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Safety and Immunogenicity of a DNA Vaccine Encoding Carcinoembryonic Antigen and Hepatitis B Surface Antigen in Colorectal Carcinoma Patients¹

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Abstract

Despite an abundance of preclinical data, relatively little is known regarding the efficacy of DNA vaccination in humans. Here, we present results from a dose-escalation clinical trial of a dual expression plasmid encoding carcinoembryonic antigen (CEA) and hepatitis B surface antigen (HBsAg) in 17 patients with metastatic colorectal carcinoma. CEA was selected as a prototypic tumor-associated self-antigen, and the HBsAg cDNA was included as a positive control for immune response to the DNA vaccine without relying upon breaking tolerance to a self-antigen. Groups of 3 patients received escalating single i.m. doses of the DNA vaccine at 0.1, 0.3, and 1.0 mg. Subsequent groups of 3 patients received three repetitive 0.3- or 1.0-mg doses at 3-week intervals. A final group of 2 patients received three repetitive 2.0 mg doses at 3-week intervals. Toxicity was limited to transient grade 1 injection site tenderness, fatigue, and creatine kinase elevations, each affecting a minority of patients in a non-dose-related manner. Repetitive dosing of the DNA vaccine induced HBsAg antibodies in 6 of 8 patients, with protective antibody levels achieved in four of these patients. CEA-specific antibody responses were not observed, but 4 of 17 patients developed lymphoproliferative responses to CEA after vaccination. No objective clinical responses to the DNA vaccine were observed among this population of patients with widely metastatic colorectal carcinoma. Nevertheless, this pilot trial has provided encouraging human immune response data in support of this vaccine technology.

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Introduction

Between 1990 and 1993, the administration of “naked” plasmid DNA encoding a specific protein antigen was shown to induce expression of the protein in mouse myocytes, elicit antibodies against the protein, and protect against influenza challenge via cytolytic T-cell responses against the expressed protein (1–3). Hundreds of publications since have reported the efficacy of DNA vaccines in small and large animal models of infectious diseases and cancer (4, 5).

DNA vaccination provides the following advantages over protein vaccines: (a) greater chemical stability; (b) relative ease of purification and characterization; (c) inherent adjuvant effects of unmethylated CpG dinucleotide motifs; (d) direct entry of antigen into intracellular MHC class I processing pathways facilitating CTL induction; and (e) intracellular antigen synthesis with posttranslational modification producing native tertiary antigen structure. Similarly, DNA vaccination provides advantages compared with recombinant viral vaccines as follows: (a) relative ease of construction, production and quality control; (b) less risk of insertional mutagenesis; (c) absence of vector-specific immune responses that limit the efficacy of booster immunizations; and (d) absence of risk related to recombinational events leading to pathogenic viruses.

Despite an abundance of preclinical data, relatively little is known regarding the efficacy of DNA vaccination in humans. Early clinical trials of DNA vaccines against infectious pathogens have provided mixed results. Trials conducted in HIV-infected individuals have been difficult to interpret because of preexisting immunity (6–8). A subsequent Phase I trial of a DNA vaccine encoding HIV antigens in 39 healthy volunteers induced antigen-specific lymphoproliferative responses but no antibody responses and only rare CTL responses (9). In the largest DNA vaccine trial reported to date, 219 healthy volunteers were randomized to receive a plasmid encoding influenza hemagglutinin or placebo (10). Virus-neutralizing and hemagglutination-inhibiting antibodies developed in only a minority of subjects. More encouraging results have emerged from the initial clinical trial of a DNA vaccine encoding a malarial antigen, the *Plasmodium falciparum* circumsporozoite protein (11). Persons receiving vaccines unequivocally developed antigen-specific, CD8-positive CTLs restricted by multiple HLA alleles, providing a foundation for further human trials of this potentially revolutionary vaccine technology.

In this report, we present results from a dose-escalation clinical trial of a dual-expression plasmid encoding CEA³ and HBsAg in 17 patients with metastatic colorectal carcinoma.

³ The abbreviations used are: CEA, carcinoembryonic antigen; HBsAg, hepatitis B surface antigen; PHA, phytohemagglutinin; S.R., stimulation ratio; rV-CEA, recombinant vaccinia virus encoding CEA.

CEA was selected as a prototypic tumor-associated self-antigen, and HBsAg cDNA was included as a positive control for immune response to the DNA vaccine uncomplicated by immunological tolerance to a self-antigen. CEA is a M_r 180,000 membrane-anchored glycoprotein expressed on the great majority of colorectal, gastric, and pancreatic carcinomas as well as ~50% of breast cancers and 70% of non-small cell lung cancers (12). CEA is also expressed, to a limited extent, in normal colonic mucosa and fetal digestive organs.

Our group has demonstrated that i.m. administration of plasmid DNA encoding human CEA induces CEA-specific humoral and cellular immune responses in mice and nonhuman primates as well as protection of mice against challenge with syngeneic colon carcinoma cells expressing human CEA (13–15). However, it is important to emphasize that human CEA is a foreign antigen in both mice and nonhuman primates, whereas humans should be immunologically tolerant to CEA because of its expression in fetal and normal adult tissues.

