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Peptide-based vaccines for cancer therapy

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Interest for cancer vaccination started more than 30 years ago after the demonstration that both in animal models and later on in patients it is possible to generate anti-tumor immune responses. The clinical application of this knowledge, however, was disappointing.

In this review we summarize results on peptides epitopes recognized by T cells that have been studied thanks to their easy synthesis and the lack of significant side effects when administered in-vivo. To improve the clinical efficacy, peptides were modified in their aminoacid sequence to augment their immunogenicity. Peptides vaccines were recently shown to induce a high frequency of immune response in patients that were accompanied by clinical efficacy. These data are discussed at the light of recent progression of immunotherapy caused by the addition of check-point antibodies thus providing a general picture of the potential therapeutic efficacy of the peptide-based vaccines and their combination with other biological agents.

Background and Introduction

Immunotherapy of cancer has represented an active area of anti-cancer therapeutic investigation during the last 2–3 decades world-wide. This interest was generated early in the fifties through the sixties of the last century by the fathers of tumor immunology (i.e., Richmond Prehn, Philadelphia; George Klein, Stockholm; Lloyd J. Old/Edward Boyse, New York; Robert Baldwin, Nottingham). These investigators demonstrated in different animal models of tumors induced by a variety of carcinogens (e.g., chemicals, UV, viruses) that both unique tumor-associated antigens (TAAs) and shared self TAAs can coexist in the same tumor. Particularly the unique, mutation-derived TAAs were found to express a strong immunogenicity that can generate transplantation immunity leading to rejection of a challenge of the same tumor cells that will otherwise take and kill 100% mice.[1,2]

These studies unequivocally demonstrated that experimental tumors can be recognized and rejected by the immune system though mechanisms explaining such features were not worked out at that time. In the subsequent 30 y or so numerous attempts were done to translate in the clinical arena such a concept without much success until recently (see below).

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The Issue of TAAs to Be Used in Clinical Trials (Shared vs. Mutated)

The main explanation for this limited clinical outcome, in our opinion, lies in the type of TAAs that were targeted in these early clinical trials. In fact, the immunization of cancer patients with the aim of generating tumor cytotoxic immune responses started with the use of whole irradiated autologous tumor cells, then transduced with different cytokine genes (reviewed in[3] with the hope that such cells could express immunogenic TAAs recognized by the immune system as determined by in vitro assays with autologous T cells.

The self TAAs

The clinical outcome of these trials, however, while proving the concept that under certain conditions tumor-derived peptides can elicit a tumor-restricted T cell response[4] remained limited (10–20% response rate).

A clear progress in the field was the finding that TAAs of melanoma patients could be molecularly identified as normal protein involved in melanin production and melanocyte differentiation.[5,6] Many similar self TAAs were described in different human tumors[7] and several of them were shown to be immunogenic thanks to the lack of complete tolerance of the body to these normal proteins. The next step in the study of human TAAs was the identification of the aminoacid sequences (9–13 aa long peptides) that were recognized by T cell receptor in the context of MHC molecules (epitope).

This evidence was also obtained by biochemical techniques that allowed the separation of the peptide/epitope from the MHC/peptide complex directly eluting them from tumor cells.[8,9] Thus these peptides were studied as potential immunogens in animal models and in cancer patients, particularly in those bearing metastatic melanoma.

Based on the identification of these shared peptides recognized by T cells in the context of HLA class I- and/or HLA-Class II several clinical studies were performed showing that tumor-specific T cell response can be activated even in the majority of patients without, however, a parallel induction of clinical response.[10-12]

However, antigen peptides remained a focus of many investigations aimed at improving their immunogenicity and clinical efficacy. This occurred even because, peptide-based vaccines have distinct practical advantages and known manageable disadvantages in comparison with other vaccine formulation (e.g., tumor cells, viral vectors, DNA/RNA), and that are summarized as follows.

Advantages: (1) Known aminoacid sequence and length that allows the prediction of molecular interactions with the given MHC allele; (2) unlimited availability owing to their easy synthesis; (3) possibility to assess the T cell response *ex-vivo* against a known, target peptide/antigen.

