Editor's Summary

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Dendritic cells are the matchmakers of the immune system: They introduce T cells to antigen, providing the right context for the T cell to react. However, tumor alters the nearby microenvironment in such a way as to block immune activation. Dhodapkar et al. attempt to overcome this inhibition by targeting a tumor antigen directly to dendritic cells.

The authors tested a vaccine that consisted of a human antibody targeted to the dendritic cell receptor DEC-205 fused with the tumor antigen NY-ESO-1 in a cohort of patients with tumors refractory to other therapies. They also added Toll-like receptor ligands as adjuvants in a dose-escalating study. They found that treatment induced both humoral and cellular immunity in these patients, with no dose-limiting toxicities. What's more, a subset of patients had either stable disease or disease regression, particularly those who had received immune checkpoint inhibitors. If these data can be reproduced in larger trials, this study suggests that targeting antigen to dendritic cells could be an additional avenue to boost the immune response to cancer.

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Immune-based therapies for cancer are generating substantial interest because of the success of immune checkpoint inhibitors. This study aimed to enhance antitumor immunity by exploiting the capacity of dendritic cells (DCs) to initiate T cell immunity by efficient uptake and presentation of endocytosed material. Delivery of tumor-associated antigens to DCs using receptor-specific monoclonal antibodies (mAbs) in the presence of DC-activating agents elicits robust antigen-specific immune responses in preclinical models. DEC-205 (CD205), a molecule expressed on DCs, has been extensively studied for its role in antigen processing and presentation. CDX-1401 is a vaccine composed of a human mAb specific for DEC-205 fused to the full-length tumor antigen NY-ESO-1. This phase 1 trial assessed the safety, immunogenicity, and clinical activity of escalating doses of CDX-1401 with the Toll-like receptor (TLR) agonists resiquimod (TLR7/8) and Hiltonol (poly-ICLC, TLR3) in 45 patients with advanced malignancies refractory to available therapies. Treatment induced humoral and cellular immunity to NY-ESO-1 in patients with confirmed NY-ESO-1–expressing tumors across various dose levels and adjuvant combinations. No dose-limiting or grade 3 toxicities were reported. Thirteen patients experienced stabilization of disease, with a median duration of 6.7 months (range, 2.4+ to 13.4 months). Two patients had tumor regression (~20% shrinkage in target lesions). Six of eight patients who received immune-checkpoint inhibitors within 3 months after CDX-1401 administration had objective tumor regression. This first-in-human study of a protein vaccine targeting DCs demonstrates its feasibility, safety, and biological activity and provides rationale for combination immunotherapy strategies including immune checkpoint blockade.

INTRODUCTION

Generation of both humoral and cellular immune responses is a desired goal of protective vaccines against cancer and certain pathogens. Although protein-based vaccines have several potential advantages in terms of ease of manufacture and the presence of multiple antigenic epitopes, they have suffered in the past from weak immunogenicity, inefficient uptake by antigen-presenting cells (APCs) for presentation to T and B cells, and lack of targeting to appropriate APCs. DCs are highly specialized APCs that play a central role in initiating and regulating immunity. Pioneering preclinical studies from Steinman and Nussenzweig demonstrated that antibody-mediated targeting of proteins to surface receptors on DCs leads to a marked increase in immunogenicity of protein antigens in vivo (1–3). One of the first DC receptors targeted via this approach is deca-lectin, DEC-205 (CD205), which is highly expressed by both human and murine DCs and can mediate antigen uptake, processing, and presentation (4). Targeting antigens to DEC-205–expressing DCs in steady state has been shown to induce tolerance in mice (5, 6). However, this can be overcome by coadministration of an adjuvant providing DC activation, leading to induction of immune responses (7, 8).

Ligands for Toll-like receptors (TLRs) are attractive adjuvants because they not only promote maturation of DCs to an immunogenic state but also lead to release of cytokines and chemokines to create an inflammatory milieu (9). Differences between specific TLR ligands may relate not only to the pattern of TLR expression in DC subsets and other immune cells (10, 11) but also to MyD88-dependent (TLR8) or MyD88-independent (TLR3) downstream signaling aided by their intracellular distribution in the early (TLR3) and late (TLR7 and TLR8) endosomes (12).

