

Expert Opinion

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Allovectin-7 therapy in metastatic melanoma

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Background: Patients with metastatic melanoma are immunosuppressed by the growing tumor. Allovectin-7 therapy is a form of active immunotherapy that aims at immunization of the host with substances designed to elicit an immune reaction that will eliminate or slow down the growth and spread of the cancer. **Objective:** to describe the rationale for immunotherapy with Allovectin-7 and assess its safety profile and efficacy based on the results of completed melanoma clinical trials. **Methods:** we reviewed both the published medical literature and the results of trials pending publication. **Results/conclusion:** Allovectin-7 is a safe and active immunotherapeutic agent. It induces local and systemic durable responses in patients with metastatic melanoma.

Keywords: Allovectin-7, DNA vaccination, immunotherapy, melanoma

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1. Introduction

The incidence of cutaneous melanoma has been rapidly increasing worldwide, with 59,940 estimated new cases in 2007 in the United States of America, thus being the solid tumor highest in incidence among young adults [1]. While patients with melanoma diagnosed early and surgically resected have excellent prognosis, the prognosis in the metastatic phase remains very poor. Currently, disseminated melanoma is not curable. In fact, the median survival of stage IV patients is about 6 – 8 months [2].

Dacarbazine is the most frequently used single chemotherapeutic agent for treatment of melanoma, with a historical response rate (RR) of 20% but actually < 10% (7 – 10%) in most of the Phase III clinical trials, where it has been used as a control treatment arm [3]. Combination chemotherapy has increased the response rates up to 25 – 35% without having a significant effect on the overall survival. Cytokines such as interferons and IL-2, induced durable complete response (CR) rates of about 4% with an overall response rate of 15%. The modest clinical activity of single-agent treatments prompted the use of combined biochemotherapy or chemoimmunotherapy regimens which, despite their higher response rates, had no significant effect on survival [4].

In view of that, alternative therapies to stimulate the immune system or find biological targeted therapies are being investigated because melanoma is an immunogenic tumor. This notion is supported by: i) The rare cases of spontaneous regression of both the primary melanoma as well as distant metastases, and biopsies of regressing lesions showing lymphocytic infiltration, overexpression of T helper (Th)-1, cytokines (IFN- γ , IL-2) [5-7]. The degree of tumor infiltration by lymphocytes was an independent predictor of melanoma-specific mortality [8]; ii) The observation that expression of melanoma-associated antigens in the context of MHC molecules, elicits T cell mediated responses [9]; iii) The complete responses observed with cytokines, biochemotherapy and vaccine therapy of melanoma [4]; and iv) The development of autoimmune phenomena such as like

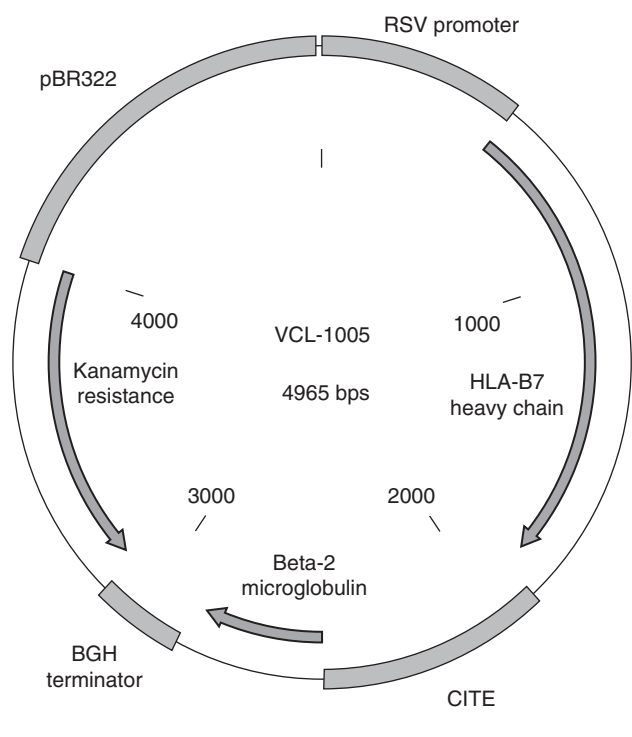


Figure 1. Diagram of plasmid with HLA-B7 and β 2 microglobulin.