Disadvantages*:* (1) high cost, due to the requirement of synthesizing the peptides under GMP conditions; (2) peptide stability may last for only 2–3 y and this may requires a re-synthesis and/or re-testing of peptides; (3) immunogenicity is variable according to each peptides since some of them may even induce immunosuppression.

Unique TAA-derived peptides

Recent advances in proteomic/genomic technologies (DNA sequencing) is allowing the identification of peptides deriving from non-synonymous somatic mutations that represent the truly tumor specific TAAs (previously defined as unique TAAs) absent in normal tissues and endowed with a higher immunogenicity as compared with self peptides.[9,13] Random mutagenesis throughout the genome is the hallmark of neoplastic transformation and occurs by nucleotide substitutions, deletions, insertions or gross chromosomal events (amplifications, deletions, inversions, translocations).[14,15] Mutations that confer a growth advantage are retained in the tumor genome by expansion of the clone bearing the mutation at the expense of other clones.[14] This process generates many tumor-specific proteins bearing aminoacid substitutions, which frequently differ from tumor to tumor, therefore forming potential neo-antigens for the host's immune system as exemplified in melanoma and GBM patients.[16,17] There is evidence both in mice and humans that TAAs that result from mutations in cancer cell genes induce strong and specific anti-tumor immune responses though this may not occur for some mutations owing to the rapid elimination of the subpopulation of tumor cells by the immune system.

Peptide-Based Clinical Trials

It was soon realized, however, that *ex-vivo* immune response and clinical response of cancer patients to immunizing self peptides was limited in frequency, strength and/or duration thus making these vaccine of limited value in the clinics.[11,18,19]

Polyspecific multipeptide approach

Since many different peptide/proteins TAAs may be express by cancer cells, a possible increase of anti-tumor response could be obtained using a vaccine containing several peptides and targeting them on cancer cells thus increasing the likelihood of inducing a T cell response able to destroy tumor cells.[12, 18, 20–23] Some of these trials were conducted as phase II randomized studies resulting also in a trend for clinical response.[24]

The modified peptides

In addition, to overcome such weak tumor immunogenicity, altered peptide ligands containing single aminoacid substitution were constructed and used since they can improve immunogenicity by a higher affinity binding to HLA molecules. Such a strategy was shown to significantly increase T cell responses but without resulting in a parallel clinical benefit.[25] This clinical inefficiency was attributed to the low frequency of effective cross-recognition of melanoma cells TAAs. The use of a modified peptide from CEA interestingly showed a dose-dependent induction of anti-CEA (CAP-1) response with the high dose of 1mg causing T cell response in 100% of pancreatic cancer patients.[26]

The long peptides

An additional modification of peptides was proposed by the group of Melief (Leiden) after an analysis of the structural modification and in vitro testing suggesting that such long (>13 aa) peptides appear to have e better immunogenicity and better efficacy most likely due to a higher affinity with the MHC molecules. This was proved in vivo in patients vaccinated against HPV peptides.[27]

The Issue of Adjuvants

Immunological adjuvants are an heterogeneous group of compounds (e.g.Montanide, GM-CSF, AS15/MAGE-A3) that may increase the immune response of the immunizing antigen by different mechanism sharing, however, the capacity to recruit pro-inflammatory factors at the vaccination site and that have been used for a long time to improve the effect of vaccines in infectious diseases and, more recently in cancer. However, there is a limited evidence from phase III studies that one adjuvant may be superior to another in the clinical response to cancer vaccines. Adjuvants may include non-specific local stimulatory molecule like Montanide (an incomplete Freunds adjuvant like molecule),or chemokines that can recruit pro-inflammatory cells that may help the patient immune system to mount a systemic tumor cytotoxic response.[28-30]

However, in a recent work Kruit and coworkers[29] have shown the superiority in overall survival of AS15 as compared with $AS02_B$ in a randomized multicenter MAGE-A3-based vaccine in NSCLC patients.

We learned also how tumor cells defend themselves from the attack by the immune response (immune escape)[31] an issue that needs to be addressed in each type of human tumors to be treated by immunobiotherapy.