The goals of the present study were 3-fold: (a) to examine the safety of single and repetitive administration of the DNA vaccine over a range of 0.1–2.0 mg/dose; (b) to examine the efficacy of DNA vaccination in humans based upon immune response to the HBsAg control antigen; (c) to evaluate any CEA-specific immune responses or antitumor effects induced by the vaccine.

Materials and Methods

DNA Vaccine. We obtained the cDNA encoding full-length human CEA from J. Kanter, National Cancer Institute (16) and cDNA for the small and middle (S2.S) proteins of HBsAg from Robert Whalen (Centre National de la Recherche Scientifique, Paris, France; Refs. 17, 18). These two cDNAs were inserted into a eukaryotic expression vector that uses separate cytomegalovirus intermediate-early promoter/enhancers to regulate transcription of CEA and HBsAg. The plasmid was derived from the commercially available eukaryotic expression vector pcDNA3 (Invitrogen, San Diego, CA). The plasmid contains two cytomegalovirus intermediate-early promoter/enhancers and bovine growth hormone polyadenylation signals flanking two multiple cloning sites. The pcDNA3 plasmid was modified by deletion of the neomycin resistance gene, the ampicillin resistance gene, and nonessential viral sequences (19). The Tn903 kanamycin resistance gene from pUC4K (Pharmacia, Piscataway, NJ) was inserted to allow selective propagation in *Escherichia coli*. The resulting plasmid was designated pCEA/HBsAg. *In vitro* expression and *in vivo* immunogenicity of pCEA/HBsAg in nonhuman primates has been reported previously (15). Clinical supplies of this construct (IND 6892) were produced under good manufacturing practices by Centocor, Inc. (Malvern, PA). The DNA vaccine was formulated in a citrate-buffered saline solution containing 0.25% bupivacaine-HCl, an amide-type anesthetic, as described previously (20). Three DNA vaccine concentrations of 0.05, 0.15, and 0.5 mg/ml were stored as 1-ml vials at -20°C and thawed at room temperature for 30 min immediately before injection.

Treatment Regimen. Seventeen patients with metastatic colorectal carcinoma, shown to express CEA by immunoperoxidase staining or by an elevated serum CEA level, were selected

(21). Patients had an Eastern Cooperative Oncology Group performance status of 0–2 and no serological evidence of antibody to HBsAg or active hepatitis B infection. Therapy was initiated at least 4 weeks after prior chemotherapy or radiotherapy. All patients gave informed consent.

Each DNA vaccine dose consisted of bilateral i.m. injections using a volume of 1–2 ml into each deltoid muscle. Groups of 3 patients received escalating single doses of pCEA/HBsAg at 0.1, 0.3, and 1.0 mg total dose. Subsequently, groups of three patients received three repetitive 0.3 or 1.0-mg doses at 3-week intervals. A final group of two patients received three repetitive 2.0 mg doses at 3-week intervals. All patients underwent 9 weeks of clinical and immunological monitoring after their first DNA vaccine dose. Patients were examined 1 and 3 weeks after each immunization to assess the degree of inflammation at the inoculation site, regional adenopathy, or other signs of toxicity. A complete blood count, serum chemistries, and C-reactive protein level were obtained before each immunization as well as 7 and 28 days after each immunization. Serum CEA levels were obtained at 3-week intervals, and anti-double-stranded DNA antibody titers were checked before immunization and during the off-study evaluation on day 64. All patients were evaluated for evidence of objective antitumor response 9 weeks after the primary immunization.

Lymphoproliferative Assay. Fresh peripheral blood mononuclear cells obtained by Ficoll density gradient centrifugation were resuspended in complete medium consisting of RPMI 1640 supplemented with 10% pooled normal human AB serum, 2 mM L-glutamine, 50 μM 2-mercaptoethanol, and antibiotics. Cells were added at 1.5×10^5 per well to 96-well, flat-bottomed plates. Stimulated cells were incubated in quadruplicate wells with baculovirus-derived recombinant human CEA (MicroGeneSys, Meriden, CT) over a range of concentrations (1–30 $\mu\text{g}/\text{ml}$); yeast-derived recombinant HBsAg (kindly provided by Merck, Sharp, and Dohme, West Point, PA) over a range of concentrations (1–30 $\mu\text{g}/\text{ml}$); BSA (30 $\mu\text{g}/\text{ml}$) as a negative control antigen; tetanus toxoid (Wyeth-Ayerst Laboratories, Paoli, PA) as a positive control antigen; PHA at 5 $\mu\text{g}/\text{ml}$ as a positive control mitogen; or baculovirus-derived recombinant HIV gp160 (MicroGeneSys) as a control for proliferation induced by trace contaminants within the baculovirus recombinant CEA preparation. Control cells were cultured in complete medium alone. All cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air for 6 days, followed by an overnight pulse with 1 $\mu\text{Ci}/\text{well}$ of tritiated thymidine. Cells were harvested, and incorporated radioactivity was quantitated using a solid-phase beta scintillation counter (Matrix 9600; Packard Instrument Co., Downers Grove, IL). This solid-phase counter is ~3-fold less efficient than a liquid-phase scintillation counter such that raw cpm are 3-fold fewer than those seen with liquid counting. The S.R. was calculated as the mean cpm of the stimulated cells divided by the mean cpm of the control cells. A positive response was defined as a postimmunization S.R. >3 and at least 2-fold greater than the pre-immunization S.R. for a given antigen.

