

Phase I Study Utilizing a Novel Antigen-Presenting Cell-Targeted Vaccine with Toll-like Receptor Stimulation to Induce Immunity to Self-antigens in Cancer Patients

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Abstract

Purpose: The use of tumor-derived proteins as cancer vaccines is complicated by tolerance to these self-antigens. Tolerance may be broken by immunization with activated, autologous, *ex vivo* generated and antigen-loaded, antigen-presenting cells (APC); however, targeting tumor antigen directly to APC *in vivo* would be a less complicated strategy. We wished to test whether targeted delivery of an otherwise poorly immunogenic, soluble antigen to APC through their mannose receptors (MR) would induce clinically relevant immunity.

Experimental Design: Two phase I studies were conducted with CDX-1307, a vaccine composed of human chorionic gonadotropin beta-chain (hCG- β) fused to an MR-specific monoclonal antibody, administered either locally (intradermally) or systemically (intravenously) in patients with advanced epithelial malignancies. An initial dose escalation of single-agent CDX-1307 was followed by additional cohorts of CDX-1307 combined with granulocyte-macrophage colony-stimulating factor (GM-CSF) and the Toll-like receptor (TLR) 3 agonist polyinosinic-polycytidylic acid (poly-ICLC) and TLR7/8 agonist resiquimod to activate the APC.

Results: CDX-1307 induced consistent humoral and T-cell responses to hCG- β when coadministered with TLR agonists. Greater immune responses and clinical benefit, including the longest duration of stable disease, were observed with immunization combined with local TLR agonists. Immune responses were induced equally efficiently in patients with elevated and nonelevated levels of serum hCG- β . Antibodies within the serum of vaccinated participants had tumor suppressive function *in vitro*. Toxicity consisted chiefly of mild injection site reactions.

Conclusions: APC targeting and activation induce adaptive immunity against poorly immunogenic self-antigens which has implications for enhancing the efficacy of cancer immunotherapy. *Clin Cancer Res*; 17(14); 4844–53. ©2011 AACR.

Introduction

Cancer vaccines activating T cells and antibodies against tumor-associated antigens (TAA) have shown promising activity in some patients; however, the magnitude of the immune response is frequently limited because many TAAs are self-proteins, frequently elevated in the circulation, to which the host may be tolerant. Vaccination with activated,

autologous antigen-presenting cells (APC), loaded with TAAs, may break tolerance and has been associated with clinical activity as suggested by the survival benefit achieved in prostate cancer patients receiving sipuleucel-T (1). Therefore, vaccine strategies that exploit the unique antigen uptake and processing functions of APCs are desirable. APCs possess several molecules that promote antigen internalization, among which are the c-type lectins including mannose receptor (MR, CD206; ref. 2). Targeting MRs on APCs results in delivery of exogenous proteins for MHC class I presentation and induction of CTLs, and greatly enhances the efficiency of MHC class II-restricted processing and presentation and induction of T helper responses (3). In humans, MRs have been identified in dermal dendritic cells (DC; ref. 4), and in cells lining splenic venous sinuses (5). Therefore, antigens could potentially be directed to MRs by either intradermal or intravenous routes. Although mannosylated antigen could be used to target MRs on APCs, such antigens could also be bound by nonendocytic mannose-binding proteins (e.g., scavenger

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

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doi: 10.1158/1078-0432.CCR-11-0891

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Translational Relevance

Soluble, tumor-associated self-antigens are often found abundantly in the peripheral blood of cancer patients but fail to activate a significant immune response likely due to limited access to antigen-presenting cells (APC) and poor costimulation of immune responses. This study tests the hypothesis that targeting hCG- β , a soluble, tumor-associated self-antigen, to APCs *in vivo* through the mannose receptor (MR) in combination with APC activation by toll-like receptor (TLR) agonists would lead to more potent antigen-specific immune responses in humans with advanced malignancies. This work has clear applications to the current and future practice of medicine in that the results have supported further testing of this vaccine strategy in an ongoing phase II study which if successful, could lead to a new strategy for vaccinating patients with diverse malignancies that express otherwise poorly immunogenic tumor antigens.

proteins and mannose-specific lectins) that have little or no role in antigen presentation. A more specific strategy exploits antibodies specific for MRs that are fused with TAA to generate a readily produced and APC-targeted vaccine. Potent cellular and humoral immune responses were activated *in vitro* and in human MR transgenic mice using antigens fused to anti-human MR antibody B11 compared with the antigen alone (3, 6).

To test this strategy in humans, we elected to target hCG- β , the β subunit of human chorionic gonadotropin (hCG), which is best known as a hormone necessary to support pregnancy but has a number of attractive features as an antigen for cancer immunotherapy (7). hCG- β is over-expressed in a variety of common cancers including those of the colon, lung, pancreas, esophagus, breast, bladder, ovarian, cervix, stomach, and prostate, as well as trophoblastic and testicular neoplasms. Elevated hCG- β serum levels and/or tissue expression are independent predictors of disease outcome and are associated with a more aggressive disease course in renal, colorectal, bladder, and pancreatic cancers (8–13). Mechanistically, hCG may facilitate cancer progression at several levels: as a transforming growth factor, an immunosuppressive agent, an inducer of metastasis, and/or as an angiogenic factor (14). hCG- β is structurally similar to TGF- β and *in vitro* studies suggest that it may help prevent apoptosis in bladder cancer cells (15). Therefore, several anticancer activities could be achieved if immune responses against an hCG- β -based vaccine could be induced.