Combination of Peptide Vaccines with Other Biological Agents

A new and promising area of studies is that of the combination of peptide vaccines with other biotherapeutics like immunomodulating or anti-vascular antibodies,[32,33] and even radio/chemotherapay.[34] The best combination appears to be that involving immunomodulatory agents (e.g., Ipilimumab, anti-PD-1, etc.)[35] which can amplify T cell expansion over time and increase the duration of the effect of vaccination.[32] In fact, the

Table 1. Phase II and III peptide-based vaccination trials with either clinical or immunological statistically significant response

Peptide	Tumor	N. patients	Clinical response	Immune response	Adjuvant	Year of publication
E75/HER2/Neu	Breast cancer	182	DFS 94vs86% ($P < 0.04$)	Yes, dose-dependent	GM-CSF	Mittendorf et al. Cancer 118: 2594, 2012
Telomerase GV1001	Pancreas, breast ca. NSCLC	1062	Not significant	ND	GM-CSF	Brunsvig et al. Clin Cancer Res 17: 6847, 2011
Idiotype peptide+KLH	NH Lymphoma	177	PFS 44 vs. 30mos $P = 0.047$	ND	GM-CSF	Schuster et al. JCO 29: 2787, 2011
GP100	MetastaticMelanoma	185	OS 17*vs*. Eleven mos $P < 0.06$	$P = 0.01$ pts with clinical response	IL2, Montanide	Schwartzentruber et al. NEJM 364: 2119, 2011
Survivin	Melanoma Stage IV	61	OS 9mos	32%	Cyclo+ Montanide	Becker et al. Cancer Immunol Immunother 61: 2001, 2012
MUC-1 Stimuvax(L-BLP25)	NSCLC Stage IIIB	34	Chemo+ vax *vs* vax $P = 0.016$	47%	Cyclo+MPL	Wu et al. BMC 11: 430, 2011
CIMAVAX (EGFR)	NSCLC Stage IIIB, IV	80	OS: NS	11 vs 5% $P < 0.024$	Montanide+ Cyclo	Neninger et al. JCO 26: 1452, 2008
GP2-Her2Neu	Breast cancer	172	NS	DTH 21vs 6mm $P < 0.01$	GM-CSF	Trappey et al., JCO; 31: Abstr 3005, 2013
IMA901	RCC	68	OS 23*vs*14 mos $P = 0.023$	74%	GM-CSF +Cyclo	Walter et al. Nature Med 18: 1254, 2012

Cyclo, Cyclophosphamide; DFS, disease-free survival; DTH, Delayed-type hypersensitivity; GM-CSF,Granulocyte Macrophage-Colony Stimulating Factor; KLH, Keyhole Limpet Hemocyanin; HER2, Human epidermal growth factor 2; MPL, Monophosphoryl Lipid A; ND, Not done; NS, Not Significant; NSCLC, Non small cell lung cancer; OS, Overall Survival; PFS, Progression-free Survival; Trials features as listed from *clinicaltrials.gov* of May 2014 and from Melero et al.[12]

only peptide-based successful phase III trial included a combination of the gp100 peptide and high dose of IL-2 in metastatic melanoma patients[36] that was crucial for the in vivo maintenance and expansion of T cells induced by the peptide.

These immunotherapeutic combinations are being tested in several phase I-II trials by different groups of researchers worldwide in the hope of increasing the clinica efficacy of cancer vaccination.

Gene signatures for immunotherapy

The availability of gene signatures to identify cancer patients that can respond to vaccination has been the focus of many researches during the last few years. Recent work of Wang and Bertognetti however, has identified a gene signature that may predict a T cell response to the MAGE.A3 of immunotherapy-treated patients.[37]

Conclusions

The peptide-based vaccines have been used in the past with a limited clinical success. However, during the last few years new knowledge has been provided on the biological characteristics of the peptides and their interaction with the immune system to be used in the clinic. New protocols have allowed to obtain significant immune and clinical responses in patients vaccinated with multiple class I and II peptides particularly by combining the peptides with a variety of other biological therapeutics in phase II and III trials (**Table 1**). This situation is now even more promising then before and we predict that such new peptide-based trials will provide other clinical success in a variety of human tumors.