Cancer cells express several antigens that can be targeted by the immune system. The cancer-testis antigen NY-ESO-1 is expressed in diverse cancer types but is not detected in nonmalignant tissues with the exceptions of germ cells and trophoblasts (13). Although the function of NY-ESO-1 is unknown, it has been speculated that its expression by cancers might reflect acquisition of properties that cancers find useful, such as immortality, self-renewal, migratory ability, and capacity to invade (14). Spontaneous immunity to NY-ESO-1, consisting of both cellular and humoral responses, is often detected in patients with advanced cancers, attesting to the immunogenicity of the molecule (15–17). These characteristics make NY-ESO-1 an attractive target for cancer vaccine development, and the antigen has been extensively studied in many clinical trials. NY-ESO-1 has been confirmed to be immunogenic in melanoma (13), and its expression in many cancers suggests its relevance in the generation of humoral and cellular responses (18–21). With regard to NY-ESO-1–specific therapy alone, much progress is reflected in the art of protein-based...
therapeutics that have focused on delivery using lipid matrices (22, 23), harnessing innate and adaptive immunity with nonspecific bacterial products like Coley’s toxin (24) or specific TLR-targeted agonists (25, 26), novel NY-ESO-1–derived promiscuous human leukocyte antigen–DR (HLA-DR) and HLA-DP epitopes (27), and antigen-specific and engineered T cell receptors for adoptive immunotherapy (28).

CDX-1401 is a fully human anti–DEC-205 monoclonal antibody (mAb) (3G9) genetically fused to the full-length NY-ESO-1 tumor antigen. In preclinical studies, NY-ESO-1 was more efficiently cross-presented to T cells when fused to 3G9 than NY-ESO-1 protein alone (29). In a human DEC-205 transgenic mouse model, the administration of 3G9-HIV-Gag together with poly-ICLC (polyinosinic-polycytidylic acid complexed with poly-L-lysine and carboxymethylcellulose) and anti-CD40 antibody led to robust anti-Gag cellular and humoral immunity (30). The combination of a DC-targeted vaccine, CDX-1401, with resiquimod (TLR7/8 agonist) and Hiltonol (poly-ICLC; TLR3 agonist) was shown to enhance antigen–specific T cell immunity in nonclinical studies (9, 31). Together, these data suggest that the DEC-205–targeted protein vaccines, in combination with TLR agonists, may be an effective platform for testing the induction of immunity against pathogens as well as cancers.

### RESULTS

**NY-ESO-1 expression screening**

Immunohistochemistry (IHC) and polymerase chain reaction (PCR) testing of archived tumor tissue for 280 screened patients showed that melanoma and synovial sarcoma frequently expressed NY-ESO-1, and results were highly concordant between the two assays (Table 1). However, colorectal, ovarian, breast, and bladder cancers were rarely strongly positive and showed poor correlation between the assays for the target antigen based on the relatively small number of samples tested.

**Patient enrollment and disposition**

A total of 45 patients were enrolled and treated on study. Figure 1 illustrates the allocation of patients to each treatment cohort. In cohorts 1 to 3, 23 patients received escalating doses of CDX-1401 (0.1, 1, or 3 mg) in combination with topical resiquimod. A dose of 1 mg was subsequently chosen for cohorts 4 to 6, in which 23 patients received CDX-1401 with subcutaneously administered adjuvant, either poly-ICLC, resiquimod, or both.

**Table 1. NY-ESO-1 tissue analysis.** NY-ESO-1 analysis was performed on all archived paraffin-fixed tumor tissues submitted for screening. N/A, not applicable.

