigen and marker. Variations in hTERT function in normal self-renewing tissues and tumors exist that can be taken advantage of for vaccine development. hTERT was first characterized as a widely shared tumor associated antigen by demonstrating the induction of specific CTLs against more than 85% of human cancers, and by the identification of an HLA-A2 peptide vaccine candidate, 1540 (ILAKFLHWL). Circulating 1540-specific CTLs were detected in nearly 91% of HLA-A2+ patients with 6 different cancers supporting vaccine development. Additional CTL-specific hTERT- peptides include peptides restricted by additional HLA.

Survivin
Survivin is an inhibitor of apoptosis and acts upstream of the main effector proteases of apoptosis, caspase 3 and 7, and is active in a cell-cycle regulated manner during the G2/S phase, as a safeguard against activated cell death during successive rounds of cell division. Like hTERT, survivin is expressed at low levels in normal, differentiated adult tissues but is overexpressed in cancers originating from a variety of tissues including lung, colon, breast, pancreas, and prostate cancer and several hematopoietic cancers. The immunogenic potential of survivin was initially demonstrated by the induction of specific HLA-A2-restricted CD8+ cytotoxic T cell responses by dendritic cells that had processed and presented recombinant survivin protein. CTLs specific for survivin were also detected in the peripheral blood of cancer patients supporting its potential as a vaccine target. A survivin peptide vaccine administered with IFNα demonstrated efficacy and benefit for patients with advanced pancreatic cancer, although contrasting clinical benefits were observed for similar vaccine approaches in advanced melanoma patients.

Tumor protein D52
A third example of a new overexpressed oncogenic tumor self antigen with widely shared tumor expression is tumor protein D52 (TPD52) and like p53, survivin and hTERT is an intracellular protein. Early clinical support for the promise of TPD52 as a vaccine target antigen came from the identification of circulating serum antibodies with specificity for TPD52 in patients with breast cancer.

Expression of TPD52 in cancer
TPD52 is an overexpressed tumor self-protein actively involved in transformation, leading to increased proliferation and metastasis. TPD52 overexpression has been demonstrated in several human malignancies including breast, prostate, and ovarian carcinomas. Expression microarray and other analyses predict TPD52 overexpression in many other cancers including multiple myeloma, Burkitt’s lymphoma, pancreatic cancer, testicular germ cell tumors, as well as multiple other adult and pediatric cancers.

Murine ortholog of TPD52
The murine ortholog of TPD52 (mD52) parallels normal tissue expression patterns and known functions of human TPD52 (hD52), with 86% amino acid identity. mD52 induced anchor-age independent growth and spontaneous lung metastasis when overexpressed in normal, non-tumorigenic cells. Reduction of hD52 expression via RNAi resulted in increased apoptosis in human breast cancer cells, and hD52 overexpression was associated with decreased overall survival in human breast cancer patients. These studies demonstrate that TPD52 overexpression is important for initiating and maintaining an oncogenic and metastatic phenotype and may be important for tumor cell survival. TPD52 is naturally expressed and involved in tumor formation and metastasis in human cells (hD52) and in mouse cells (mD52). This makes mD52 a unique and powerful overexpressed tumor-self antigen for study as a cancer vaccine target in murine models of cancer.

TPD52 as a vaccine target
The first demonstration that tumor protective immunity could be induced against TPD52 involved a recombinant protein-based mD52 vaccine that induced protection against tumor challenge when administered with CpG-ODN as a molecular adjuvant. mD52 protein administered without CpG-ODN failed to elicit an immune response, indicating that the TLR agonist was necessary to break tolerance. Subcutaneous injection of mD52 protein with CpG-ODN required concomitant CD4+CD25+ T regulatory (Treg) cell depletion to improve tumor protection. DNA-based vaccine approaches using the TRAMP model of prostate cancer demonstrated that mD52 DNA vaccination induced an immune response that prevented tumors with increased efficacy when administered with GM-CSF and induced long-term immunologic memory. When mD52 DNA vaccination was compared head-to-head with hD52 DNA vaccination, the partial xenogenic (hD52) was more effective at protecting against tumor challenge, however both strategies induced durable responses that rejected secondary tumor challenge months later. The T cell cytokine secretion patterns for all the TPD52 vaccine studies demonstrated that a Th1-type cellular immune response was responsible for tumor rejection and that a complete response may be hindered by a potentially unique subset of CD8+ IL-10+ regulatory T cells. An overlapping peptide-based mD52 vaccine, evaluated independently, demonstrated efficacy in a murine breast cancer model. Important facts have been revealed by preclinical TPD52 vaccine studies to date (summarized in Table 1).