Disclosure of Potential Conflicts of Interest

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References

1. Prehn RT, Main JM. Immunity to methylcholanthrene-induced sarcomas. J Natl Cancer Inst 1957; 18:769-78; PMID:13502695
2. Duan F, Lin Y, Liu C, Engelhorn ME, Cohen AD, Curran M, Sakaguchi S, Merghoub T, Terzulli S, Wolchok JD, et al. Immune rejection of mouse tumors expressing mutated self. Cancer Res 2009; 69:3545-53; PMID:19351857; http://dx.doi.org/10.1158/0008-5472.CAN-08-2779
3. Parmiani G, Pilla L, Maccalli C, Russo V. Autologous versus allogeneic cell-based vaccines? Cancer J 2011; 17:331-6; PMID:21952283; http://dx.doi.org/10.1097/PPO.0b013e3182337a76
4. Marchand M, Weynants P, Rankin E, Arienti F, Belli F, Parmiani G, Cascinelli N, Bourlond A, Vanwijck R,

Humblet Y, et al. Tumor regression responses in melanoma patients treated with a peptide encoded by gene MAGE-3. Int J Cancer 1995; 63:883-5; PMID:8847150; http://dx.doi.org/10.1002/ijc.2910630622
5. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science 1991; 254:1643-7; PMID:1840703; http://dx.doi.org/10.1126/science.1840703
6. Coulie PG, Van den Eynde BJ, van der Bruggen P, Boon T. Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy. Nat Rev Cancer 2014; 14:135-46; PMID:24457417; http://dx.doi.org/10.1038/nrc3670
7. Novellino L, Castelli C, Parmiani G. A listing of human tumor antigens recognized by T cells: March 2004 update. Cancer Immunol Immunother 2005; 54:187-207; PMID:15309328; http://dx.doi.org/10.1007/s00262-004-0560-6
8. Hunt DF, Henderson RA, Shabanowitz J, Sakaguchi K, Michel H, Sevilir N, Cox AL, Appella E, Engelhard VH. Characterization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry. Science 1992; 255:1261-3; PMID:1546328; http://dx.doi.org/10.1126/science.1546328
9. Rammensee HG, Singh-Jasuja H. HLA ligandome tumor antigen discovery for personalized vaccine approach. Expert Rev Vaccines 2013; 12:1211-7; PMID:24090147; http://dx.doi.org/10.1586/14760584.2013.836911
10. Parmiani G, Castelli C, Dalerba P, Mortarini R, Rivoltini L, Marincola FM, Anichini A. Cancer immunotherapy with peptide-based vaccines: what have we achieved? Where are we going? J Natl Cancer Inst 2002; 94:805-18; PMID:12048268; http://dx.doi.org/10.1093/jnci/94.11.805
11. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med 2004; 10:909-15; PMID:15340416; http://dx.doi.org/10.1038/nm1100
12. Melero I, Gaudernack G, Gerritsen W, Huber C, Parmiani G, Scholl S, et al. Advances in cancer immunotherapy: Active immunotherapy. Nature Rev Clin Oncology 2014; (Forthcoming)
13. Parmiani G, Russo V, Marrari A, Cutolo G, Casati C, Pilla L, Maccalli C, Rivoltini L, Castelli C. Universal and stemness-related tumor antigens: potential use in cancer immunotherapy. Clin Cancer Res 2007; 13:5675-9; PMID:17908956; http://dx.doi.org/10.1158/1078-0432.CCR-07-0879
14. Leary RJ, Lin JC, Cummins J, Boca S, Wood LD, Parsons DW, Jones S, Sjöblom T, Park B-H, Parsons R, et al. Integrated analysis of homozygous deletions, focal amplifications, and sequence alterations in breast and colorectal cancers. Proc Natl Acad Sci U S A 2008; 105:16224-9; PMID:18852474; http://dx.doi.org/10.1073/pnas.0808041105
15. Pleasance ED, Cheetham RK, Stephens PJ, McBride DJ, Humphray SJ, Greenman CD, Varela I, Lin ML, Ordóñez GR, Bignell GR, et al. A comprehensive catalogue of somatic mutations from a human cancer genome. Nature 2010; 463:191-6; PMID:20016485; http://dx.doi.org/10.1038/nature08658