Antibody Response to HBsAg. Anti-HBsAg antibody responses were evaluated by the commercially available AUSAB ELISA (Abbott Laboratories, Chicago, IL), which detects antibodies to the small S protein of HBsAg. Anti-HBsAg antibody levels

Table 1 Patient characteristics

| Patient no. | Age/Sex | Dosage (mg) | Performance status ^a | Metastatic disease | Serum CEA Pre-study (ng/ml) | Serum CEA week 9 (ng/ml) | Response ^b |
|-------------|---------|-------------|---------------------------------|--------------------|-----------------------------|--------------------------|-----------------------|
| 1 | 71/M | 0.1 × 1 | 1 | Liver | 960 | 3,923 | P |
| 2 | 62/F | 0.1 × 1 | 0 | Liver, Lymph Node | 127 | 180 | S |
| 3 | 61/M | 0.1 × 1 | 1 | Lung, Lymph Node | 893 | 1,819 | P |
| 4 | 62/M | 0.3 × 1 | 1 | Liver, Peritoneum | 293 | 554 | P |
| 5 | 55/M | 0.3 × 1 | 2 | Liver, Peritoneum | 3 | 7 | P |
| 6 | 59/F | 0.3 × 1 | 1 | Liver, Peritoneum | 7 | 15 | P |
| 7 | 56/F | 1.0 × 1 | 1 | Lung, Liver | 214 | 371 | P |
| 8 | 70/F | 1.0 × 1 | 1 | Lymph Node, Skin | 166 | 332 | S |
| 9 | 54/M | 1.0 × 1 | 1 | Liver | 311 | 446 | P |
| 10 | 49/F | 0.3 × 3 | 1 | Peritoneum | 33 | 41 | S |
| 11 | 67/M | 0.3 × 3 | 1 | Liver | 501 | 666 | P |
| 12 | 53/M | 0.3 × 3 | 1 | Liver, Lymph Node | 822 | 969 | P |
| 13 | 73/F | 1.0 × 3 | 1 | Liver, Peritoneum | 159 | 480 | P |
| 14 | 69/F | 1.0 × 3 | 1 | Lung, Liver | 2,807 | 3,978 | S |
| 15 | 51/F | 1.0 × 3 | 0 | Lung | 62 | 106 | S |
| 16 | 51/M | 2.0 × 3 | 0 | Lung, Liver | 53 | 93 | P |
| 17 | 50/F | 2.0 × 3 | 0 | Lung, Liver | 10 | 87 | P |

^a Performance status on Eastern Cooperative Oncology Group Scale (0–4).

^b Response based upon the day 64 off-study evaluation. S, stable disease; P, progressive disease.

≥10 mIU/ml in the Abbott ELISA correlate with protective immunity in humans (22). CEA-specific antibody responses were evaluated by ELISA and Western blot using methods described previously (23).

Results

Patient Characteristics. The clinical aspects of the trial are summarized in Table 1. The patients included 8 men and 9 women with a median age of 59 years (range, 49–73). All patients had metastatic colorectal carcinoma, and 16 of 17 had elevated serum CEA levels (>3 ng/ml) at the time of study entry. Five of 17 patients had stable disease at 9 weeks of follow-up, whereas the remaining 12 patients demonstrated disease progression. None of the patients demonstrated a sustained decrease in serum CEA levels after vaccination.

Toxicology. i.m. injection of the DNA vaccine produced transient grade 1 tenderness at the injection site in a minority of patients unrelated to dose. No signs of local inflammation, regional lymphadenopathy, or allergic reactions occurred. A few patients reported mild, transient fatigue, which was not dose related.

Transient grade 1 elevations of creatine kinase occurred in two patients 1–2 weeks after immunization in a non-dose-related manner. None of the patients developed anti-double-stranded DNA antibodies. C-Reactive protein levels, a marker of the hepatic acute phase protein response, were elevated in 8 of 17 patients before immunization, consistent with the diagnosis of advanced carcinoma (24). Elevated postimmunization values, which were greater than twice the pre-immunization value, occurred in 7 patients. C-Reactive protein levels typically peaked at or near the time of off-study evaluation, when patients were experiencing progressive metastatic carcinoma. Thus, the observed increase in acute phase reactants is consistent with progressive carcinoma and does not necessarily implicate inflammation or tissue damage as a consequence of DNA vaccination.