Table 1
that a tumor self-protein can be immunogenic when delivered as a simple protein, peptides or plasmid DNA. Second, TPD52 vaccines prevent tumor formation without inducing autoimmunity, even when classical CD4+CD25+Treg cells were depleted. These studies suggest that TPD52-specific T cells are present and not completely eliminated by central tolerance, and that peripheral tolerance is involved in obstructing complete tumor rejection to include suppression by an as yet undefined but potentially unique subset of CD8+ Treg cells. An additional note-worthy observation from our preclinical vaccine studies is that DNA-based vaccines (most notably xenogeneic hD52 DNA) appear to be more potent and effective suggesting that TLR-9 plays a role as a molecular adjuvant. This is supported by the requirement for the inclusion of CpG ODN with recombinant protein to induce protective immunity.

As a first step to human studies and eventual clinical trials, we generated CTLs specific for hD52 from the peripheral blood of an HLA-A2 male normal donor by in vitro stimulation with a synthetic peptide (QLFHSFSV; modified at P2 and P9 to increase affinity for HLA-A2) derived from the amino acid sequence of hD52 using established protocols. These CTLs only lysed HLA-A2 prostate cancer cell lines that naturally overexpressed hD52 (Fig. 2). This initial experiment further supports the potential use of TPD52 as a vaccine target in humans.

### Concluding Remarks

Employing the immune system to fight cancer has long been viewed as promising, as is evidenced by the extensive list of publications attesting to the hard work of many. This promise is now being realized by FDA approval of two of the newest treatments for prostate cancer and melanoma, Sipuleucel T and CTLA-4 blockade. Both of these immunotherapies rely on eliciting T cell-mediated immunity which requires antigen recognition and specificity. Sipuleucel T is a therapeutic vaccine, and CTLA-4 (T...
cell checkpoint) blockade is being explored in combination with vaccination. Most vaccine trials focus on patients with advanced cancer (therapeutic vaccines) and have largely produced disappointing results. Historically vaccines have been successful only when applied to prevent disease. Perhaps immunization to prevent primary cancer, recurrence and/or metastasis as opposed to eliminating already existing primary and metastatic tumors is the next step in cancer vaccine development.

Regardless of the application, cancer vaccines are only as good as the antigens they target. In this context it is logical to argue that the antigen content of the vaccine formulation is more important than the delivery vehicle. A review of the literature will reveal that much of the past vaccine development work has focused on generating new delivery vehicles and formulations to make a few well-studied antigens more potent in murine models of cancer and clinical trials. This focus on a minority of antigens is also true for the study of oncogenes in general. The recent prioritization of vaccine antigens for translational studies supports the notion that it is time to find new, better antigens on which to focus vaccine efforts.

What constitutes an ideal cancer vaccine antigen? Many would argue, and logically so, that completely foreign (e.g., viral) non-self proteins would be the most immunogenic (Fig. 1) and therefore effective. Much of the early preclinical experimental literature and the FDA approval of preventive cervical cancer vaccines would be in agreement. Unfortunately most clinically significant cancers are not (or at least have yet) to be associated with viruses. This truth led to the TAA discovery movement and the development of various technologies for its accomplishment. This produced many candidate vaccine antigens that with further effort, could yield ideal targets for the next generation of cancer vaccines, among them being the overexpressed tumor-self antigens. This large group of understudied antigens more potent in murine models of cancer and clinical trials. This focus on a minority of antigens is also true for the study of oncogenes in general. The recent prioritization of vaccine antigens for translational studies supports the notion that it is time to find new, better antigens on which to focus vaccine efforts.

No potential conflicts of interest were disclosed.

Disclosure of Potential Conflicts of Interest

This work was supported by a grant from the DOD CDMRP PCRP: Award Number W81XWH–08–1–0660; and by funds from Texas Tech University Health Sciences Center.