16. Wölfel T, Hauer M, Schneider J, Serrano M, Wölfel C, Klehmann-Hieb E, De Plaen E, Hankeln T, Meyer zum Büschenfelde KH, Beach D. A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. Science 1995; 269:1281-4; PMID:7652577; http://dx.doi.org/10.1126/science.7652577
17. Johnson BE, Mazor T, Hong C, Barnes M, Aihara K, McLean CY, Fouse SD, Yamamoto S, Ueda H, Tatsuno K, et al. Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. Science 2014; 343:189-93; PMID:24336570; http://dx.doi.org/10.1126/science.1239947
18. Jalali SA, Parmiani G. Pre-clinical and clinical aspects of peptide-based vaccine against human solid tumors. Recent Pat Biotechnol 2011; 5:108-17; PMID:21707528; http://dx.doi.org/10.2174/187220811796365716
19. Parmiani G, Cimminiello C, Maccalli C. Increasing immunogeni city of cancer vaccines to improve their clinical out come. Exp Rev Vaccines 2013; 10.1586/14760584
20. Valmori D, Dutoit V, Ayyoub M, Rimoldi D, Guillaume P, Liénard D, Lejeune F, Cerottini JC, Romero P, Speiser DE. Simultaneous CD8+ T cell responses to multiple tumor antigen epitopes in a multipeptide melanoma vaccine. Cancer Immun 2003; 3:15-23; PMID:14580186
21. Slingluff CL Jr., Petroni GR, Yamshchikov GV, Barnd DL, Eastham S, Galavotti H, Patterson JW, Deacon DH, Hibbitts S, Teates D, et al. Clinical and immunologic results of a randomized phase II trial of vaccination using four melanoma peptides either administered in granulocyte-macrophage colony-stimulating factor in adjuvant or pulsed on dendritic cells. J Clin Oncol 2003; 21:4016-26; PMID:14581425; http://dx.doi.org/10.1200/JCO.2003.10.005
22. Slingluff CL Jr., Petroni GR, Yamshchikov GV, Hibbitts S, Grosh WW, Chianese-Bullock KA, Bissonette EA, Barnd DL, Deacon DH, Patterson JW, et al. Immunologic and clinical outcomes of vaccination with a multiepitope melanoma peptide vaccine plus low-dose interleukin-2 administered either concurrently or on a delayed schedule. J Clin Oncol 2004; 22:4474-85; PMID:15542798; http://dx.doi.org/10.1200/JCO.2004.10.212
23. Slingluff CL Jr., Petroni GR, Olson W, Czarkowski A, Grosh WW, Smolkin M, Chianese-Bullock KA, Neese PY, Deacon DH, Nail C, et al. Helper T-cell responses and clinical activity of a melanoma vaccine with multiple peptides from MAGE and melanocytic differentiation antigens. J Clin Oncol 2008; 26:4973-80; PMID:18809608; http://dx.doi.org/10.1200/JCO.2008.17.3161
24. Slingluff CL Jr., Lee S, Zhao F, Chianese-Bullock KA, Olson WC, Butterfield LH, Whiteside TL, Leming PD, Kirkwood JM. A randomized phase II trial of multiepitope vaccination with melanoma peptides for cytotoxic T cells and helper T cells for patients with metastatic melanoma (E1602). Clin Cancer Res 2013; 19:4228-38; PMID:23653149; http://dx.doi.org/10.1158/1078-0432.CCR-13-0002
25. Filipazzi P, Pilla L, Mariani L, Patuzzo R, Castelli C, Camisaschi C, Maurichi A, Cova A, Rigamonti G, Giardino F, et al.. Limited induction of tumor cross-reactive T cells without a measurable clinical benefit in early melanoma patients vaccinated with human leukocyte antigen class I-modified peptides Clin Cancer Res 2012; 18:6485-96; PMID:23032742; http://dx.doi.org/10.1158/1078-0432.CCR-12-1516