There is evidence that the human immune system may recognize hCG- β . Multiple human leukocyte antigen (HLA) class I and class II-restricted synthetic hCG- β epitopes with the capacity to induce CD4⁺ and CD8⁺ T-cell responses *in vitro* have been identified (16). Tolerance to hCG- β was broken in studies in which hCG vaccines were tested as contraceptive agents (17) and in patients with

malignancies. For example, a synthetic vaccine targeting hCG- β composed of the COOH terminal peptide of hCG- β (CTP37) conjugated to diphtheria toxoid induced anti-hCG antibodies in most patients with advanced colorectal cancer and anti-hCG antibody induction was associated with longer overall survival (18).

CDX-1307, a fusion protein composed of an MR-specific human immunoglobulin IgG1 and hCG- β , was designed to deliver the entire hCG- β protein to APCs and to induce hCG- β -specific cellular and humoral immune response. Preclinical studies showed that APCs such as DCs pulsed with CDX-1307 elicit potent, HLA-restricted, proliferative and cytolytic T-cell responses, including killing of hCG- β -expressing cancer cell lines (19). CDX-1307 is specific for MR and there has been no significant cross-reactivity with other tissues. In human MR transgenic mice (hMR-Tg), there was no significant toxicity attributable to the drug (unpublished data). In the majority of the human tissues, CDX-1307 specifically stained spindle/DCs and tissue macrophages, in addition to vascular endothelium, hepatic sinusoidal endothelium, and some bone marrow hematopoietic progenitor cells. Importantly, CDX-1307 was not observed to bind to ovarian cells and testicular Leydig cells known to express the luteinizing hormone (LH)/hCG receptor, suggesting that this molecule lacks hCG agonist properties (19). This result was anticipated as the monomeric forms of the α and β subunits of hCG cannot independently act as functional agonists (20).

Despite this exquisite capacity to deliver antigen to APCs, we recognized that a second event may prove crucial to generate immune responses in patients. A considerable historical database suggests that cancer vaccines are more immunogenic if administered with adjuvants including cytokines and pathogen-related molecules that provide "danger signals." The cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) is involved in recruitment and maturation of APCs (21–23) and has been incorporated into numerous clinical studies with cancer vaccines (reviewed in ref. 23) to enhance immune responses. There is also considerable interest in the use of Toll-like receptor (TLR) agonists in vaccine strategies. TLR-mediated recognition of microbial components triggers maturation and activation of APCs, enhancing expression of costimulatory signals and release of cytokines (24). TLR3 and TLR7/8 agonists in combination lead to the generation of the most desirable milieu for T-cell activation such as interleukin (IL) 12 secretion and Th1 polarization (25–27). In our preclinical experiments, combining CDX-1307 with polyinosinic-polycytidylic acid stabilized with poly-L-lysine and carboxymethyl cellulose (poly-ICLC), a synthetic double-stranded ribonucleic acid (dsRNA) agonist of TLR3, and resiquimod, an imidazoquinoline synthetic agonist of TLR7/8, significantly enhanced the stimulation of hCG- β -specific T cells (28).

We hypothesized that APC targeting of hCG- β using CDX-1307 along with GM-CSF, poly-ICLC, and/or resiquimod would lead to enhanced immune responses against hCG- β in humans. To test this hypothesis, we conducted 2

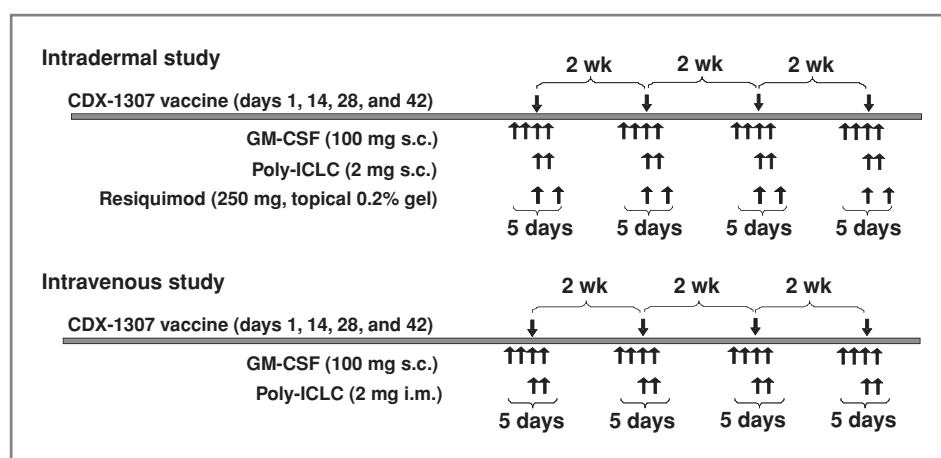


Figure 1. CDX-1307 vaccine regimen. When required for a particular cohort, adjuvants were administered as shown. All cohorts allowed retreatment in the event of stable disease. i.m., intramuscularly.

phase I studies of CDX-1307 administered either locally (intradermally) or systemically (intravenously). An initial dose-escalation of CDX-1307 as a single agent was followed by additional cohorts receiving CDX-1307 in combination with GM-CSF, GM-CSF plus poly-ICLC, GM-CSF plus resiquimod, and finally, all agents combined. Our goal was to determine the safety and immunologic and clinical efficacy of these combined regimens.