Funding

References

1. American Cancer Society. Cancer Facts & Figures 2013. Atlanta: American Cancer Society; 2013.
2. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature 2011; 480:480-9; PMID:22193102; http://dx.doi.org/10.1038/nature10673.
3. Couzin-Frankel J. Breakthrough of the year 2013. Cancer immunotherapy. Science 2013; 342:1432-3; PMID:24357284; http://dx.doi.org/10.1126/science.1248615.
4. Weber JS, Kahler KC, Hauschald A. Management of immune-related adverse events and kinetics of response with ipilimumab. J Clin Oncol 2012; 30:2691-7; PMID:22641989; http://dx.doi.org/10.1200/JCO.2012.41.6790.
5. Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol 2002; 2:269-78; PMID:11973939; http://dx.doi.org/10.1038/nri1319.
6. Lewis JD, Reilly BD, Bright RK. Tumor-associated antigens: from discovery to immunotherapy. Int Rev Immunol 2003; 22:811-12; PMID:12962271; http://dx.doi.org/10.1080/0883803035221.
7. Schiller JT, Castellau X, Garland SM. A review of clinical trials of human papillomavirus prophylactic vaccines. Vaccine 2012; 30(Suppl 5):F123-38; PMID:22319956; http://dx.doi.org/10.1016/j.vaccine.2012.04.108.
8. Oggi C, Ariga A. Clinical evaluation of therapeutic cancer vaccines. Hum Vaccin Immunother 2013; 9:1049-57; PMID:2345867; http://dx.doi.org/10.4161/hv.23917.
9. Weiner LM, Sutana R, Murray J. Vaccine prevention of cancer: can endogenous antigens be targeted? Cancer Prev Res (Phila) 2010; 3:140-5; PMID:20332297; http://dx.doi.org/10.1158/1940-6207.CAPR-10-0040.
10. Michael A, Kelch K, Annels N, Pandha H. Prostate cancer vaccines. Expert Rev Vaccines 2013; 12:523-6; PMID:23496665; http://dx.doi.org/10.1586/erv.13.27.
11. Aradao F, Vaccielli E, Eggermont A, Galon J, Sautès-Fridman C, Tartour E, Ziółkowski I, Kroemer G, Galluzzi L, Trial Watch: Peptide vaccines in cancer therapy. Oncoimmunology 2013; 2:e26621; PMID:24498550; http://dx.doi.org/10.4161/onci.26621.
12. Mesi EA, Feltolse MA, Mungur K. Human viral oncogenicis: a cancer hallmark analysis. Cell Host Microbe 2014; 15:266-82; PMID:24629334; http://dx.doi.org/10.1016/j.chom.2014.02.011.
13. Coggins JH Jr., Barsoum AL, Rohrer JW, Thurnher M, Zitzi M. Contemporary definitions of tumor specific antigens, immunogens and markers as related to the adaptive responses of the cancer-bearing host. Anticancer Res 2009; 29(5c):2345-55; PMID:16080461.
14. Yang JC. The adoptive transfer of cultured T cells for patients with metastatic melanoma. Clin Dermatol 2013; 31:209-19; PMID:24338864; http://dx.doi.org/10.1016/j.clindermatol.2012.08.019.
15. Kalkani P, Shiraiishi T, Rajagopalan K, Kim R, Mooney SM, Getzenber GH. Cancer/testis antigens and urogenital malignancies. Nat Rev Urol 2012; 9:368-86; PMID:22710665; http://dx.doi.org/10.1038/nruro.2012.117.
16. Coulie PG, Van den Eynde BJ, van de Bruggen P, Boon T. Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy. Nat Rev Cancer 2014; 14:135-46; PMID:24475147; http://dx.doi.org/10.1038/nrnc.2014.76.
17. Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, Mellman I, Prindiville SA, Viner JL, Weiner LM, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. Clin Cancer Res 2009; 15:5323-37; PMID:19729653; http://dx.doi.org/10.1158/1078-0432.CCR-09-0737.
18. Angello MA, Den RB, Knoedsen KE. AR function in promoting metastatic prostate cancer. [epub ahead of print]. Cancer Metastasis Rev 2014; PMID:24425228; http://dx.doi.org/10.1007/s10549-014-9972-3.
19. McNamara KM, Yoda T, Nutani AM, Shibahara Y, Miki Y, Wang L, Nakamura Y, Suzuki K, Yang Y, Abe E, et al. Androgenic pathways in the progression of triple-negative breast carcinoma: a comparison between aggressive and non-aggressive subtypes. [epub ahead of print]. Breast Cancer Res Treat 2014; 145:281-93; PMID:24715382; http://dx.doi.org/10.1007/s10549-014-2942-6.
20. Olson BM, McNeel DG. Antibody and T-cell responses specific for the androgen receptor in patients with prostate cancer. Prostate 2007; 67:1729-39; PMID:17879963; http://dx.doi.org/10.1002/pros.20652.
21. Olson BM, McNeel DG. CD8+ T cells specific for the androgen receptor are common in patients with prostate cancer and are able to lyse prostate tumor cells. Cancer Immunol Immunother 2011; 60:781-92; PMID:21350948; http://dx.doi.org/10.1007/s00262-011-1363-9.
22. Olson BM, Johnson LE, McNeel DG. The androgen receptor: a biologically relevant vaccine target for the treatment of prostate cancer. Cancer Immunol Immunother 2013; 62:585-96; PMID:23018626; http://dx.doi.org/10.1007/s00262-012-1363-9.
23. Seliger B, Kono K, Rongcan Y, Kieselung R. Cytotoxic T cell epitopes and tissue distribution of the HER-2/
new proto-oncogene: Implications for vaccine development. In W.M. Katz (Ed.) Peptide-based Cancer Vaccines. Georgetown, TX: Eurekam.com & Landes Bioscience, 2000.