Courtesy of Vical Incorporated.

vitiligo with cytokines and enterocolitis, dermatitis, hypophysitis or auto-antibodies (anti-thyroglobulin, anti-cardiolipin or antinuclear) in patients responsive to anti-cytotoxic T lymphocyte antigen 4 (CTLA4) monoclonal antibodies [10,11].

Many malignant tumors, including melanoma, possess several different mechanisms that enable them to escape immunosurveillance [12]. These mechanisms include a specific immunological tolerance induced by tumor antigens, the intervention of negative regulatory T cells, loss of antigens recognized by tumor-specific cytotoxic T cells (CTLs) because of downregulation of antigen gene expression, down-modulation of class I/II MHC molecules, β 2-microglobulin, or components of the antigen-processing and expressing machinery, lack of co-stimulatory molecules, or tumor cell (or tumor microenvironment) production of immunosuppressive molecules.

Allovectin-7 attempts to reverse the crucial downmodulation of class I/II MHC molecules that has been reported in 10 – 30% of biopsies of human melanoma lesions [13-16].

2. Allovectin-7

Allovectin-7[®], developed by Vical, Inc., is a DNA plasmid containing the genes coding for foreign human HLA-B7 (an allogeneic MHC class I protein) heavy chain and β 2-microglobulin, inserted into a simplified eukaryotic expression vector (pBR322) [17].

The VCL-1005 plasmid DNA length is 4965 base pairs and the molecular mass is 3.15×10^6 . The expression of both genes is driven by the Rous Sarcoma Virus-long-terminal repeat promoter. The two genes are separated by a cap-independent translational enhancer (CITE), an internal ribosomal entry site that permits coexpression of both genes from a single promoter in eukaryotic cells. The bovine growth hormone (BGH) transcription terminator and polyadenylation signal (BGH P(A)), terminates transcription. The kanamycin resistance gene expresses only in the bacterial host strain (Figure 1). Allovectin-7 is complexed with a cationic lipid mixture, 1,2-dimyristyloxypropyl-3-dimethylhydroxyethyl ammonium bromide/dioleoyl-phosphatidylethanolamine (DMRIE/DOPE).

DMRIE is a cationic lipid with a molecular mass of 636.89, whereas DOPE is a zwitterionic lipid, with a molecular mass of 744.04, important for the uptake of the DNA by the tumor [18]. The advantages of using such a preparation include the expression of a foreign and highly immunogenic cell surface protein (HLA-B7) which is expressed in only 20% the US population.

Replacement of β 2-microglobulin increases surface expression of the patient's own MHC-class I molecules on tumor cells which stimulates immunity against both the transfected cells, as well as previously unrecognized antigens present in unmodified tumor cells. Tumor cell modification with the HLA-B7 gene also could stimulate immunity toward cells that express tumor-associated antigens in association with the patient's own HLA antigens. In fact, in the clinical trial carried-out by Nabel *et al.*, the expression of HLA-B7 in tumor cells caused the recognition of the foreign antigens by the immune system, stimulating the release of cytokines locally which in turn induced a T cell response against the unmodified tumor [19].

In murine models, such local immune stimulation enhanced recognition of tumor antigens previously undetected by the immune system. In animals, direct gene transfer into established tumors could attenuate tumor growth or, in some cases, lead to complete tumor regression [20].

3. DNA vaccination advantages

DNA vaccination provides several advantages over protein or peptide vaccines [21]. First, DNA vaccines have greater chemical stability and are more easily sequenced and purified than proteins/peptides. Second, plasmid vaccines are inherently immunogenic and generally do not require coadministration of adjuvants. Finally, in preclinical studies, DNA vaccines have elicited a broad range of antigen-specific immune responses, including antibodies, CTL activation, and delayed-type hypersensitivity (DTH). Immunization with plasmid preparations provides certain advantages over recombinant viral vaccination. First, DNA vaccines are easier to prepare, produce and control for quality than viral recombinants.