<table>
<thead>
<tr>
<th>n</th>
<th>IHC, n (% positive)*</th>
<th>PCR, n (% positive)†</th>
<th>Concordance, n (% positive)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;5%</td>
<td>&gt;30%</td>
<td>&gt;5%</td>
</tr>
<tr>
<td>All</td>
<td>280</td>
<td>69 (25)</td>
<td>44 (16)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>55</td>
<td>17 (31)</td>
<td>11 (20)</td>
</tr>
<tr>
<td>Colorectal</td>
<td>41</td>
<td>12 (29)</td>
<td>7 (17)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>41</td>
<td>16 (39)</td>
<td>15 (37)</td>
</tr>
<tr>
<td>Synovial</td>
<td>14</td>
<td>9 (64)</td>
<td>9 (64)</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>6</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Not specified/other</td>
<td>21</td>
<td>7 (33)</td>
<td>6 (29)</td>
</tr>
<tr>
<td>Lung</td>
<td>38</td>
<td>8 (21)</td>
<td>7 (18)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>23</td>
<td>5 (22)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Bladder/urothelial</td>
<td>13</td>
<td>3 (23)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>Breast</td>
<td>13</td>
<td>1 (8)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>Other</td>
<td>56</td>
<td>7 (13)</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

*Results are shown for NY-ESO-1 expression either at >5% of cells with ≥1+ intensity (cutoff used for study eligibility) or at >30% of cells with ≥1+ intensity. Threshold for positive was determined on the basis of negative and positive control samples. Concordance between PCR and IHC positivity was determined at each IHC cutoff.

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Fig. 1. Patient allocation to treatment cohorts. i.c., intracutaneous; s.c., subcutaneous.
As shown in Table 2, nearly half of the enrolled patients had melanoma. Additional cancer types included ovarian, sarcoma, non–small cell lung, and colorectal cancers. Distant metastases were noted in 87% of the treated patients, consistent with the presence of advanced disease, as required for enrollment. Expression of the NY-ESO-1 antigen in tumor specimen was confirmed by IHC or PCR in 27 of 42 (64%) of the treated patients with available tissue. In cohorts 1 to 4, 12 of 30 (40%) had confirmed tumor NY-ESO-1 expression, whereas revised entry criteria required that all patients in cohorts 5 and 6 had confirmed tumor NY-ESO-1 expression.

Dosing, toxicity, and pharmacokinetics
Dose escalation proceeded as planned with no dose-limiting toxicities (DLTs). Of the 45 enrolled patients, 41 completed at least one cycle of CDX-1401 treatment, defined as a 6-week course of treatment with CDX-1401 and applicable adjuvant(s). Ten patients with stable disease were re-treated, with a median of 10 CDX-1401 doses (range, 6 to 20).

There were no cases of treatment discontinuation due to toxicity, and treatment-related toxicities were all of grade 1 or 2 severity. As shown in Table 3, the most frequent treatment-related adverse events were administration site reaction, fatigue, nausea, and chills.

As expected from these relatively small doses (up to 3 mg), pharmacokinetic analysis revealed no detectable levels of circulating CDX-1401 (limit of detection, 0.08 μg/ml) in samples obtained at 2, 4, or 24 hours after administration of CDX-1401.

Induction of humoral immunity to NY-ESO-1
As shown in Fig. 2, most patients (79%) had NY-ESO-1–specific immunoglobulin G (IgG) titers after vaccination, with high titers (≥1:10,000) in 52% and very high titers (≥1:100,000) in 33% of patients. Similarly, strong humoral immunity developed in each cohort and in patients with or without confirmed NY-ESO-1 expression in their tumor. Patients with NY-ESO-1+ tumors frequently (54%) had anti–NY-ESO-1 antibodies present at baseline, including 5 patients with titers above 1:100,000. Isotype analysis of patient’s samples with anti–NY-ESO-1 responses after treatment revealed a predominant IgG1 response, with several patients also showing IgG2, IgG3, and IgG4 anti–NY-ESO-1 antibodies (table S1).

Table 2. Pretreatment patient characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All patients (N = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>23 (51)</td>
</tr>
<tr>
<td>Age (years), median (range)</td>
<td>64 (38–90)</td>
</tr>
<tr>
<td>Cancer type, n (%)</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>21 (47)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>6 (13)</td>
</tr>
<tr>
<td>Sarcoma*</td>
<td>5 (11)</td>
</tr>
<tr>
<td>Non–small cell lung cancer</td>
<td>4 (9)</td>
</tr>
<tr>
<td>Colorectal</td>
<td>4 (9)</td>
</tr>
<tr>
<td>Other†</td>
<td>5 (11)</td>
</tr>
<tr>
<td>Distant metastases, n (%)</td>
<td>39 (87)</td>
</tr>
<tr>
<td>Previous anticancer treatment regimen, median (range)</td>
<td>3 (0–10)</td>
</tr>
<tr>
<td>NY-ESO-1+ tumor, n (%)</td>
<td>27 (64)†</td>
</tr>
</tbody>
</table>