46. Zitvogel L, Chastanier M. Clinical trials of HER-2/neu peptide-based vaccines. In W.M. Katz (Ed.) Peptide-based Cancer Vaccines. Georgetown, TX: Eurekam.com & Landes Bioscience, 2000.

47. Wiedermann U, Davis AB, Zielinski CC. Vaccination for the prevention and treatment of breast cancer with special focus on Her-2/neu peptide vaccines. Breast Cancer Res Treat 2013; 138:1-12; PMID:23340862; http://dx.doi.org/10.1007/s10549-013-2410-8

48. Levine AJ. p53, the cellular gatekeeper for growth and suppression of cancer and a new therapy for breast cancer. J Natl Cancer Inst 2003; 95:1466-70; PMID:14579847; http://dx.doi.org/10.1093/jnci/dkg324

49. Beatty GL, Vonderheide RH. Telomerase as a universal target for cancer vaccines. Expert Rev Vaccines 2008; 7:867-84; PMID:19388882; http://dx.doi.org/10.1517/neo.05277

50. Su Z, Dannull J, Yang BK, Dahm P, Coleman D, Litvak E, et al. Telomerase mRNA-transfected dendritic cells stimulate antigen-specific CD8+ and CD4+ T cell responses in patients with metastatic breast cancer. J Immunol 2008; 187:2466-73; PMID:19074825; http://dx.doi.org/10.4161/onci.7.6.17831
3304 Volume 10 Issue 11 Human Vaccines & Immunotherapeutics

62. Venika EK, Konsolakis G, Aggourakos D, Kotsakis A, Papadimitriou E, Christos S, Menez-Jamer S, Kosmatopoulou K, Georgoulas V, MAVroudis D. Immuno- logical responses in cancer patients after vaccination with the influenza and human papillomavirus vaccine. Virulence 2010; 1:41-52; PMID:20606694; http://dx.doi.org/10.4161/viru.1.1.16886

63. Ballinette R, Feuj MH, d’Astous N, Basset P, Clarke CL. Byrne JA. The hD52 (TPD52) gene is a candidate target gene for events resulting in increased 8q24 copy number in human breast carcinoma. Genes Chromosomes Cancer 2000; 29:48-57; PMID:10918933; http://dx.doi.org/10.1002/(SICI)1099-0704(20000999)29:1<48::AID-GICC1050>3.0.CO;2-O

64. Byrne JA, Tomasetto C, Garnier JM, Rouyer N, Mattei MG, Bellocco JP, Rio MC, Basset P. A screening method to identify genes commonly overexpressed in carcinomas and the identification of a novel complement- dependent RNA sequence. Cancer Research 1995; 55:2896-903; PMID:7796418

65. Pollack JR, Sorlie T, Perou CM, Rees CA, Jeffrey SS, Lonning PE, Tibshirani R, Botstein D, Bengtsson T, et al. Molecular classification of breast cancer: classification of breast cancer: discovery and validation of new phenotype using gene expression profiling. Proc Natl Acad Sci U S A 2002; 99:12962-6; PMID:12297621; http://dx.doi.org/10.1073/pnas.192097799