Table 1. Phase I studies with Allovectin-7 in metastatic melanoma.

Study number ref.	Allovectin-7 dosage	Number of patients (HLA-B7 status)	Number of responses in injected lesions	Number of responses in non-injected lesions	Overall response
PI IND [19]	0.29 – 0.86 µg/injection	5 (HLA-B7-)	1	4 skin and 1 lung in 1 patient	1 PR
PI IND [24]	10 – 300 µg/injection	10 (HLA-B7-)	2	1	1 PR
1005-101 [23]	10 – 250 µg/injection	17 (HLA-B7-)	5	0	1 CR, 2 PRs
PI IND [25]	5 – 10 µg/injection	7(Included HLA-B7+)	3	0	2 PRs
All studies	overall	39	11	5	7 (19%)

CR: Complete response; PR: Partial response.

Second, recombinant viral vaccines frequently evoke vector-specific immune responses. Third, DNA vaccines carry less risk of insertional mutagenesis than viral vectors. Fourth, unlike adenoviral and vaccinia recombinants, DNA vaccines are not known to downregulate MHC class I gene expression in transfected cells [22]. Finally, DNA vaccines do not confer a risk of producing recombinant pathogenic viruses.

4. Pharmacokinetic and pharmacodynamic properties in preclinical models

In a mouse model, following a single intravenous dose of Allovectin-7, plasmid DNA was rapidly degraded, with a terminal half-life ($t_{1/2}$) of < 5 min, and was undetectable in the blood within 1 hour after administration. Ten minutes after administration, plasmid DNA was identified in all tissues analyzed. It was detectable also at 1 h and 24 h after administration and disappeared 7 days after administration.

In spite of the homogeneous distribution of the plasmid into the body, the expression of HLA-B7 protein was not identified in any tissue by immunostaining [17]. The effect of transfection of healthy tissue was studied in mice following the direct intrahepatic injection of plasmid DNA. No adverse events on the histopathology and clinical biochemistry of the mice were observed [17,20]. In preclinical (animal) models, the safety of the preparation was evaluated in mice and monkeys. The administration of a single intravenous dose as well as daily repeated intravenous injections at 1400 times the maximum single human dose of Allovectin-7, were not associated with any signs of acute toxicity or organ pathology during the 14 – 28-day trial period. Only transient effects on hematochemistry were observed in cases of repeated administrations for 14 consecutive days. In *Cynomolgus* monkeys, the repeated intravenous injections up to a cumulative dose of 720 µg plasmid DNA did not show any signs of toxicity [20].

5. Immune system

In an initial Phase I study in patients affected by cutaneous metastases from melanoma, the direct intratumor injection of Allovectin-7 elicited specific cytotoxic T cell responses to tumor antigens, with no detectable plasmid DNA in the patients' sera at any time and no development of anti-DNA antibodies [19]. In a large open-label Phase I Allovectin 7 clinical trial conducted by Stopeck *et al.*, the preparation was tested in patients bearing melanoma, renal carcinoma and colorectal adenocarcinoma, including as inclusion criteria a normal T lymphocyte response to phytohemagglutinin stimulation [23]. The efficiency of gene transfer was determined by PCR, flow cytometry and immunohistochemistry on tissue biopsies. There was evidence of gene uptake and HLA-B7 expression in 98% of the patients' tissue samples. CD8 T cell infiltration was identified in 22 out of 23 tumor biopsies studied. Anti-HLA-B7 CTLs were found in the peripheral blood of 23 out of 43 post-gene-therapy samples with an enhanced tumor-specific release of cytokines.

6. Clinical trials

6.1 Phase I studies

The first human Phase I study was conducted in 1993 by Dr Gary Nabel. A cumulative dose up to 2.58 µg of DNA was administered in 3 divided doses into the tumors of five patients with skin melanoma (Table 1) [19]. HLA-B7 gene expression was detectable in treated nodules of all five treated patients. In one patient, injected cutaneous nodules, four non-injected skin nodules and a 3-cm pulmonary lesion regressed completely. The number of HLA-B7-reactive CTLs increased after gene therapy in two patients. No auto-antibodies were detected in the blood. Following this study, the β 2-microglobulin gene was added to the plasmid to improve the expression of the HLA-B7 on the cell surface [19].