* Sarcoma subtypes were leiomyosarcoma (n = 2); endometrial stromal sarcoma, adipocytic, and round cell liposarcoma. † Other tumor types included cholangiocarcinoma; carcinoid tumor of liver, anal, and bladder; and myeloma. ‡ Tumor NY-ESO-1 expression status is unknown for three patients.

Table 3. Adverse events considered related to study treatment (CDX-1401, resiquimod, and/or poly-ICLC). Table includes all adverse events reported with a possible, probable, or definite relationship to any of the study treatments, occurring in more than one patient. No grade 3, 4, or 5 events have been reported as potentially related to study treatment.

<table>
<thead>
<tr>
<th>Study Treatment</th>
<th>CDX-1401 plus topical resiquimod (cohorts 1–3; n = 23), n (%)</th>
<th>CDX-1401 plus poly-ICLC (cohort 4; n = 7), n (%)</th>
<th>CDX-1401 plus subcutaneous resiquimod (cohort 5; n = 7), n (%)</th>
<th>CDX-1401 plus poly-ICLC and subcutaneous resiquimod (cohort 6; n = 8), n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administration site reaction</td>
<td>18 (78)</td>
<td>5 (71)</td>
<td>5 (71)</td>
<td>7 (88)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>5 (22)</td>
<td>3 (43)</td>
<td>2 (29)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Nausea</td>
<td>2 (9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Chills</td>
<td>1 (4)</td>
<td>1 (14)</td>
<td>0 (0)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Influenza-like illness</td>
<td>2 (9)</td>
<td>1 (14)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>3 (13)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>2 (9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (29)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>1 (4)</td>
<td>1 (14)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hemoglobin decreased</td>
<td>2 (9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>1 (4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (13)</td>
</tr>
</tbody>
</table>
### Induction of cellular immunity to NY-ESO-1

Cellular immunity to NY-ESO-1 was determined by interferon-γ (IFN-γ) enzyme-linked immunospot (ELISPot) on purified blood mononuclear cells from patient samples collected before and after CDX-1401 administration. Patient samples were qualified by demonstrating responsiveness to CD3 stimulation and to a pool of major histocompatibility complex class I peptides derived from human viruses (fig. S1). We detected NY-ESO-1–specific T cell responses in 56% patients with evaluable pre- and posttreatment samples (Fig. 3A). As seen with humoral immunity, significant NY-ESO-1–specific T cell responses were observed in most cohorts, suggesting that even low doses of CDX-1401 were immunogenic and both poly-IICLC and resiquimod can provide appropriate immune activation to drive T and B cell responses. Durability of the T cell response was demonstrated in two patients from whom samples from additional cycles of CDX-1401 treatment were available. In these patients, the induction of NY-ESO-1–specific T cells was maintained through three cycles (about 7 months) of treatment (Fig. 3B).

In a few patients with sufficient lymphocytes, it was possible to further characterize NY-ESO-1 T cell responses by intracellular cytokine and HLA pentamer staining. Figure 4A shows a patient sample with an NY-ESO-1–specific response by both CD4+ and CD8+ cells as demonstrated by intracellular cytokine staining for IFN-γ and TNF-α (tumor necrosis factor–α). Of the five patients tested, three had both CD8+ and CD4+ NY-ESO-1 T cell responses, whereas two patients only showed a dominant CD4+ response (Fig. 4B).

Peripheral blood mononuclear cells (PBMCs) from HLA-A2.1+ patients were stained directly (that is, no in vitro stimulations) with an NY-ESO-1 peptide–specific pentamer or irrelevant control pentamer. Of the 16 patients stained with the pentamers, only 3 showed NY-ESO-1 pentamer–positive CD8 T cells that increased in numbers after vaccination (fig. S2), suggesting a low abundance of circulating T cells reactive to the well-known immunodominant epitope, NY-ESO-157–165, and possible reactivity against epitopes presented by other HLA class I/II alleles.