66. Wang R, Xu J, Sarmah M, Zihui M, Zhou H, Jin S, Lin SM, Mahdavi J, Amin N, et al. P63, a novel prostate-specific and androgen-responsive gene of the TDP52 family, amplified in chromosomal domain 8q24.12 in human prostate cancer. Cancer Research 2004; 64:1589-94; PMID:14949714; http://dx.doi.org/10.1158/0008-5472-CAN-03-3331

67. Rubin MA, Rubin SB, Hornstra M, Tomlina SL, Rhodes DR, Paris PL, Hofer MD, Storz-Schweizer M, Kuefer R, Fletcher JA, et al. Overexpression, amplification, and androgen regulation of TPD52 in prostate cancer. Cancer Research 2004; 64:3814-22; PMID:15151278; http://dx.doi.org/10.1158/0008-5472-CAN-03-3881

68. Byrne JA, Ballene RL, Schoneberg Fejzo M, Mercieca J, Chien YE, Livnat Y, Ste Haeps L, Peters GB, Bykh K, Kardel SA, et al. Tumor protein D52 (TPD52) is overexpressed and a gene amplification target in ovarian cancer. Int J Cancer 2005; 117:1049-54; PMID:15986628; http://dx.doi.org/10.1002/ijc.21250

69. Largo C, Alvarez S, Blesa D, Martin-Subero JI, González-García I, Brieva JA, Dopazo J, Siebert R, Ullrich A, et al. Nonredundant functions for tumor protein D52 (TPD52) gene: analysis of multiple chromosome regions in multiple myeloma. Haematologica 2006; 91:184-91; PMID:16461302

70. Tiacci E, Ovierian PL, Biggera B, Aquilani R, Torricelli GL, Pettinotti V, Martelli MP, Liso A, Benedetti R, Pacini R, et al. Tumor protein D52 (TPD52): a novel B-cell/plasma-cell molecule with unique expression pattern and Ca(2+)-dependent association with annexin VI. Blood 2005; 105:2812-20; PMID:15576473; http://dx.doi.org/10.1182/blood-2004-07-2630

71. Dave SS, Fu K, Wright GW, Lam LT, Klain P, Boerma EJ, Grenier TC, Weisburger DD, Rosewold A, Oddoux C. OX19 in the Molecula- R Profiling Project. Molecular diagnosis of Burkitt’s lymphoma. N Engl J Med 2006; 354:2341-42; PMID:16704435; http://dx.doi.org/10.1056/NEJMoa057590

72. Hummel M, Bentink S, Berger H, Klapper W, Wend- sendorf S, Barth TF, Bernd HW, Cogliatti SB, Dier- lamm J, Feller AC, et al.; Molecular Mechanisms in Chromosome Rearrangements Project Group. A biologic definition of Burkitt’s lymphoma from transcriptional and genomic profiling. N Engl J Med 2006; 354:2341-93; PMID:16704442; http://dx.doi.org/10.1056/NEJMoa053511

73. Loukopoulos P, Shibata T, Katoh H, Koku K, Sakamoto M, Yamazaki K, Kousuge T, Kanai Y, Houedard F, Buttaro I, et al. Genome-wide based comparative genomic hybridization analysis of pancreatic adenocarcinoma: identification of genetic indicators that predict patient outcome. Cancer Sci 2007; 98:392-400; PMID:17323815; http://dx.doi.org/10.1111/j.1349-7006.2007.00395.x

74. Skorheim RI, Auuto R, Lind GE, Kragemard S, Andrews PW, Monni O, Kallioniemi O, Loathe RA. Novel genomic aberrations in testicular germ cell tumors by array-CGH, and associated gene expression changes. PLoS ONE 2006; 28:315-26; PMID:17167184

75. Koekela JE, Beck S, Oshlen AR, Reuter VE, Bod GJ, Houdouvard J, Chagliani RS. In vivo differentiation and genomic evolution in adult male germ cell tumors. Genes Chromosomes Cancer 2008; 47:43-55; PMID:17943742; http://dx.doi.org/10.1002/gcc.20504

76. Mcnichire A, Summergill B, Lu YJ, Missiaglia E, Kizawa S, Osterhui JS, Loojenga LH, Shipley J. Genome copy number number and gene expression patterns in tes- ticular germ cell tumours. Br J Cancer 2007; 97:1707-12; PMID:18095402; http://dx.doi.org/10.1038/bjc.6000479