In the second Phase I study by Nabel *et al.*, 10 HLA-B7–negative patients with stage IV melanoma received escalating

Table 2. Phase II studies with Allovectin-7 in metastatic melanoma.

Study number ref.	Allovectin-7 dosage	Number of patients (HLA-B7 status)	Local response (number)	Response in uninjected lesions (number)	Overall response (number)
VCL-1005-201 [27]	10 µg/injection × 4	25 (HLA-B7-)	2, (7 ≥ 25%)	1 ≥ 70%, 2 ≥ 25%	1 PR
VCL-1005-203 [28]	10 µg/injection × 6	52 (Included HLA-B7+)	1CR, 3 PR, (5 MR)	4 (21 – 78%)	2 PR
VCL-1005-205 [29]	10 µg/injection × 6	77 (Included HLA-B7+)	7	0	2CR, 5PR
VCL-1005-208 [30,31]	2 mg weekly × 6	127	30	9	4CR, 11 PR
All studies	overall	281	43 (15.3%)	9	25 (8.9%)

CR: Complete response; MR: Minor response; PR: Partial response.

doses of Allovectin-7 at 10 – 300 µg/injection [24]. Expression of plasmid DNA was seen in the injected tumors of nine patients. There was T cell migration into treated lesions in six of seven and enhanced tumor-infiltrating lymphocyte reactivity in two of two tumor samples analyzed. One patient with skin and visceral metastasis had a partial response (PR).

In the VCL-1005-101 trial, 17 patients received intralésional injection of Allovectin-7, up to 250 µg of plasmid DNA every 3 weeks [23]. Five patients received two or three injections of 10 µg of plasmid DNA into a single tumor site on days 1, 15, 29. Reverse-transcriptase polymerase chain reaction (RT-PCR) analysis of tumor biopsies documented transfection of tumor cells by Allovectin-7 and the expression of allogeneic HLA-B7 protein on the surface of tumor cells. Evidence of gene uptake and/or HLA-B7 expression was found in 93% of the patients evaluated. Objective tumor regression in the injected nodules occurred in 7 of 14 evaluable patients of which 5 had ≥ 50% regression. CD8 T cell infiltrates were detected in 96% of 23 tumor biopsies evaluated. Anti-HLA-B7 CTLs were found in 3 of 14 patients' post therapy peripheral blood samples.

In a fourth Phase I study, 7 patients with melanoma were treated using multiple injections, 5 – 10 µg/injection for six injections over an 8 week period [25]. Three of the seven had visceral metastases. Three patients had local responses in the injected lesions, and two had systemic partial response. A general review of the Phase I trials with Allovectin-7 completed at University of Arizona has been summarized in an earlier presentation [26].

6.2 Phase II/III studies

The effects of Allovectin-7 were investigated in Phase II/III clinical trials in patients with stage III, IVM1a, or IVM1b melanoma. Eligible patients for the study had good performance status, limited metastatic disease measuring ≥ 1 cm² and ≤ 25 cm² (skin, lymph nodes, or lung), and a normal serum level of lactate dehydrogenase (LDH).

In the first Phase II study (VCL-1005-201), 10-µg-dose Allovectin-7 was administered as four intratumoral injections

over an eight-week period [27]. Sites of Allovectin-7 injections included: skin or nodal (26/38), lung (4/38) and other viscera (8/38). Twenty-five of 38 patients who received at least two of the four planned injections and were evaluated at a 6-weeks were evaluable for response (Table 2). Nine patients had objective tumor regression including two patients (8%) with ≥ 70% regression in the injected tumor lesions, one of whom also had a systemic tumor response. Five patients had local response of their injected tumor, two had regression of at least one non-injected lesion. The median survival of 25 evaluable patients was 12.6 months.