Grade 1–2 anemia developed in a non-dose-related fashion in 6 patients and was consistent with anemia of chronic disease in this patient population with metastatic colorectal carcinoma. Granulocytes have been shown to express non-specific cross-reacting antigen, raising the possibility that immunization against CEA could lead to autoimmune neutropenia (12, 25). Despite this theoretical concern, no consistent change in WBC counts or neutrophil counts occurred after vaccination. No deterioration in renal or hepatic function attributable to immunization was observed.

Lymphoproliferative Responses. Lymphoproliferative responses to CEA occurred in 4 of 17 patients after DNA immunization. The quotient of the postimmunization S.R. divided by the pre-immunization S.R. for baculovirus recombinant CEA and baculovirus recombinant HIV gp160 control protein is illustrated in Fig. 1 for these 4 patients. Postimmunization responses to CEA ranged from 2.6- to 44-fold greater than pre-immunization values. These lymphoproliferative responses appear CEA specific, because responses to HIV gp160 control protein did not increase after immunization. Details of the postimmunization lymphoproliferative responses in these 4 patients are provided in Table 2. CEA produced stimulation ratios ranging from 3.5 to 78. BSA and baculovirus recombinant HIV gp160 control proteins produced negative results with S.R.s ranging from 0.5 to 1.1 and from 0.5 to 2.7, respectively. PHA mitogen produced stimulation ratios >200 in all patients at all studied time points. CEA-specific lymphoproliferative responses occurred 6–9 weeks after primary immunization and showed no clear relationship to the dose or schedule of plasmid DNA immunization.

No detectable lymphoproliferative responses to HBsAg were observed in any of the 17 patients studied. Lymphoproliferative responses to tetanus toxoid and PHA mitogen were analyzed to determine the effect of progressive metastatic colorectal carcinoma upon lymphocyte function *in vitro*. Proliferative responses to PHA did not change significantly over 9 weeks

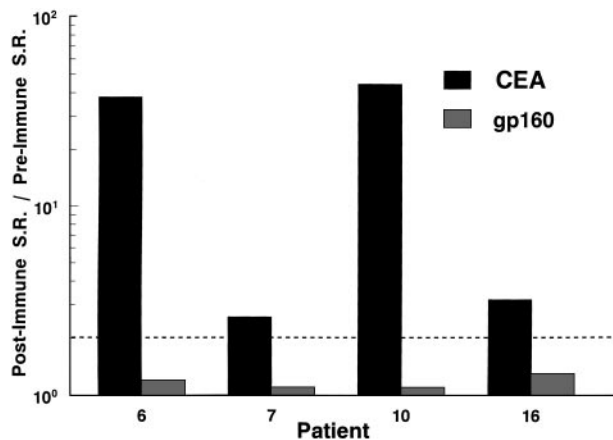


Fig. 1 Lymphoproliferative responses to CEA of patients immunized with pCEA/HBsAg. Values represent the quotient of the postimmunization stimulation ratio (S.R.) divided by the pre-immunization S.R. for a given antigen. The dashed line at 2 represents the threshold for a positive response. ■, baculovirus recombinant CEA protein; ▨, baculovirus recombinant HIV gp160 as a control protein.

of follow-up, despite disease progression in 12 of 17 patients. The mean \pm SE S.R. in response to PHA pre-study and 9 weeks after primary immunization were 1060 ± 220 and 880 ± 130 , respectively. Conversely, proliferative responses to a recall antigen, tetanus toxoid, diminished significantly during 9 weeks of follow-up. Six of 17 patients demonstrated lymphoproliferative responses to tetanus toxoid pre-study, but none of these 6 patients responded to tetanus toxoid at the time of off-study evaluation (day 64).

Antibody Responses. Anti-HBsAg antibody responses were evaluated by the commercially available AUSAB ELISA (Abbott Laboratories), which detects antibodies to the small S protein of HBsAg. All patients were negative for anti-HBsAg antibody pre-immunization as a criterion for study entry. Three of 9 patients receiving a single dose of pCEA/HBsAg developed HBsAg antibodies, but only 1 achieved a protective level exceeding 10 mIU/ml (patient 5, 157 mIU/ml at week 9). Repetitive dosing of pCEA/HBsAg induced HBsAg antibodies in 6 of 8 patients, and 4 of these patients achieved protective antibody levels. Among patients receiving repetitive doses of the DNA vaccine, HBsAg antibody responses showed a trend toward dose dependence, occurring in only 1 of 3 patients in the 300- μ g dose group compared with 5 of 5 patients receiving 1–2-mg doses. None of the patients developed detectable CEA-specific antibodies.