Patients and Methods

Patient population

This study (which consisted of 2 separate phase I clinical trials) was conducted only after approval by the appropriate Institutional Review Boards, and informed consent was obtained from each subject prior to the initiation of screening procedures for the study. Patients with advanced epithelial malignancies including breast, colorectal, pancreatic, ovarian, or bladder cancer with measurable or evaluable disease were permitted to enroll. Although hCG- β testing was not required for eligibility, each of these indications has been associated with expression of hCG- β (14). Patients were required to have had progressive disease after standard therapy for their malignancy. ECOG status of 0 or 1, life expectancy ≥ 16 weeks, and adequate organ function were also required, whereas patients receiving immunosuppressive therapy or with immunosuppressive disease were excluded.

Study drugs and dosing

CDX-1307 was supplied by Celldex Therapeutics, Inc. at 1 mg/mL, 2.5 mg/mL, and 5.0 mg/mL. GM-CSF (Leukine; sargramostim) was obtained from commercial sources. Poly-ICLC (Hiltonol) was supplied by Oncovir, Inc. in 1 mL of 2 mg/mL single-dose vials. Resiquimod (R-848; S-28463; 4-amino-2ethoxymethyl- α, α -dimethyl-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol) supplied by 3M Company. Topical resiquimod was solubilized in a single-phase gel formulation composed of propylene glycol, colloidal silicon dioxide, and triacetin at a concentration of 0.2%.

CDX-1307 was administered once every 2 weeks for 4 total doses, by intradermal injection in study CDX1307-01, or a 20-minute intravenous infusion in study CDX1307-02 (Fig. 1). In later cohorts, the volume of the CDX-1307 was great enough that the local injections were given as a combination of intradermal and subcutaneous if necessary. Additional adjuvants were administered as follows: GM-CSF (100 μ g) once daily subcutaneously for 4 days, beginning 2 days prior to each CDX-1307 administration. For the intradermal study, the GM-CSF was injected at the CDX-1307 injection site. Poly-ICLC, at a dose of 2 mg, was given either subcutaneously (CDX1307-01 study) immediately below the CDX-1307 injection or intramuscularly (CDX1307-02 study), on 2 consecutive days beginning on the day of CDX-1307 administration. Resiquimod, at a dose of 0.5 mg (250 mg of 0.2% gel) was applied topically at the injection site on the day of CDX-1307 administration and then 2 days later. Because injectable resiquimod was not available, it was not used in the intravenous study.

Study design

In each study, dose-escalation of CDX-1307 as a single agent was first conducted to either a maximum tolerated dose (MTD) or the maximum planned dose. Criteria for dose-escalation required that less than 33% of the patients in each dose cohort experience dose-limiting toxicity (DLT), defined as a treatment-related adverse event or new laboratory abnormality of NCI CTCAE grade 3 or greater severity. The initial dose-escalation phase was followed by additional cohorts evaluating CDX-1307 combined with GM-CSF, GM-CSF plus the TLR3 agonist poly-ICLC, GM-CSF plus the TLR7/8 agonist resiquimod, and GM-CSF plus both TLR agonists (Table 1).

Assessment of safety was determined by vital sign measurements, clinical laboratory tests (including pituitary and other endocrine assessments), human anti-human antibody (HAHA) assessment, physical examinations, chest radiography, electrocardiograms (ECG), and the incidence and severity of treatment-emergent adverse events.

Table 1. Patient characteristics at study entry

	Study CDX1307-01 local delivery (n = 57)	Study CDX1307-02 intravenous delivery (n = 30)	All patients (n = 87)
Median age, y	61	59	60
Male, n (%)	32 (56)	6 (20)	38 (44)
ECOG, n (%)			
0	22 (39)	16 (53)	38 (44)
1	35 (61)	14 (47)	49 (56)
Primary cancer, n (%)			
Pancreatic	12 (21)	1 (3)	13 (15)
Colorectal	33 (58)	9 (30)	42 (48)
Breast	7 (12)	20 (67)	27 (31)
Other (bladder/ureteral, ovarian, and testicular)	5 (9)	0	5 (6)
No. of sites/organ systems of disease (median)	3	3	3
No. of lesions (median)	5	5	6
No. prior anticancer regimens (median)	4	4	4
No. prior cytotoxic chemotherapy regimens (median)	3	3	3
Prior radiotherapy, n (%)	29 (51)	22 (73)	51 (59)
Elevated serum hCG- β , ^a n (%)	26 (46)	12 (46)	38 (46)

^aBaseline hCG- β data were not available for 4 patients treated in the iv study (all received CDX-1307 as single-agent).

To obtain preliminary evidence of clinical activity, we used computed tomography (CT) scans available from before and after all the immunizations and used Response Evaluation Criteria in Solid Tumors (RECIST) criteria to determine responses and progressive disease. Serum hCG- β levels were measured and elevations (above the average of the laboratories' upper limit of normal, 5 IU/mL) were considered "positive."