The VCL-1005-203 dose-escalation Phase II study was designed to evaluate the safety and efficacy of Allovectin-7 in patients with melanoma unresponsive to standard therapy [28]. Six weekly intratumoral injections of 10 µg of Allovectin-7 were given every nine weeks. Fifty-two patients were enrolled and 92% had received prior systemic therapy including chemotherapy or immunotherapy or radiation treatment. There were nine responses in injected lesions including one complete, three partial and five minor responses. Two of 52 (4%) patients had partial overall response.

The third Phase II trial, VCL-1005-205, was an open-label, multicenter study designed to evaluate the safety and efficacy of Allovectin-7 in patients whose disease was recurrent or unresponsive to standard therapy [29]. A total of 77 patients received Allovectin-7 (10 µg) as six weekly intratumoral injections followed by three weeks of rest. Sixty-nine of the patients (90%) had stage IV disease, 44% had visceral metastasis, and 22% per cent were HLA-B7 positive. There were seven responses, including two CR, for an overall response rate of 9.1%. The median duration of response was 4.9 months, and the median time to progression (TTP) and survival were 2.1 months and 14 months, respectively. Twenty-six (31%) of the 84 injected lesions had a PR or CR, while 38 (15%) of the 254 non-injected lesions had a PR or CR.

In the next Phase II trial (VCL-1005-208), patients with two or more injectable lesions were randomized to receive

Allovecitin-7 injected into as many as five lesions [30,31]. Patients received the intratumoral injections, maximum total dose 2 mg, weekly for six weeks, followed by three weeks of observation and evaluation. Overall response was assessed using response evaluation criteria in solid tumors (RECIST) guidelines. One hundred and thirty-three patients enrolled and 127 were evaluated for tumor response. Fifteen patients had either PR (3.1%) or CR (8.7%), for an overall response rate of 11.8%. The response rate was significantly ($p = 0.02$) higher in the patients with 1 injectable lesion than in patients with multiple injectable lesions. The response rate was 19.4% for patients who had injection of the only lesion they had, 26.9% for those who had 1 lesion injected out of ≥ 2 injectable lesions and 12.4% for those who had multiple lesions injected. Five patients who developed vitiligo had PRs and also had regression of non-injected lesions. Systemic non-injected lesion response was observed in 3 out of 15 (20%) responders. Three of the 15 responders had a response that included lung metastases. Overall, 7% of evaluable patients had a systemic non-injected lesion response. The median duration of response, TTP and median survival were 12.7, 1.6 and 21.3 months respectively.

The objective of the Phase III trial (VCL-1001-301) was to compare Allovecitin-7 plus dacarbazine to dacarbazine alone in chemotherapy-naïve patients with advanced melanoma ([32], unpublished data). Patients were stratified by sex, site of metastatic disease and age, and then were randomized to receive either dacarbazine alone (800 mg/m²) given intravenously every 28 days, or dacarbazine at the same dose plus Allovecitin-7 (10 µg) given by intratumoral injection into a single tumor nodule on days 3 and 10 of each 28-day cycle. The objectives of the study included improvement in the median TTP by ≥ 2 months and/or 15% improvement in tumor response rate. One hundred and four patients received dacarbazine alone and 98 received the combination regimen. The response rates were 11.6% for dacarbazine and 13.2% for the combination. The median survival durations were 9.24 and 10.75 months, respectively, and TTPs were 1.6 and 1.9 months, respectively (pers. commun., unpublished results). It was concluded that adding low-dose Allovecitin-7 to dacarbazine yielded no significant clinical benefit.

Currently a Phase III clinical trial designed to compare the safety and efficacy of Allovecitin-7 to Dacarbazine or Temozolomide is accruing patients. Chemo-naïve patients with stage III and stage IV M1a/M1b melanoma are stratified by number of injectable lesions, Eastern Cooperative Oncology Group (ECOG) performance status and serum lactate dehydrogenase (LDH) level and then randomized to the Allovecitin-7 or to the control group treated with

dacarbazine or Temozolomide. The primary end point is objective clinical response rate at ≥ 24 weeks.

6.3 Allovecitin-7 therapy-related side effects

The safety profile of Allovecitin-7 has been very favorable. At low doses, most of the reported adverse effects were related to complications of tumor biopsy or injection procedures. Allovecitin-7-associated transient generalized side effects including paresthesias, asthenia, myalgias and flu-like syndrome. There was no Grade 3 or 4 toxicity. When allovecitin-7 was injected in visceral metastases, procedure-related complications such as ecchymosis (1.3%) and pneumothoraces (2.3%) occurred.