Discussion

A major aim of the study was to examine the safety of single and repetitive administration of the DNA vaccine at total doses ranging from 0.1 to 6.0 mg. Toxicity was limited to transient grade 1 injection site tenderness, fatigue, and creatine kinase elevations, each affecting a minority of patients in a non-dose-related manner. This is consistent with other Phase I trials of DNA vaccines reporting occasional injection site erythema or tenderness and no systemic toxicity (5, 6, 8, 11, 26, 27). Thus, the upper limit of dosing for i.m. DNA vaccines

appears to be determined predominantly by practical limits on the production of clinical grade plasmid DNA rather than toxicity.

A second aim of the study was to examine the efficacy of DNA vaccination in humans based upon immune response to the HBsAg control antigen. Repetitive dosing of pCEA/HBsAg induced HBsAg antibodies in 6 of 8 patients, with protective antibody levels achieved in 4 of these patients. HbsAg DNA immunization has had mixed success to date (28, 29). Particle-mediated delivery of another DNA vaccine encoding HBsAg to the epidermis failed to induce primary immune responses to HBsAg or provide a priming event for a memory response to the licensed recombinant protein vaccine when a low dose was used (0.25 μ g, two doses; Ref. 28). This is consistent with our observations in pig-tailed macaques, where only one of three animals receiving repetitive doses of pCEA/HBsAg by gene gun developed HBsAg antibodies, whereas all three animals receiving the DNA vaccine i.m. seroconverted (15). However, a subsequent study in humans using particle-mediated delivery of higher doses of DNA resulted in seroconversion of all 12 healthy volunteers (29).

In the present study, HBsAg antibodies were induced within 9 weeks of primary immunization, a constraint imposed by vaccination of patients with limited life expectancy because of metastatic colorectal carcinoma. We have reported previously that only one of three pig-tailed macaques receiving repetitive 1-mg doses of pCEA/HBsAg by i.m. injection developed HBsAg antibodies within 19 weeks after primary immunization, whereas all three seroconverted to protective levels of antibody by week 28 (15). Thus, a more protracted vaccination schedule may increase the frequency of seroconversion to protective levels of HBsAg antibodies, particularly when lower doses of DNA are used. Indeed, in the most promising clinical study to date using multiple DNA immunizations by PowderJect, 11 of 12 patients required >12 weeks before protective levels of anti-HbsAg antibodies were achieved (29). Another factor which may have diminished the frequency of anti-HBsAg seroconversion in the present study was the use of a dual expression plasmid with two separate CVM promoter/enhancers. Promoter interference may have reduced the expression level of each antigen as compared with the use of two separate plasmids (30). A lack of detectable *in vitro* lymphoproliferative responses to HBsAg in the present study was not unexpected, because such responses are weak or absent after viral infection or repetitive immunization with the commercial HBsAg protein vaccine (31–33). Nevertheless, the present study has demonstrated the efficacy of i.m. DNA vaccination in humans regarding induction of protective serological responses to HBsAg.

The third aim of the study was to evaluate any CEA-specific immune responses or antitumor effects induced by the vaccine. CEA-specific antibody responses were not observed. However, 4 of 17 patients developed lymphoproliferative responses to CEA after vaccination. The low frequency of responses may have resulted in part from the compromised immune status of these patients with advanced colorectal carcinoma. This premise is supported by the observation that proliferative responses to a recall antigen, tetanus toxoid, diminished significantly in these patients during their period of study participation. We have reported previously a similar decrease in

Table 2 Specificity of the lymphoproliferative response of selected patients immunized with pCEA/HBsAg

| Patient | Weeks after primary immunization | Media | CEA | gp160 ^a | BSA | PHA |
|---------|----------------------------------|---------------------|-------------|--------------------|---------|-----------------|
| 6 | 6 | 40 ± 4 ^b | 1,300 ± 200 | 110 ± 70 | 42 ± 8 | 21,000 ± 1,000 |
| 7 | 9 | 30 ± 6 | 110 ± 10 | 28 ± 10 | 28 ± 7 | 38,000 ± 7,000 |
| 10 | 6 | 32 ± 8 | 2,500 ± 700 | 17 ± 2 | 17 ± 5 | 51,000 ± 10,000 |
| 10 | 9 | 55 ± 8 | 420 ± 20 | 49 ± 20 | 30 ± 7 | 65,000 ± 4,000 |
| 16 | 6 | 84 ± 10 | 290 ± 60 | 106 ± 10 | 68 ± 30 | 17,000 ± 700 |

^a Baculovirus recombinant HIV gp160 protein.

^b Values are mean cpm of quadruplicate wells ± SE.

recall antigen responsiveness in patients with metastatic adenocarcinoma (34).

An increasing body of literature has been reported, exploring different vaccination strategies targeting CEA (reviewed in Ref. 35). Our group has recently reported induction of CEA-specific autoantibodies by a recombinant vaccinia virus encoding CEA (rV-CEA; Ref. 23). Schlom's group has also described the derivation of CEA-specific cytolytic T-cell lines after rV-CEA immunization (36). However, lymphoproliferative responses to CEA were not observed in either trial (34, 36). T-cell responses have been reported in subsequent pox-based CEA immunization strategies that included costimulatory or immunostimulatory molecules (35). CEA-specific antibody and lymphoproliferative responses have been reported after vaccination with an anti-idiotypic monoclonal antibody mimicking a portion of the CEA molecule in patients with colorectal carcinoma (37). Finally, dendritic cell-based vaccines have also shown T-cell and clinical responses (35). Collectively, these CEA immunization strategies demonstrate that breaking immunological tolerance to this molecule is feasible, generally safe, and shows some clinical promise.