Pharmacokinetics

Circulating CDX-1307 was measured prior to and 120 minutes after each CDX-1307 administration, 1 and 3 days after the first CDX-1307 dose (days 2 and 4), and 2 weeks after the last CDX-1307 dose (day 56). Blood levels of CDX-1307 were determined using a functional ELISA. Plasma or serum samples were incubated on plates coated with a recombinant fragment of MR containing the B11 epitope. Bound CDX-1307 was detected using anti-hCG- β c-terminal peptide mAb (Wako Chemicals USA, Inc.) followed by goat anti-human IgG horseradish peroxidase (HRP) and tetramethylbenzidine (TMB). The assay has a lower limit of quantitation of 40 ng/mL.

Immunohistochemistry

Punch biopsy samples were placed in foil and embedded in optimum cutting temperature (OCT). Samples were snap frozen and sealed with parafilm for storage at -80°C until use. Sections of skin biopsies from the CDX-1307 injection site and a distant site were blocked using a peroxidase block (DAKO Envision Kit) and non-specific binding block (DAKO Protein Block). Rabbit anti-hCG (DAKO) or negative control normal rabbit immuno-

globulin (DAKO) was then added. Sections were incubated with peroxidase-labeled polymer (DAKO Envision Kit). 3,3'-Diaminobenzidine (DAB) substrate was added (DAKO). Slides were counterstained with hematoxylin. For MR staining, fluorescein isothiocyanate (FITC)-labeled B11 monoclonal antibody (mAb) and a rabbit-anti-FITC (Dako North America, Inc.) were used.

Immune monitoring

Anti-hCG- β -specific antibodies were detected by adding serially diluted serum to ELISA plates coated with commercially available hCG- β and then HRP-conjugated goat anti-human antibody. Plates were developed with a TMB substrate system and read using an automatic microplate reader (OD = 450 nm). A posttreatment sample was considered positive for the presence of anti-hCG- β antibodies if the mean OD value was greater than the mean value of the predose sample + 3 stable disease (or +0.1, whichever was higher). The titer of the sample was defined as the inverse of the dilution that yields an OD closest to a preset value for background as 0.1. Anti-hCG- β antibody isotypes were determined using isotype-specific HRP-conjugated goat anti-human IgG, IgM, and IgG1. The analysis for humoral response was conducted on samples from patients who had received at least 3 doses of the CDX-1307. Statistical analysis was conducted on the titers comparing the groups with various adjuvant regimens using Student's t test, unpaired.

To measure T-cell responses, autologous CD4⁺ T cells isolated from peripheral blood mononuclear cells (PBMC) using the Dynabeads Untouched Human CD4⁺ T cell Kit and cultured for at least 10 days in complete medium

[RPMI 1640 supplemented with 10% FBS and penicillin-streptomycin-neomycin (PSN) antibiotic mixture] with added phytohemagglutinin (PHA; 5 µg/mL; Sigma), IL-2 (10 U/mL, Cell Sciences), and IL-7 (20 ng/mL, Cell Sciences) were pulsed for at least 3 hours with an hCG-β peptide pool (custom peptide by ProImmune), irradiated for 5 minutes (dose 5,000 rads by a Faxitron Cabinet X-ray Irradiation System) and used as APCs to stimulate T cells (CD4 and CD8 combined) isolated from previously cryopreserved PBMCs at a T cell:APC ratio of 10:1. IL-2 (10 ng/mL) was changed every 3 to 4 days. After 10 days of *in vitro* stimulation, 5×10^4 T cells were tested in an ELISPOT assay (MabTech) with stimulators consisting of CD4⁺ APCs pulsed with the hCG-β peptide pool (10 µg/mL), actin peptide pool (10 µg/mL, ProImmune) as negative control, and CEF peptides (1 µg/mL, Panatecs; Axxora LLC) or anti-CD3 mAb as positive control. ELISPOT plates were subsequently evaluated by a Zeiss Elispot plate reader (Zellnet Consulting Inc.). The negative peptide pool spot numbers were subtracted from the hCG-β peptide pool to determine hCG-β-specific responses. The negative peptide pool varied significantly in donor samples ranging from 20 to 100 spots per 5×10^4 T cells.

Cell growth studies with SCaBER cell line

The hCG-β-secreting bladder cancer cell line, SCaBER (American Type Culture Collection) was seeded into 24 well plates at 5×10^4 cells per well in RPMI 1640 with 10% fetal calf serum (FCS) and 1% antibiotic antimycotic and incubated at 37°C in the presence of 5% CO₂ for 24 hours. The media was replaced with new media containing 1:50 of normal human serum or pre- and posttreatment samples from patient 5028 who received CDX-1307 (2.5 mg) with GM-CSF and poly-ICLC. Recombinant hCG-β was added to

the same patient samples at 1 µg/mL to determine the specificity of the anti-hCG-β responses. Flow cytometry was used to analyze the cell viability using the BD FACSCalibur following labeling of cells with propidium iodide stain and a minimum of 10,000 cells were counted for each well tested.

Results

Patients with advanced epithelial malignancies have elevated serum levels of hCG-β

These studies enrolled patients with advanced, pretreated colorectal, breast, pancreatic, bladder/ureteral, ovarian, and testicular cancers (Table 1). Because the intravenous dosing study was initiated in breast cancer patients before being expanded to other patient populations, the number of women in this study is substantially greater. In general, patients had multiple sites of metastases (median number of involved organ systems = 3) and had been heavily pretreated (median number of prior cytotoxic chemotherapy regimens = 3). Serum levels of hCG-β were elevated (above the average of the 2 laboratories' upper limit of normal, 5 IU/mL) in 46% of patients (Table 1).