### Clinical activity

Stable disease was noted for 13 patients, distributed among treatment cohorts (Fig. 2). Seven of these patients had melanoma, two had colorectal cancer, and the remaining four had myeloma, bladder cancer, cholangiocarcinoma, and non–small cell lung cancer. Median duration of stable disease was 6.7 months (range, 2.4+ to 13.4 months). Remarkably, two of the melanoma patients were also observed to have some tumor shrinkage after one cycle of CDX-1401 treatment, with both patients having about 20% shrinkage in the sum of the diameters of target lesions. One patient with regression of a tonsil lesion subsequently showed clinical progression after additional treatment with CDX-1401, but was still alive at the 2-year follow-up. The second patient had regression in a lung lesion and received a second cycle of treatment before progression indicated by the appearance of a new lesion.

The detection of NY-ESO-1 expression in tumor tissue did not appear to correlate with patient outcome. Stable disease was seen in 7 of 27 (26%) of the patients with NY-ESO-1 expression, and in 5 of 15 (33%) of those lacking NY-ESO-1 expression. Note that these tested tissue samples represented archival specimens obtained at a median of 17.5 months (range, 2.3 months to 10.2 years) before study entry. Similarly, the rate of disease stabilization was similar for most patients who developed or maintained NY-ESO-1–specific humoral response (10 of 34, 29%) and for the few patients who did not (3 of 8, 38%).

However, the proportion of patients with stable disease was higher for those who maintained or developed NY-ESO-1–specific T cell responses (Fig. 3). Stable disease was seen in three of six (50%) patients who entered the study with preexisting cellular immunity to NY-ESO-1, and all three had increased responses while on study. For the remaining 13 patients who developed cellular immunity while on treatment, 6 (46%) experienced stable disease. In contrast, stable disease was seen in only 2 (13%) of the 15 patients who did not develop cellular immunity to NY-ESO-1. Four of six (67%) patients who displayed the strongest responses (>50 IFN-γ spots per 2 × 10⁶ PBMCs) also experienced stable disease. The association of cellular response and stable disease does not appear to be a consequence of extended duration of therapy. Peak responses were observed in the first treatment cycle for seven of the nine patients with stable disease who developed cellular immunity.
Eleven patients completed study follow-up at 2 years, whereas one patient remained in follow-up. During study follow-up, six melanoma patients went on to receive anti–CTLA-4 mAb within 3 months of the last CDX-1401 dose, and four of these patients were reported to experience a partial response (PR) or complete response (CR) by the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1) (32) or irResponse (33) criteria (table S2). In addition, two non–small cell lung cancer patients who received investigational checkpoint blockade within 2 months of discontinuing CDX-1401 were also reported to experience PR. All six of these responding patients had tumors confirmed to express NY-ESO-1. Five also developed or maintained NY-ESO-1–specific immunity in most patients including those with tumors that tested negative for this antigen.

The magnitude of the humoral response was particularly impressive, with a third of the patients achieving titers greater than 1:100,000. NY-ESO-1–specific T cells were observed in more than half of the patients using an in vitro restimulation assay. We demonstrated evidence of both CD8 and CD4 responses, suggesting that this protein-based vaccination approach can generate a broad spectrum of immunity in advanced cancer patients.