The incidence of CEA-specific lymphoproliferative responses was low in this pilot Phase I trial. Also, these immune responses were not associated with objective tumor regression or sustained declines in circulating CEA, and there was not a good correlation between positive lymphoproliferation and stable disease. This is consistent with the finding of others demonstrating a lack of correlation between *in vitro* assays and clinical outcome (38–40). Several factors may have contributed to this suboptimal immune response. As discussed above, an extended immunization period may favor a more robust immune response. Alternatively, prime-boost immunization strategies combining different vaccines may further enhance immune responses (41). Thus, additional Phase I/II trials of CEA polynucleotide immunization examining augmentation strategies such as cytokine or costimulatory molecule cDNA codelivery with or without priming by rV-CEA or recombinant canarypox are warranted in an effort to increase the frequency and magnitude of CEA-specific T-cell responses before Phase III trials. Also, given the favorable safety profile of DNA vaccines thus far, future trials of CEA immunization should focus perhaps on patients with less advanced disease.

This and other first-generation clinical trials have indicated that DNA vaccination is safe and well tolerated. Furthermore, the present study has unequivocally demonstrated induction of primary immune responses to HBsAg in the majority of patients

receiving repetitive doses. This study, together with the other Phase I trials of a malarial and hepatitis B DNA vaccines (11, 29), provides the most encouraging human immune response data to date in support of DNA vaccination. The next decade promises to provide much insight into the clinical utility of this novel vaccine technology in the fields of infectious disease and cancer.

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References

- Wolff, J. A., Malone, R. W., Williams, P., Chong, W., Acsadi, G., Jani, A., and Felgner, P. L. Direct gene transfer into mouse muscle *in vivo*. *Science* (Wash. DC), 247: 1465–1468, 1990.
- Tang, D. C., DeVit, M., and Johnston, S. A. Genetic immunization is a simple method for eliciting an immune response. *Nature* (Lond.), 356: 152–154, 1992.
- Ulmer, J. B., Donnelly, J. J., Parker, S. E., Rhodes, G. H., Felgner, P. L., Dworki, V. J., Gromkowski, S. H., Deck, R. R., DeWitt, C. M., Friedman, A., *et al.* Heterologous protection against influenza by injection of DNA encoding a viral protein. *Science* (Wash. DC), 259: 1745–1749, 1993.
- Donnelly, J. J., Ulmer, J. B., Shriver, J. W., and Liu, M. A. DNA vaccines. *Annu. Rev. Immunol.*, 15: 617–648, 1997.
- White, S. A., and Conry, R. M. DNA vaccines. In: S. A. Rosenberg (ed.), *Principles and Practice of the Biologic Therapy of Cancer*: pp. 674–686, Ed. 3. Philadelphia: Lippincott Williams & Wilkins, 2000.
- MacGregor, R. R., Boyer, J. D., Ugen, K. E., Lacy, K. E., Gluckman, S. J., Bagarazzi, M. L., Chattergoon, M. A., Baine, Y., Higgins, T. J., Ciccirelli, R. B., Coney, L. R., Ginsberg, R. S., and Weiner, D. B. First human trial of a DNA-based vaccine for treatment of human immunodeficiency virus type 1 infection: safety and host response. *J. Infect. Diseases*, 178: 92–100, 1998.
- Boyer, J. D., Chattergoon, M. A., Ugen, K. E., Shah, A., Bennett, M., Cohen, A., Nyland, S., Lacy, K. E., Bagarazzi, M. L., Higgins, T. J., Baine, Y., Ciccirelli, R. B., Ginsberg, R. S., MacGregor, R. R., and Weiner, D. B. Enhancement of cellular immune response in HIV-1 seropositive individuals: a DNA-based trial. *J. Applied Biomaterials*, 90: 100–107, 1999.
- Calarota, S., Bratt, G., Nordland, S., Hinkula, J., Leandersson, A. C., Sandstrom, E., and Wahren, B. Cellular cytotoxic response induced by DNA vaccination in HIV-1-infected patients. *Lancet*, 351: 1320–1325, 1998.
- Goepfert, P., Mulligan, M., Corey, L., Graham, B., Evans, T., Weinhold, K., Stablein, D., and Ginsberg, R. AVEG 031: Phase I evaluation of a gag-pol facilitated DNA vaccines for HIV-1 prevention. Twelfth World AIDS Conference, Geneva, June, 1998.