Patient disposition

Of the 89 patients enrolled, 87 received CDX-1307 (57 in the intradermal study and 30 in the intravenous study; Table 2). In the intradermal study, 10 patients did not complete all 4 vaccinations due to rapid disease progression and/or declining performance, whereas 2 patients treated with CDX-1307 at 2.5 mg with GM-CSF and TLR agonists (resiquimod or resiquimod + poly-ICLC) received multiple courses of vaccination due to stable disease (5 and 3 courses, respectively). In the intravenous study, 5 patients

Table 2. Treatment allocation and clinical outcome

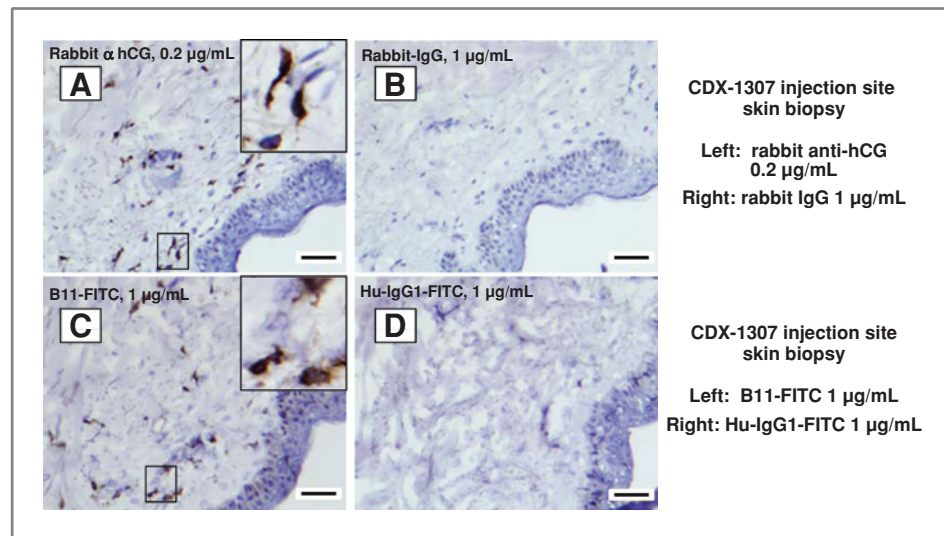
Treatment cohort	Study CDX1307-01 local delivery (n = 57) ^a	Clinical outcome for stable disease (mo)	Study CDX1307-02 intravenous delivery (n = 30) ^a	Clinical outcome for stable disease (mo)
CDX-1307 as single agent	0.3 mg i.d. (n = 6)	1 (3.5)	1 mg i.v. (n = 4)	1 (2.3+)
	1.0 mg i.d. (n = 6)		3 mg i.v. (n = 4)	
	2.5 mg i.d. (n = 6)		10 mg i.v. (n = 3)	
CDX-1307 + GM-CSF	2.5 mg i.d. (n = 7)		30 mg i.v. (n = 5)	1 (6.4)
			10 mg i.v. (n = 5)	1 (5.3)
			30 mg i.v. (n = 3)	2 (5.2, 5.2)
CDX-1307 + GM-CSF + poly-ICLC	2.5 mg i.d. (n = 6)	1 (18.2)	30 mg i.v. (n = 6) ^b	
CDX-1307 + GM-CSF + resiquimod	2.5 mg i.d. (n = 7) ^b			
CDX-1307 + GM-CSF + poly-ICLC + resiquimod	2.5 mg i.c. (n = 10)		1 (8.8)	
	15.0 mg i.c. (n = 9)			

^aData represent CDX-1307 dose levels and administration routes evaluated in each treatment cohort (n).

^bTwo additional patients, one in each of these cohorts, were enrolled and received GM-CSF but did not receive CDX-1307. These patients are therefore excluded from this table.

Abbreviations: i.d., intradermal injection; i.c., intracutaneous (a combination of intradermal and subcutaneous) injection; i.v., infusion.

Figure 2. Accumulation of hCG- β in dermal APCs of a patient treated with 1 mg of CDX-1307. Skin biopsy samples were obtained near the injection site 48 hours after intradermal injection of CDX-1307 for analysis of hCG- β . Samples were stained with anti-hCG- β or control rabbit IgG (A and B). Sequential sections were stained for MR using B11-FITC or isotype-FITC control and then detected using a rabbit anti-FITC probe (C and D). The black scale bar = 50 μ m.



discontinued treatment prematurely due to rapid disease progression, whereas 3 (2 treated with CDX-1307 alone and 1 treated with CDX-1307 at 30 mg plus GM-CSF) received a second course of treatment due to stable disease.