26. Geynisman DM, Zha Y, Kunnavakkam R, Aklilu M, Catenacci DVT, Polite BN, Rosenbaum C, Namakydoust A, Karrison T, Gajewski TF, et al. A randomized pilot phase I study of modified carcinoembryonic antigen (CEA) peptide (CAP1-6D)/montanide/GM-CSF-vaccine in patients with pancreatic adenocarcinoma. J Immunother Cancer 2013; 1:8; PMID:24829746; http://dx.doi.org/10.1186/2051-1426-1-8
27. Welters MJP, Kenter GG, Piersma SJ, Vloon APG, Löwik MJG, Berends-van der Meer DMA, Drijfhout JW, Valentijn ARPM, Wafelman AR, Oostendorp J, et al. Induction of tumor-specific CD4+ and CD8+ T-cell immunity in cervical cancer patients by a human papillomavirus type 16 E6 and E7 long peptides vaccine. Clin Cancer Res 2008; 14:178-87; PMID:18172269; http://dx.doi.org/10.1158/1078-0432.CCR-07-1880
28. Schaed SG, Klimek VM, Panageas KS, Musselli CM, Butterworth L, Hwu W-J, Livingstone PD, Lewis WL, Houghton AN, Chapman PB, et al. T cell responses against tyrosinase 368-376 (370D) peptide in HLA*A201+ melanoma patients: randomized trial comparing incomplete Freund's adjuvant, GM-CSF and QS21 as immunological adjuvants. Clin Cancer Res 2002; 20:2610-5
29. Kruit WHJ, Suciu S, Dreno B, Mortier L, Robert C, Chiarion-Sileni V, Maio M, Testori A, Dorval T, Grob J-J, et al. Selection of immunostimulant AS15 for active immunization with MAGE-A3 protein: results of a randomized phase II study of the European Organisation for Research and Treatment of Cancer Melanoma Group in Metastatic Melanoma. J Clin Oncol 2013; 31:2413-20; PMID:23715572; http://dx.doi.org/10.1200/JCO.2012.43.7111
30. Ali OA, Verbeke C, Johnson C, Sands RW, Lewin SA, White D, Doherty E, Dranoff G, Mooney DJ. Identification of immune factors regulating antitumor immunity using polymeric vaccines with multiple adjuvants. Cancer Res 2014; 74:1670-81; PMID:24480625; http://dx.doi.org/10.1158/0008-5472.CAN-13-0777
31. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol 2012; 12:253-68; PMID:22437938; http://dx.doi.org/10.1038/nri3175
32. Fourcade J, Sun Z, Pagliano O, Chauvin J-M, Sander C, Janjic B, Tarhini AA, Tawbi HA, Kirkwood JM, Moschos S, et al. PD-1 and Tim-3 regulate the expansion of tumor antigen-specific $CD8^+$ T cells induced by melanoma vaccines. Cancer Res 2014; 74:1045-55; PMID:24343228; http://dx.doi.org/10.1158/0008-5472.CAN-13-2908
33. Okuyama R, Aruga A, Hatori T, Takeda K, Yamamoto M. Immunological responses to a multi-peptide vaccine targeting cancer-testis antigens and VEGFRs in advanced pancreatic cancer patients. Oncoimmunology 2013; 2:e27010; PMID:24498547; http://dx.doi.org/10.4161/onci.27010
34. Vacchelli E, Senovilla L, Eggermont A, Fridman WH, Galon J, Zitvogel L, Kroemer G, Galluzzi L. Trial watch: Chemotherapy with immunogenic cell death inducers. Oncoimmunology 2013; 2:e23510; PMID:23687621; http://dx.doi.org/10.4161/onci.23510
35. Maio M, Di Giacomo AM, Robert C, Eggermont AMM. Update on the role of ipilimumab in melanoma and first data on new combination therapies. Curr Opin Oncol 2013; 25:166-72; PMID:23299197; http://dx.doi.org/10.1097/CCO.0b013e32835dae4f
36. Schwartzentruber DJ, Lawson DH, Richards JM, Conry RM, Miller DM, Treisman J, Gailani F, Riley L, Conlon K, Pockaj B, et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. N Engl J Med 2011; 364:2119-27; PMID:21631324; http://dx.doi.org/10.1056/NEJMoa1012863
37. Wang E, Bedognetti D, Marincola FM. Prediction of response to anticancer immunotherapy using gene signatures. J Clin Oncol 2013; 31:2369-71; PMID:23715576; http://dx.doi.org/10.1200/JCO.2013.49.2157