10. Clements-Mann, M. L., Eichelberger, M., Boslego, J. W., Harro, C., Evans, R. K., Ullmer, J. B., Friedman, A., Chan, I. S. F., Liu, M., and Donnelly, J. J. Safety and immunogenicity of influenza hemagglutinin DNA vaccine alone or with aluminum adjuvant in adult volunteers. *Am. Soc. Virol.*, 17: 115, 1998.
11. Wang, R., Doolan, D. L., Le, T. P., Hedstrom, R. C., Coonan, K. M., Charoenvit, Y., Jones, T. R., Hobart, P., Margalith, M., Ng, J., Weiss, W. R., Sedegah, M., de Taisne, C., Norman, J. A., and Hoffman, S. L. Induction of antigen-specific cytotoxic T lymphocytes in humans by a malaria DNA vaccine. *Science (Wash. DC)*, 282: 476–480, 1998.
12. Thompson, J. A., Grunert, F., and Zimmermann, W. Carcinoembryonic antigen gene family: molecular biology and clinical perspectives. *J. Clin. Lab. Anal.*, 5: 344–366, 1991.
13. Conry, R. M., LoBuglio, A. F., Kantor, J., Schlom, J., Loechel, F., Moore, S. E., Sumerel, L. A., Barlow, D. L., Abrams, S., and Curiel, D. T. Immune response to a carcinoembryonic antigen polynucleotide vaccine. *Cancer Res.*, 54: 1164–1168, 1994.
14. Conry, R. M., LoBuglio, A. F., Loechel, F., Moore, S. E., Sumerel, L. A., Barlow, D. L., and Curiel, D. T. A carcinoembryonic antigen polynucleotide vaccine has *in vivo* antitumor activity. *Gene Ther.*, 2: 59–65, 1995.
15. Conry, R. M., White, S. A., Fultz, P. N., Khazaeli, M. B., Strong, T. V., Allen, K. O., Barlow, D. L., Moore, S. E., Coan, P. N., Davis, I., Curiel, D. T., and LoBuglio, A. F. Polynucleotide immunization of non-human primates against carcinoembryonic antigen. *Clin. Cancer Res.*, 4: 2903–2912, 1998.
16. Robbins, P. F., Kantor, J. A., and Salgaller, M. Transduction and expression of the human carcinoembryonic antigen gene in a murine colon carcinoma cell line. *Cancer Res.*, 51: 3657–3662, 1991.
17. Davis, H. L., Michel, M-L., and Whalen, R. G. DNA-based immunization induces continuous secretion of hepatitis B surface antigen and high levels of circulating antibody. *Hum. Mol. Genet.*, 2: 1847–1851, 1993.
18. Davis, H. L., Michel, M-L., Mancini, M., Schleaf, M., and Whalen, R. G. Direct gene transfer in skeletal muscle: plasmid DNA-based immunization against the hepatitis B virus surface antigen. *Vaccine*, 12: 1503–1509, 1994.
19. Conry, R. M., LoBuglio, A. F., Loechel, F., Moore, S. E., Sumerel, L. A., Barlow, D. L., Pike, M. J., and Curiel, D. T. A carcinoembryonic antigen polynucleotide vaccine for human clinical use. *Cancer Gene Ther.*, 2: 33–38, 1995.
20. Danko, I., Fritz, J. D., Jiao, S., Hogan, K., Latendresse, J. S., and Wolff, J. A. Pharmacological enhancement of *in vivo* foreign gene expression in muscle. *Gene Ther.*, 1: 114–121, 1994.
21. Conry, R. M., LoBuglio, A. F., and Curiel, D. T. Clinical protocol: Phase Ia trial of a polynucleotide anti-tumor immunization to human carcinoembryonic antigen in patients with metastatic colorectal cancer. *Hum. Gene Ther.*, 7: 755–772, 1996.
22. Mushahwar, I. K., Dienstag, J. L., Polesky, H. F., McGrath, L. C., Decker, R. H., and Overby, L. R. Interpretation of various serological profiles of hepatitis B virus infection. *Am. J. Clin. Pathol.*, 76: 773–777, 1981.
23. Conry, R. M., Allen, K. O., Lee, S-w., Moore, S. E., Shaw, D. R., and LoBuglio, A. F. Human autoantibodies to carcinoembryonic antigen induced by a vaccinia-CEA vaccine. *Clin. Cancer Res.*, 6: 34–41, 2000.
24. Dinarello, C. A. The acute phase response. *In: J. C. Bennett and F. Plum (eds.), Cecil Textbook of Medicine*, Ed. 20, Vol. 2, pp. 1535–1537. Philadelphia: W. B. Saunders Company, 1996.
25. Schwartz, K., Bruckel, N., Schwaibold, H., von Kleist, S., and Grunert, F. Non-specific cross-reacting antigen: characterization of specific and cross-reacting epitopes. *Mol. Immunol.*, 26: 467–475, 1989.
26. Shroff, K. E., Smith, L. R., Baine, Y., and Higgins, T. J. Potential for plasmid DNAs as vaccines for the new millennium. *Pharm. Sci. Tech. Today*, 2: 205–212, 1999.