Toxicity

No DLTs, grade 4/5 treatment-related events, or treatment-related deaths occurred during the studies. Injection site reactions (erythema, pain, pruritus, induration) occurred in 70% of patients receiving CDX-1307 locally and were more frequent and severe (but still mild to moderate) for the groups receiving adjuvants (Supplementary Table S1). In the intravenous study, pain and erythema at the GM-CSF and poly-ICLC injection sites were also frequent. In the intradermal study, additional treatment-related toxicities included fatigue (39%), pyrexia (25%), chills (16%), pain (11%), and diarrhea (11%), whereas fatigue (20%), influenza-like illness (20%), bone pain (13%), and myalgia (13%) occurred in the intravenous study. The majority of treatment-related adverse events were mild to moderate in severity. However, 5 patients experienced grade 3 events that were assessed with a potential relationship to study treatment. In the intradermal study, these events included (i) weakness (2.5 mg CDX-1307 + GM-CSF + poly-ICLC), (ii) fatigue, disequilibrium, hyponatremia (2.5 mg CDX-1307 + GM-CSF + resiquimod), (iii) leukocytosis (2.5 mg CDX-1307 + GM-CSF + resiquimod), and (iv) 1 serious adverse event of generalized allergic reaction with fatigue (1 mg CDX-1307), whereas in the intravenous study, 1 patient experienced grade 3 abdominal pain (30 mg CDX-1307 + GM-CSF + poly-ICLC).

Pharmacokinetics of CDX-1307

The local administration of up to 15 mg of CDX-1307 did not result in detectable circulating levels. In the intravenous study, significant circulating levels of CDX-1307 were observed only at the intravenous doses of 10 and 30 mg. At 30 mg, a level of 0.84 ± 0.75 μ g/mL (mean \pm

SD) was observed 2 hours after dosing and rapidly cleared with no detectable CDX-1307 in circulation at 24 hours. Among the patients with detectable serum levels of CDX-1307, there was no correlation between serum levels of the vaccine and immune responses to hCG- β .

Accumulation of CDX-1307 in dermal APCs

We obtained skin biopsies from a patient at the injection site and a distal site (opposite arm) 48 hours after receiving 1 mg CDX-1307. As shown in Figure 2, hCG- β accumulates in MR-expressing cells (C) with morphology consistent with dermal DC and macrophages (A). hCG- β staining was not observed in the distant site biopsy (data not shown) suggesting that systemic levels of CDX-1307 were not high enough to bind to APCs at other sites.

Induction of humoral immunity to hCG- β

We analyzed the humoral responses to purified hCG- β by ELISA for each patient who received at least 3 doses of vaccine. Although soluble hCG- β , and pre-existing antibodies to hCG- β could possibly interfere with the assessment of the hCG- β immune response, anti-hCG- β antibodies were detectable in 8 of 27 (30%) evaluated patients who received CDX-1307 intravenously. There was a relatively low response in patients who received CDX-1307 alone (4 of 14; mean titer = 1:125), or in combination with GM-CSF (1 of 7; titer = 1:200). In the cohort that received CDX-1307 and poly-ICLC, there is a trend toward higher response (3 of 6; mean titer = 1:1,133) compared with CDX-1307 alone.

In patients receiving CDX-1307 intradermally, anti-hCG- β antibodies were shown in 23 of 49 (47%) evaluable patients but none developed antibodies to hCG- β following CDX-1307 treatment alone. The highest rate of antibody response and geometric mean titer occurred with the combination of CDX-1307 (either 2.5 or 15 mg) with GM-CSF, poly-ICLC, and resiquimod ($P = 0.032$ compared with CDX-1307 + GM-CSF; Fig. 3A). The antibodies were