In the Phase II trials and in the largest dose-escalation Phase III trial the common Allovecitin-7-related reported adverse events were Grade 1 and 2. The adverse events related to Allovecitin-7 included fatigue (48% of patients), injection site pain (47%), and rigors (28%). Generalized pain such as arthralgia or myalgia constituted one-third of the adverse events. Injection-site reactions such as pain, bleeding or bruising, constituted roughly a third of the top 20% of events.

7. Expert opinion

Despite decades of clinical investigation, a therapy that prolongs survival in patients with metastatic melanoma remains elusive. Current therapies have low response rates and complete responses are rare. Patients with Stage III/IV M1a/b often have higher responses to systemic immunotherapy and survive longer, thus they are more likely to give an immunotherapeutic agent a chance to show its potential. Initial trials with investigational agents help define the safety, dosing schedules, optimal patient population and synergy with other agents that could enhance the benefits from the drug. The Allovecitin-7 trials completed thus far showed that it is a safe drug with mild and self-limited adverse effects. It induces local and systemic durable responses in melanoma patients. The highest dose level, 2 mg/dose, gave superior local and systemic responses in injected and non-injected metastatic lesions. The overall response rate was equivalent to Dacarbazine, the FDA approved standard for metastatic melanoma. Further study of Allovecitin-7 in combination with other cytotoxic and immunotherapeutic agents is needed to determine its proper place in the management of metastatic melanoma.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2007. *CA Cancer J Clin* 2007;57:43-66
2. Lotze MT, Dallal RM, Kirkwood JM, Flickinger JC. Cutaneous melanoma. In: De Vita VT, Hellman S, Rosenberg SA, editors, *Cancer: principles and practice of oncology*. 7th edition. Philadelphia: Lippincott Williams Wilkins; 2001. p. 2012-69
3. Eggermont AM, Kirkwood JM. Re-evaluating the role of dacarbazine in metastatic melanoma: what have we learned in 30 years? *Eur J Cancer* 2004;40:1825-36
4. Bajetta E, Del Vecchio M, Bernard-Marty C, et al. Metastatic melanoma: chemotherapy. *Semin Oncol* 2002;29:427-45
5. Papac RJ. Spontaneous regression of cancer: possible mechanisms. *In Vivo* 1998;12:571-8
6. Printz C. Spontaneous regression of melanoma may offer insight into cancer immunology. *J Natl Cancer Inst* 2001;93:1047-8
7. Bulkley GB, Cohen MH, Banks PM, et al. Long-term spontaneous regression of malignant melanoma with visceral metastases. Report of a case with immunologic profile. *Cancer* 1975;36:485-94
8. Tuthill RJ, Unger JM, Liu PY, et al. Risk assessment in localized primary cutaneous melanoma: a Southwest Oncology Group study evaluating nine factors and a test of the Clark logistic regression prediction model. *Am J Clin Pathol* 2002;118:504-11
9. Kawakami Y, Rosenberg SA. Human tumor antigens recognized by T-cells. *Immunol Res* 1997;16:313-39
10. Gogas H, Ioannovich J, Dafni U, et al. Prognostic significance of autoimmunity during treatment of melanoma with interferon. *N Engl J Med* 2006;354:709-18
11. Yang JC, Hughes M, Kammula U, et al. Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. *J Immunother* 2007;30:825-30
12. Restifo NP, Wunderlich JR. Cancer immunology. In: De Vita VT, Hellman S, Rosenberg SA, editors, *Cancer: principles and practice of oncology*. 7th edition. Philadelphia: Lippincott Williams Wilkins; 2005. p. 139-61