Overall, we observed similar induction of NY-ESO-1 immune responses using various dosing regimens. No clear dose response of CDX-1401 was observed, which is consistent with preclinical studies of DEC-205 vaccines. In addition, no particular advantage of the subcutaneous formulation of resiquimod or the combination of resiquimod and poly-ICLC was noted compared to topical resiquimod or poly-ICLC as single adjuvants. However, given the small number of patients and the variability of responses among patients in each cohort, further confirmatory studies would be required. On the basis of the preclinical data supporting poly-ICLC as the most potent immune activator for combination with DEC-205, further development would be required. Our study demonstrates that treatment with CDX-1401, in combination with TLR ligands (poly-ICLC or resiquimod), provides a well-tolerated and practical approach that results in integrated humoral and cellular NY-ESO-1–specific immunity in most patients including those with tumors that tested negative for this antigen.
Despite the induction of measurable T cell responses, tumor regression was not observed in most patients. This suggests the need to consider earlier-stage patients or to combine this approach with strategies that overcome the immunosuppressive effects in the tumor bed, including combination with new antibodies that block T cell inhibitory checkpoints such as anti-PD1 or anti-PDL1. Follow-up of patients who went on to receive such therapies indicates an overall response rate of 6 of 8 (75%) for patients receiving checkpoint inhibitors after CDX-1401 treatment. Of those patients with melanoma who went on to receive anti–CTLA-4 mAb, four of six (67%) had responses, which is potentially interesting when compared to the overall response rate of 11% previously reported in metastatic melanoma patients treated with single-agent anti–CTLA-4 mAb (36).

In recent years, a variety of immunotherapeutic approaches have been tested for the induction of humoral and cellular immune responses to NY-ESO-1. In particular, studies using recombinant vaccinia and fowlpox vectors expressing NY-ESO-1 antigen (37) and synthetic long peptides derived from NY-ESO-1 (38, 39) have helped document the ability of cancer patients to develop antibodies as well as CD4 and CD8 T cell responses to NY-ESO-1. A recent study has also demonstrated that some vaccines may inadvertently lead to increases in NY-ESO-1–specific regulatory T cells that could limit clinical benefit (40). Differences in patient populations and immune monitoring preclude direct comparisons between these studies. Vaccines against NY-ESO-1 may, in principle, also lead to development of immune responses against other antigens because of epitope spreading. Further studies are needed to better evaluate the possibility of epitope spreading with this vaccine (41).

This specific in vivo targeting of DCs using CDX-1401 has advantages over alternate technologies. The collection of autologous DCs with ex vivo manipulation has shown efficacy as evidenced by the survival advantage shown with sipuleucel-T (42); however, the practical complexity of tumor NY-ESO-1 expression (>5% positive cells) by PCR and/or IHC at a central laboratory was required for cohorts 5 and 6. For cohorts 1 to 4, this analysis was performed retrospectively. Exclusionary conditions included Eastern Cooperative Oncology Group (ECOG) performance status >1, active central nervous system metastases or potential alternate malignancy, autoimmune disease, and concurrent treatment with immunosuppressive or immunomodulatory agents. Before the conduct of any protocol-specific procedures, all patients signed written informed consent after the nature and possible consequences of the studies were explained.

Study design and procedures

This phase 1 study was designed to examine the safety and tolerability profile of CDX-1401 in combination with the TLR agonists, resiquimod and/or poly-ICLC, in patients with malignancies known to express NY-ESO-1. All patients who received at least one dose of study drug were included in safety analyses. Additional objectives of this study included an evaluation of CDX-1401–induced immune responses, antitumor activity, and pharmacokinetics.

The study consisted of six sequentially enrolled (nonrandomized) open-label treatment cohorts (Fig. 1). An initial dose-escalation phase (cohorts 1 to 3; CDX-1401 in combination with topical resiquimod) was conducted to select a CDX-1401 dose level of interest for further study. Subsequent cohorts evaluated CDX-1401 in combination with poly-ICLC, escalating doses of parenteral resiquimod, or both to assess an optimal adjuvant regimen.

Safety parameters assessed at baseline and study visits included physical examination, vital signs, ECOG performance status, hematology, blood chemistry, urinalysis, thyroid function, and the incidence and severity of adverse events. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse
amino-2-ethoxymethyl-resiquimod (3M Pharmaceuticals; also known as R-848; S-28463; 4-
treatment period (Fig. 1). Dose levels were 0.1, 1.0, and 3.0 mg. Topical
given biweekly for a total of four administration sessions over the ~6-week
stimulation system and DAB as chromagen and hematoxylin as counter-
stain. Tumors were manually evaluated on the basis of the percentage of
detection system and DAB as chromagen and hematoxylin as counter-
immediate after CDX-1401 vaccination and again about 24 hours later.
Each topical resiquimod application was to remain on the treatment
site for about 8 hours, unless local toxicity warrants premature removal.
Resiquimod for injection (0.02 to 0.5 mg in a 0.25-ml volume) was
administered as a subcutaneous injection after CDX-1401 vaccina-
6.7.4-quinoline-1-
carbazole (250 mg of 0.2% gel) immediately after CDX-1401 vaccination and again about 24 hours later.