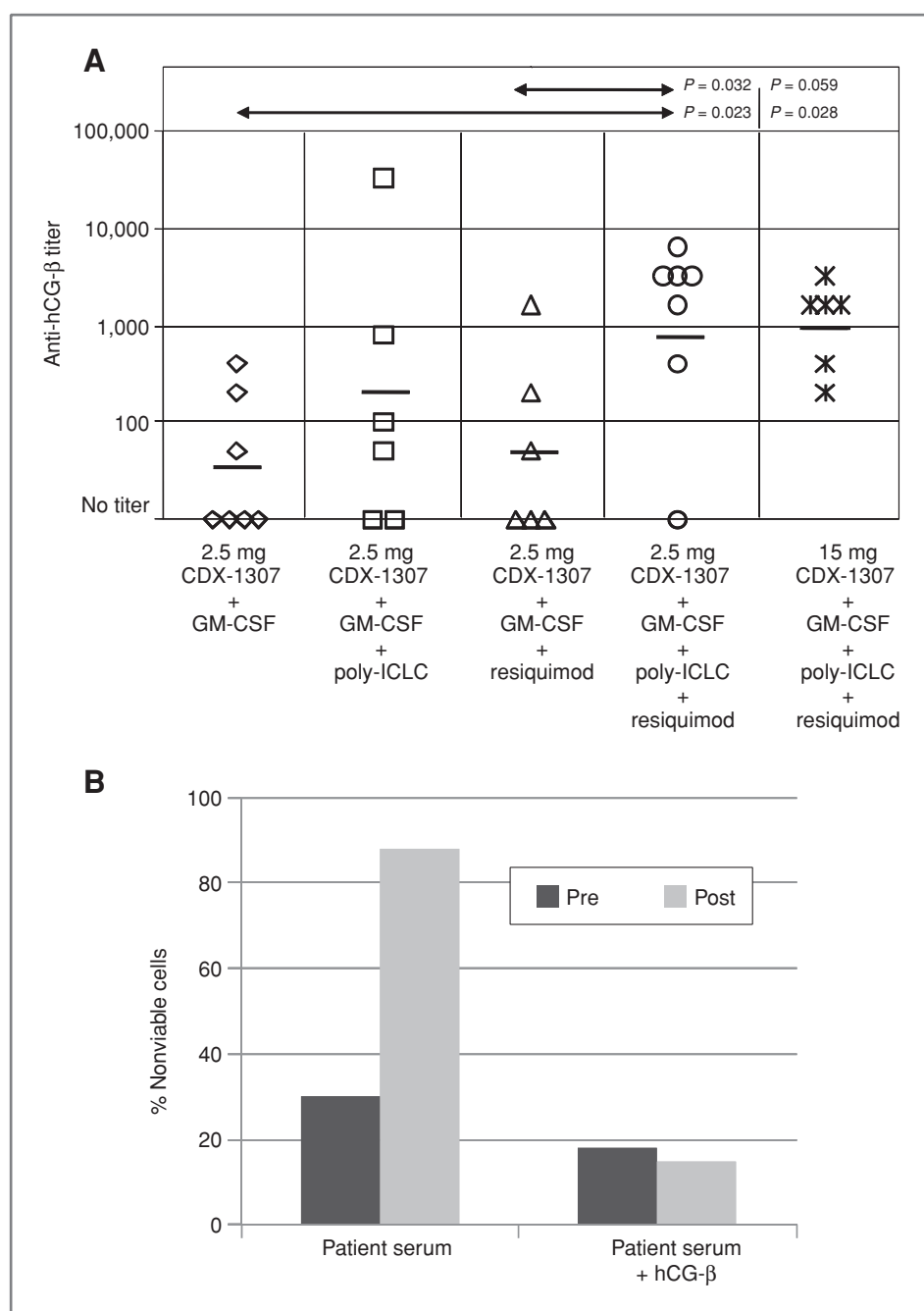


Figure 3. Induction of hCG- β -specific antibodies by intradermal CDX-1307. A, anti-hCG- β antibody responses were measured by ELISA as described in Materials and Methods. Initial cohorts with CDX-1307 alone did not have antibody responses above baseline. Values shown are the maximum titer achieved in each patient and the line represents the geometric mean reciprocal titer for patients in that cohort. *P* values are shown for analysis of the combination cohorts against GM-CSF alone or GM-CSF + resiquimod. There was no significant difference between the combination TLR cohorts and the cohort with GM-CSF and poly-ICLC. B, data from flow population analysis of SCaBER bladder cancer cells subjected to 72-hour incubation with serum from patient sample 5028 at 1:50 dilution under standard cell culture conditions with and without addition of 1 mg/mL hCG- β . All nonviable cell populations (propidium iodide positive) are indicated after incubation with pre- and post-CDX-1307 (following 3 doses).

primarily of IgG1 and IgM isotypes (data not shown). One patient (5028) receiving CDX-1307 (2.5 mg) with GM-CSF and poly-ICLC developed markedly high titer anti-hCG- β (1:32,000).

To evaluate the functional activity of the anti-hCG- β antibodies, serum from patient 5028 was incubated with SCaBER, a bladder cancer cell line expressing hCG- β and which has previously been shown to respond to anti hCG- β antibodies (15). Incubation of SCaBER cells with a 1:50 dilution of the postvaccination serum over 72 hours in culture gave rise to a significant shift in cell viability and

resulted in an increase in percentage of nonviable cells from 30% to 88% in cells cultured with the pre- and postdose serum, respectively (Fig. 3B). Furthermore, this drop in cell viability could be completely ablated by the addition of 1 μ g/mL of hCG- β showing the specificity of the response.

Induction of cellular immunity to hCG- β

Similarly to the antibody responses, significant hCG- β -specific T-cell responses were not observed in patients who received CDX-1307 alone but were seen when CDX-1307 was delivered in combination with a TLR

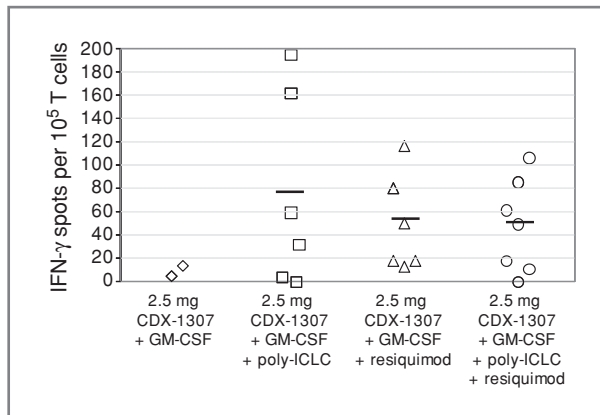


Figure 4. Induction of hCG- β -specific T-cell responses by ELISPOT in patients treated with intradermal CDX-1307 in combination with adjuvants. IFN- γ ELISPOT was used to measure T cells responding to hCG- β overlapping peptides after restimulation *in vitro* as described in Materials and Methods. Initial cohorts with CDX-1307 alone did not have T-cell responses above baseline and are not depicted. Values shown are the maximum T-cell response achieved in each patients and the line represents the mean for patients in that cohort.

agonist. T-cell responses from the cohorts that received TLR agonist in combination with the CDX-1307 vaccination is shown in Figure 4. The results did not show significant differences in the percentage of T-cell responders with the combination of TLR agonists compared with single TLRs. This is partly confounded by the variability in the quality of the processed immune cells between patients. Nevertheless, after *in vitro* restimulation, some patients showed a significant percentage of hCG- β -specific T cells at a frequency of approximately 1:300 to 1:3,000 total T cells.