13. Ruiter DJ, Mattijssen V, Broecker EB, Ferrone J. MHC antigens in human melanomas. *Semin Cancer Biol* 1991;2:35-45
14. Natali PG, Nicotra MR, Bigotti A, et al. Selective changes in expression of HLA class I polymorphic determinants in human solid tumors. *Proc Natl Acad Sci USA* 1989;86:6719-23
15. Marincola F, Shamamian P, Alexander RB, et al. Loss of HLA haplotype and B locus down-regulation in melanoma cell lines. *J Immunol* 1994;153:1225-37
16. van Duinen SG, Ruiter DJ, Broecker EB, et al. Level of HLA antigens in locoregional metastases and clinical course of the disease in patients with melanoma. *Cancer Res* 1988;48:1019-25
17. Lew D, Parker SE, Latimer T, et al. Cancer gene therapy using plasmid DNA: pharmacokinetic study of DNA following injection in mice. *Hum Gene Ther* 1995;6:553-64
18. Plautz G, Yang Z, Wu B, et al. Immunotherapy of malignancy by in vivo gene transfer into tumors. *Proc Natl Acad Sci USA* 1993;90:4645-9
19. Nabel GJ, Nabel EG, Yang Z-Y, et al. Direct gene transfer with DNA-liposome complexes in melanoma: expression, biologic activity, and lack of toxicity in humans. *Proc Natl Acad Sci USA* 1993;90:11307-11
20. Parker SE, Vahlsing HL, Serfilippi LM, et al. Cancer gene therapy using plasmid DNA: safety evaluation in rodents and non-human primates. *Hum Gene Ther* 1995;6:575-90
21. White SA, Conry RM. Cancer vaccines: clinical applications. DNA vaccines. In: Rosenberg SA, editor, *Principles and practice of the biologic therapy of cancer*. 3rd edition. Philadelphia: Lippincott Williams Wilkins; 2000. p. 674-86
22. Donnelly JJ, Ulmer JB, Liu MA. Protective efficacy of intramuscular immunization with naked DNA. *Ann NY Acad Sci* 1995;772:40-6
23. Stopeck AT, Hersh EM, Akporiaye ET, et al. Phase I study of direct gene transfer of an allogeneic histocompatibility antigen, HLA-B7, in patients with metastatic melanoma. *J Clin Oncol* 1997;15:341-9
24. Nabel GJ, Gordon D, Bishop DK, et al. Immune response in human melanoma after transfer of an allogeneic class I major histocompatibility complex gene with DNA-liposome complexes. *Proc Natl Acad Sci USA* 1996;93:15388-93
25. Silver HKB, Klassa RJ, Bally JB, et al. Phase I gene therapy of HLA-B7 transduction by direct injection in malignant melanoma. *Proceedings of AACR 37:#2337*, 1996
26. Hersh EM, Figlin R, Gutheil J, et al. Intratumoral gene transfer therapy of cancer [abstract 47]. *Mol Ther* 2001;3(5 Suppl):S19
27. Lewis KD, Humes TN, Gonzalez R. Current status and future prospects of gene therapy: Focus on Alloectin-7 in the treatment of metastatic melanoma. *Am J Cancer* 2005;4:137-44
28. Stopeck AT, Jones A, Hersh EM, et al. Phase II study of direct intralesional gene transfer of Alloectin-7, an HLA-B71/(32-microglobulin DNA-liposome complex, in patients with metastatic melanoma. *Clin Cancer Res* 2001;7:2285-91
29. Gonzalez RA, Hutchins LB, Nemunaitis JC, et al. Phase 2 trial of Alloectin-7 in advanced metastatic melanoma. *Melanoma Res* 2006;16(6):521-6
30. Bedikian AY, Gonzalez R, Richards J, et al. A phase II study of high-dose alloectin-7 in patients with advanced metastatic melanoma [Abstract] 2897. *Proc Am Soc Clin Oncol* 2003;22:720
31. Bedikian AY, Gonzalez R, Richards J, et al. E. A phase II study of high-dose Alloectin-7 in patients with advanced metastatic melanoma. (accepted, *Melanoma Research*)
32. Richards J, Thompson J, Atkins MB, et al. A controlled, randomized Phase III trial comparing the response to dacarbazine with and without Alloectin-7 in patients with metastatic melanoma [Abstract 1380]. *Proc Am Soc Clin Oncol* 2002;21

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