Pharmacokinetics
Serum samples for assessment of circulating CDX-1401 were obtained
before vaccination and at 2, 4, and 24 hours after the first vaccination,
included measures of cellular and humoral response to NY-ESO-1. Standard
disease-specific tumor evaluation was performed according to RECIST 1.1
within 28 days before each re-treatment cycle and at a minimum frequency of
12 weeks. Patients were followed for up to 2 years for assessment of safety,
response to subsequent therapy, and survival.

Study drugs and dosing
In all cohorts, a treatment cycle was defined as a 6-week course of treat-
ment with CDX-1401 and applicable adjuvant(s), followed by a 6-week rest. Additional 12-week cycles were allowed for patients who
continued to meet all eligibility criteria and who had not experienced DLT/
tolerance or progression of disease.
CDX-1401 (Cellnex Therapeutics) was administered via intra-
cutaneous injection (a combination of intradermal and subcutaneous injection, with about half of the planned dose administered by each route)
within a 5 × 5–cm area on the extremities or the abdomen. CDX-1401 was
given biweekly for a total of four administration sessions over the ~6-week
treatment period (Fig. 1). Dose levels were 0.1, 1.0, and 3.0 mg. Topical resiquimod (3M Pharmaceuticals; also known as R-848; S-28463; 4-
amino-2-ethoxymethyl-α, α-dimethyl-1H-imidazo[4,5-c]quinoline-1-ethanol) was applied topically at a dose of 500 μg (250 mg of 0.2% gel) immediately after CDX-1401 vaccination and again about 24 hours later.

NY-ESO-1 expression
The IHC method was optimized, and expression of NY-ESO-1 was ver-
tified with control sections from breast cancer, melanoma, and ovarian carcinoma sections at Clarient Inc. Briefly, 4- to 5-μm cuts of standard formalin-fixed, paraffin-embedded tumor specimens were mounted on charged glass slides, deparaffinized, and washed per standard methods. After heat-induced antigen retrieval at 100°C, samples were stained for 30 min with a murine mAb specific for NY-ESO-1 (E978, Invitrogen). Known positive cell line HT-1080 fibrosarcoma and negative cell line SiHa squamous cell carcinoma were used for staining verification. Expression of NY-ESO-1 was visualized with the Leica Bond Refine-HRP detection system and DAB as chromagen and hematoxylin as counterstain. Tumors were manually evaluated on the basis of the percentage of positive cells with a standard bright-field microscope (0 to 100%, inten-
sity 0 to 3+). A sample with greater than or equal to 5% positive cells was considered positive for NY-ESO-1 protein.

For reverse transcription PCR (RT-PCR), total RNA was prepared from
10-μm-thick, formalin-fixed, paraffin-embedded tumor sections and from control tumor cell lines with the in-house methods (Clarient Inc.) Quan-
titative RT-PCR was performed with a TaqMan assay and an ABI Prism
7900 HT system (Applied Biosystems) with a TaqMan RNA-to-Ct 1-Step kit (Life Technologies). TaqMan gene expression primers and probes for NY-ESO-1 (Hs00265824_m1) and human GUSB (HS99999908_m1) were purchased from Life Technologies. As a positive control, total RNA extracted from HT-1080 cells transformed with NY-ESO-1, and a negative control from HL-60 cell line extracts, determined not to express the target, were used in every run, as well as a negative template control to test for contamination. GUSB expression was included as a sample amplification control. For inclusion in the trial CDX1401-01, any sample with an NY-ESO-1 signal greater than the threshold was considered positive.