Clinical activity

Nine patients had stable disease for 2.3 to 18.2 months, and there was a mixed response seen in a patient with pancreatic cancer. There were reductions in tumor markers [α -fetoprotein (AFP) and carcinoembryonic antigen (CEA)] correlating with treatment, with 1 patient showing a clear decrease in detectable hCG- β at a time when the anti hCG- β titer was the greatest. Interestingly, the 2 patients with the best outcome (stable disease for 8.8 and 18.2 months) both had evidence of humoral and T-cell immunity generated by the vaccine.

Discussion

Although autologous APC-based immunotherapy has shown clinical utility (1), the requirements for *ex vivo* antigen loading and APC activation prior to administration of an autologous cellular product may limit the widespread use of this approach. Herein, we showed that a novel strategy for *in vivo* delivery of an otherwise poorly immunogenic, protein self-antigen (hCG- β) to resident APCs via mannose receptors could lead to enhanced antibody and T-cell responses to the antigen when delivered in the presence of GM-CSF and TLR3 and TLR7/8

agonists. We also wished to gain experience with this first-in-man strategy using 2 different modes of delivery, intradermal and intravenous. An important aspect of this study was the inclusion of patients with and without detectable circulating levels of hCG- β as a result of their tumor burden. Elevated serum hCG- β was observed at study entry in approximately half of the patients, a scenario in which there could be chronic exposure of APCs to the tumor antigen, potentially leading to tolerance. Because postmenopausal women may occasionally have elevated hCG not all instances of elevated serum hCG- β were necessarily tumor derived; however, elevations in men and nonmenopausal women suggest that the majority of the elevations were tumor related. In contrast to the circulating hCG- β , CDX-1307 was designed to allow uptake of the antigen by APCs in a fashion that would result in intracellular processing and presentation in both class I and II pathways. Consequently, we found that even in patients with elevated serum levels of hCG- β , hCG- β -specific immune responses could be induced by CDX-1307.

Using a method previously described with anti-hCG- β mouse serum (29), we showed the growth-inhibiting effects of anti-hCG- β antibodies elicited by CDX-1307 on an hCG- β -secreting bladder cancer cell line. This effect was shown to be specific, as addition of hCG- β eliminated the decrease in cell viability following incubation with the CDX-1307-treated patient serum sample. Thus, vaccination with CDX-1307 may have the potential to inhibit the growth-promoting effects of hCG- β on tumor cell populations *in vivo*.

We also sought to investigate the role of DC maturation and activation facilitated by GM-CSF and TLR3 and TLR7/8 agonists. GM-CSF has been shown to activate DC *in vitro* and has been widely used as an adjuvant for a number of vaccines (21). TLR3 agonists such as poly-ICLC are potent activators of DC maturation which would be important for induction of adaptive immunity (30). In addition to TLR3 activation, poly-ICLC has been shown to exert its effects via MDA5 in mice (31), a receptor shown to mediate type I IFN secretion in response to poly-I:C. TLR7/8 agonists are also relevant for activating adaptive immune responses and have been used in anticancer strategies (32) and may also have effects on functions of the humoral immune system (33). We chose the combination of TLR3 and TLR7/8 agonists based on reported synergies and our preclinical data. Specifically, stimulation of monocyte-derived DCs with resiquimod together with poly-I:C lead to synergistic expression of IFN- β and IFN- λ 1 as well as IL-6, IL-10, IL-12, and TNF- α (26).

To minimize systemic toxicity and to directly address the impact on resident DCs, we delivered GM-CSF and the TLR3 and TLR7/8 agonists locally, alone and in combination, providing both antigen delivery and costimulation to resident APCs. We showed immunogenicity of CDX-1307 that was most apparent with administration of GM-CSF and the combined TLR agonists. The impact of this additional stimulation on the generation of antigen-specific T-cell responses was profound, as at least 1 TLR agonist was required to detect T-cell responses against hCG- β . The

induction of these T-cell responses confirms that MR targeting results in efficient antigen delivery.

This study is the first to directly show the relative contributions of antigen delivery to APC, and antigen delivery plus costimulatory signals in cancer patients. The utility of TAA loading and DC activation *in vivo* has considerable clinical potential for cancer patients but may also be broadly applicable to other applications in which chronic antigen exposure has limited immunotherapy strategies in the past. Ongoing studies are now testing CDX-1307 along with TLR agonists and GM-CSF as neoadjuvant therapy for patients with hCG- β -expressing muscle invasive bladder cancer.

Disclosure of Potential Conflicts of Interest

The authors T. Keler, J. Green, R. Riggs, L.-Z. He, V. Ramakrishna, and L. Vitale declare competing financial interests. T. Keler, J. Green, R. Riggs, L.-Z.

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He, V. Ramakrishna, T. Davis, and L. Vitale are employed by and/or stockholders of Celldex Therapeutics, Inc.

Acknowledgment

The authors thank Xi-Tao Wang for his contribution to the immunohistochemistry work.

Grant Support

This study was supported by NCI-Avon foundation supplement to Duke Comprehensive Cancer Center (3 P30 CA014236-30S4) and Celldex Therapeutics, Inc.

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Received April 7, 2011; revised May 24, 2011; accepted May 26, 2011; published OnlineFirst June 1, 2011.

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