77. Hoek KS. DNA microarray analyses of melanoma gene expression: a decade in the mines. Pigment Cell Melanoma Res 2007; 20:466-84; PMID:17395490; http://dx.doi.org/10.1111/j.1600-0749.2007.00412.x

78. Roesech A, Becker B, Bentink S, Spang R, Vogl A, Hagen I, Landighthouse V, Teg T. Ataxia telengiectasia- associated gene TPD52 may be an additional gene that differentiates between leukemic and nonleukemic B-cell malignancies. Leukemia 2006; 20:1490-6; PMID:16760010; http://dx.doi.org/10.1038/sj.leu.2403798

79. Byrne JA, Frost S, Chen Y, Bright RK. Tumor protein D52 (TPD52) and cancer-oncogene understudy or understudied oncogene? Tumour Biol 2014; 35(7): 1369-76; PMID:24798974; http://dx.doi.org/10.1007/s13277-014-2006-x

80. Byrne JA, Mattei MG, Basset P. Definition of the D52 gene/protein family through cloning of D52 homologues in human (hD53) and mouse (mD52). Genomics 1996; 35:523-32; PMID:9015248; http://dx.doi.org/10.1006/geno.1996.0393

81. Lewis JD, Payton LA, Wharf JD, Byrne JA, Smith DI, Yang L, Bright RK. Induction of tumorogenesis and metastasis by the murine orthologue of tumor protein D52 (mD52). Genomics 1996; 35:523-32; PMID:9015248; http://dx.doi.org/10.1006/geno.1996.0393

82. Lewis JD, Payton LA, Wharf JD, Byrne JA, Smith DI, Yang L, Bright RK. Induction of tumorogenesis and metastasis by the murine orthologue of tumor protein D52 (mD52). Genomics 1996; 35:523-32; PMID:9015248; http://dx.doi.org/10.1006/geno.1996.0393

83. Payton LA, Lewis JD, Byrne JA, Bright RK, Vaccination with metastasis-related tumor associated antigen TPD52 and CgP/GfO/DN induces protective tumor immunity. Cancer Immunol Immunother 2008; 57:799-811; PMID:17962942; http://dx.doi.org/10.1007/s00262-007-0476-9

84. Bright JD, Schultz CH, Byrne JA, Bright RK. Injection site and regulatory T cells influence durable vac- cine-induced tumor immunity to an over-expressed self tumor associated antigen. Oncoimmunology 2013; 2:e250949; PMID:24073579; http://dx.doi.org/10.4161/onci.250949

85. Lewis JD, Sullivan LA, Byrne JA, de Riese W, Bright RK. Memory and cellular immunity induced by a DNA vaccine encoding self antigen TPD52 administered with soluble GM-CSF. Cancer
100. Bright JD, Aldrich JF, Byrne JA, Bright RK. Vaccination with the prostate cancer overexpressed tumor self-protein TPD52 elicits protective tumor immunity and a potentially unique subset of CD8+ T cells. Austin J Clin Immunol 2014; 1:1-13

101. Mirshahidi S, Kramer VG, Whitney JB, Essono S, Lee S, Dranoff G, Anderson KS, Ruprecht RM. Overlapping synthetic peptides encoding TPD52 as breast cancer vaccine in mice: prolonged survival. Vaccine 2009; 27:1825-33; PMID:19201387; http://dx.doi.org/10.1016/j.vaccine.2009.01.089

102. Bright RK, Kimchi ET, Shearer MH, Kennedy RC, Pass HE. SV40 Tag-specific cytotoxic T lymphocytes generated from the peripheral blood of malignant pleural mesothelioma patients. Cancer Immunol Immunother 2002; 50:682-90; PMID:11862420; http://dx.doi.org/10.1007/s00262-001-0240-8

103. Bright RK, Voeck CD, Emmert-Buck MR, Duray PH, Solomon D, Fetsch P, Rhim JS, Linehan WM, Topalian SL. Generation and genetic characterization of immortal human prostate epithelial cell lines derived from primary cancer specimens. Cancer Res 1997; 57:995-1002; PMID:9041206

104. Duraiswamy J, Kaluza KM, Freeman GJ, Coukos G. Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection function in tumors. Cancer Res 2013; 73:3591-603; PMID:23633484; http://dx.doi.org/10.1158/0008-5472.CAN-12-4100