Humoral immune response analysis
The magnitude and isotype of the anti–NY-ESO-1 antibody response were determined from patient serum samples. Sample time points were
day 0 (before dosing), day 28 (2 weeks after the second CDX-1401 dose in cycle 1 only), day 42 (2 weeks after the third CDX-1401 dose), and days 70 to 77 (about 1 month after the fourth CDX-1401 dose). NY-ESO-1-specific antibodies were detected by adding serially diluted serum to ELISA plates coated with NY-ESO-1 and developed with a goat anti-human IgG (H&L) peroxidase and TMB staining. A posttreatment sample was considered positive for the presence of anti–NY-ESO-1 antibodies if the mean absorbance value was greater than the mean value of the predose sample, plus 3 SDs or +0.1, whichever was greater. The titer of the sample was defined as the reciprocal of the dilution with an absorbance value closest to a preset background of 0.1. Anti–NY-ESO-1 antibody isotypes were determined with isotype-specific HRP-conjugated goat anti-human IgG1, IgG2, IgG3, IgG4, and IgM. Analysis was carried out on posttreatment samples (1:50 dilution) from all patients with positive NY-ESO-1 titers (n = 26).

Cellular immune response analysis of NY-ESO-1 synthetic peptide library
A library of HLA class I–binding peptides derived from NY-ESO-1 protein sequence previously described (29) was used to prepare high-quality and 95% pure synthetic peptides (ProImmune). Peptides were resus-
pended in anhydrous dimethyl sulfoxide at 10 mg/ml, aliquoted, flushed with nitrogen, sealed, and stored at −40°C until use.

ELISpot assay
ELISpot assay was used to screen the patient blood samples for immune responses. For every patient in the clinical trial, pre- and post-dose blood samples were collected, and multiple post-dose, in addition to the predose, samples were tested for NY-ESO-1–specific IFN-γ secretion. In each ELISpot assay, PBMCs were first presensitized in a 7-day in vitro sens-
itization culture in the presence of low-dose IL-2 (10 ng/ml). The NY-
ESO-1 peptide pool comprised 28 overlapping peptides covering the full
length of the NY-ESO-1 protein (29). In the assay, APCs (T-depleted PBMCs) were pulsed with NY-ESO-1 peptides and negative control
peptides (WRKY47, ProImmune). Anti-CD3 mAb and CEF-1 peptide pool
served as positive controls in the assay. The CEF-1 pool contains 32 HLA class I peptides from human cytomegalovirus, Epstein-Barr virus, and influenza virus (PANATecs GmbH). ELISpot plates were washed as per kit instructions. Spot counts were evaluated with the Zeiss system

Intracellular cytokine staining

Patient PBMCs were cultured for 7 days as described above and stained for intracellular cytokines. Briefly, presensitized cells were first restimulated with T-depleted APCs pulsed with various peptides and then treated for intracellular cytokines. The cells were then analyzed by flow cytometry with FACSCanto II (BD Biosciences).

Statistical analysis

For this phase 1 trial, the statistical analysis consisted of descriptive summaries of the safety and immunologic data. Adverse events were tabulated on the basis of the standardized terms assigned by the Medical Dictionary for Regulatory Activities (MedDRA). Tumor responses were evaluated and reported in accordance with RECIST (version 1.1) (32).

REFERENCES AND NOTES

www.sciencetranslationalmedicine.org/cgi/content/full/6/232/232ra51/DC1

Fig. S1. ELISpot response to CD3 stimulation and viral antigens.

Fig. S2. Detection of antigen-specific CD8+ T cells in peripheral blood.

Table S1. Isotype analysis of anti–NY-ESO-1 antibody responses.

Table S2. Melanoma patients who received anti-CTLA-4 mAb within 3 months of discontinuing CDX-1401.

SUPPLEMENTARY MATERIALS

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Fig. S1. ELISpot response to CD3 stimulation and viral antigens.

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Table S1. Isotype analysis of anti–NY-ESO-1 antibody responses.

Table S2. Melanoma patients who received anti-CTLA-4 mAb within 3 months of discontinuing CDX-1401.


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Relevant patents and patent applications:

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<tr>
<th>Title</th>
<th>Patent/application serial number</th>
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<tr>
<td>Antibodies that bind human dendritic and epithelial cell 205 (DEC-205)</td>
<td>International Patent Application No. PCT/US2008/082745 (WO2009061996); now in national and regional phases</td>
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<tr>
<td>Antibodies that bind human dendritic and epithelial cell 205 (DEC-205)</td>
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