27. Mincheff, M., Tchakarov, S., Zoubak, S., Loukinov, D., Botev, C., Altankova, I., Georgiev, G., Petrov, S., and Meryman, H. T. Naked DNA and adenoviral immunizations for immunotherapy of prostate cancer: a Phase I/II clinical trial. *Eur. Urol.*, 38: 208–217, 2000.
28. Tacket, C. O., Roy, M. J., Widera, G., Swain, W. F., Broome, S., and Edelman, R. Phase I safety and immune response studies of a DNA vaccine encoding hepatitis B surface antigen delivered by a gene delivery device. *Vaccine*, 17: 2826–2829, 1999.
29. Roy, M. J., Wu, M. S., Barr, L. J., Fuller, J. T., Tussey, L. G., Speller, S., Culp, J., Burkholder, J. K., Swain, W. F., Dixon R. M., Widera G., Vessey, R., King, A., Ogg, G., Gallimore, A., Haynes, J. R., and Fuller, D. H. Induction of antigen-specific CD8+ T cells, T helper cells, and protective levels of antibody in humans by particle-mediated administration of a hepatitis B virus DNA vaccine. *Vaccine*, 19: 764–778, 2000.
30. Lieu, F. H., Hawley, T. S., Fong, A. Z., and Hawley, R. G. Transmissibility of murine stem cell virus-based retroviral vectors carrying both interleukin-12 cDNAs and a third gene: implications for immune gene therapy. *Cancer Gene Ther.*, 4: 167–175, 1997.
31. Celis, E., Kung, P. C., and Chang, T. W. Hepatitis B virus-reactive human T lymphocyte clones: antigen specificity and helper function for antibody synthesis. *J. Immunol.*, 132: 1511–1516, 1984.
32. Bocher, W. O., Herzog-Hauff, S., Schlaak, J., Meyer zum Buschenfelde, K-H., and Loh, H. F. Kinetics of hepatitis B surface antigen-specific immune responses in acute and chronic hepatitis B or after HBs vaccination: stimulation of the *in vitro* antibody response by interferon gamma. *Hepatology*, 29: 238–244, 1999.
33. Ferrari, C., Penna, A., Bertolotti, A., Valli, A., Antoni, A. D., Giuberti, T., Cavalli, A., *et al.* Cellular immune response to hepatitis B virus-encoded antigens in acute and chronic hepatitis B virus infection. *J. Immunol.*, 145: 3442–3449, 1990.
34. Conry, R. M., Khazaeli, M. B., Saleh, M. N., Allen, K. O., Barlow, D. L., Moore, S. E., Craig, D., Arani, R. B., Schlom, J., and LoBuglio, A. F. Phase I trial of a recombinant vaccinia virus encoding carcinoembryonic antigen in metastatic adenocarcinoma: comparison of intradermal *versus* subcutaneous administration. *Clin. Cancer Res.*, 5: 2330–2337, 1999.
35. Berinstein, N. L. Carcinoembryonic antigen as a target for therapeutic anticancer vaccines: a review. *J. Clin. Oncol.*, 20: 2197–2207, 2002.
36. Tsang, K. Y., Zarella, S., Nieroda, C. A., Ming, Z. Z., Hamilton, J. M., and Schlom, J. Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. *J. Natl. Cancer Inst.*, 87: 982–990, 1995.
37. Foon, K. A., John, W. J., Chakraborty, M., Sherratt, A., Garrison, J., Flett, M., and Bhattacharya-Chatterjee, M. Clinical and immune response in advanced colorectal cancer patients treated with anti-idiotypic monoclonal antibody vaccine that mimics the carcinoembryonic antigen. *Clin. Cancer Res.*, 3: 1267–1276, 1997.
38. Rosenberg, S. A., Yang, J. C., Schwartzentruber, D. J., Hwu, P., Marincola, F. M., Topalian, S. L., Restifo, N. P., Dudley, M. E., Schwarz, S. L., Spiess, P. J., Wunmdlerlich, J. R., Parkhurst, M. R., Kawakami, Y., Seipp, C. A., Einhorn, J. H., and White, D. E. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. *Nat. Med.*, 4: 321–327, 1998.
39. Von Mehren, M., Arlen, P., Gulley, J., Rogatko, A., Cooper, H. S., Meropol, N. J., Alpaugh, R. K., Davey, M., McLaughlin, S., Beard, M. T., Tsang, K. Y., Schlom, J., and Weiner, L. M. The influence of granulocyte macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma. *Clin. Cancer Res.*, 7: 1181–1191, 2001.
40. Nielsen, M., and Marincola, M. Melanoma vaccines: the paradox of T cell activation without clinical response. *Cancer Chemother. Pharmacol.*, 46: 62–66, 2000.
41. McShane, H. Prime-boost immunization strategies for infectious disease. *Curr. Opin. Mol. Ther.*, 4: 23–27, 2002.