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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Flow Data collection: BD FACSDiva_v8; Elispot collection: ImmunoSpot® SOFTWARE_v6 Gene Expression collection: Digital Analyzer RCC files into nCounter_v4
Data analysis	Flow Data analyses: FlowJo_v9; FlowJo_v10.1; Graphpad Prism_v7.03 and _v8.3 Elispot analyses: ImmunoSpot® SOFTWARE; Graphpad Prism_v7.03 and _v8.3 ELISA analyses: MS Office Excel v2002; Graphpad Prism_v7.03 and _v8.3 Gene Expression analyses: nCounter_v4 with Advanced Analysis module 2.0

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data used in support of this manuscript are available and can be accessed at <https://ciml.labkey.com/home/project-begin.view?> by request and are available by request to the corresponding authors. Codes, raw data and analyses for gene expression analyses have been deposited at: https://github.com/patrickjdanaher/CITN07-nCounter-data-code-results.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	With 27 patients per group with evaluable data (30 patients per group, assuming 10% missing immunogenicity data), there is 87% power to detect a difference between a 75% response rate in the Flt3L arm (Cohort 1) and a 40% rate in the no Flt3L group (Cohort 2). This calculation is based on a 1-sided Fisher exact test with a significance level of 0.1; the estimated 40% response rate in the no Flt3L group is based on prior data. Citations in support of this are two clinical studies in which we used two arms, two vaccination platforms, etc: Sabado et al Can Imm Res. https://dx.doi.org/10.1158%2F2326-6066.CIR-14-0202 Pavlick et al Can Imm Res. https://dx.doi.org/10.1158%2F2326-6066.CIR-19-0545
Data exclusions	No Data were excluded
Replication	This Clinical trial was not replicated or duplicated, as the company (CellDex) producing the agents did not have the funding to proceed with additional investigations.
Randomization	Study Participants (n=30 per cohort) were enrolled randomly in at 1:1 ratio into either cohort via the OPEN system. Whole blood staining and analyses was restricted to the first n=16 subjects enrolled per cohort.
Blinding	Investigators were not blinded to group allocation (ie which cohort a subject was enrolled) as the treatment regimen for cohort 1 differed from cohort 2 in the administration of 10ds of Flt3L.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Flow Cytometry Abs:
(PBMC Panel)
Marker-Fluor: Clone: Supplier-Cat #
CD3-PE-CF594: UCHT1: BD-562280
CD4-FITC: RPA-T4: Biolegend-300506
CD8-PerCP-Cy5.5: SK1: BD-341051
CD11c-AlexaFluor700: B-ly6: BD-561352
CD14-V450: MϕP9: BD-560349
CD16-APC-H7: 3G8 BD-560195
CD19-PE-CY5: HIB19: Biolegend-302210
CD45-AmCyan: 2D1: BD-339192
CD56 -PE-CY7: NCAM16.2: BD-335791
CD122-APC: TU27 Biolegend-339008
CD123-PE: 9F5: BD-340545
HLA-DR-BV605: L243: Biolegend-307640

(DC Panel)
Marker-Fluor: Clone: Supplier-Cat #
CD3/CD19/CD14/CD20--FITC: CD3 (clonce SK7), CD19 (SJ25C1), CD20 (L27), CD14 (MϕP9): BD-643510
HLA-DR-Alexa Fluor 700: L243: Biolegend-307626
CD11c-Pacific Blue: Bu15: Biolegend-337212
CD33-PE-CF594: WM53: BD-562492
CD1c PE-Cy7: L161: Biolegend-331516
CD141-Brilliant violet 785: M80: Biolegend-344115
CLEC9A-PE: 8F9: Biolegend-353803
CD45RA-Brilliant violet 650: HI100: Biolegend-304135
AXL-APC: 108724: R&D systems-FAB154A
CD16-APC-CY7: 3G8: Biolegend-302017
CD123-BV605: 6H6: Biolegend-306026

Other Abs:
Specificity: Clone: Supplier-Cat #
IHC
anti-NYESO-1: E978: Sigma-n2038

ELISPOT and ELISA
anti-human IFNɣ: 7-B6-1: mabtech-3420-6-250
anti-human IFNγ: 1-D1K: mabtech-3420-3-250
anti-biotin alkaline phosphatase: Vector-SP-3020
goat anti-human IgG (H&L) peroxidase: Jackson ImmunoResearch Cat# 109-036-088
goat anti-mouse FLAG: Sigma F1804

PK
Anti-human-Flt3L: 40416: R&D Systems-MAB608

ICS
Anti-human IFNγ (AF700): Biolegend- # 502520
Anti-human TNFa (APC): Biolegend-# 502930
Anti-human IL2 (PE): Biolegend-# 500307

Validation

All antibodies used in this study were rated by the manufacturers as specific to human antigen and quality tested (routinely) for Flow Cytometry applications. Product Data Sheets (Biolegend) and Technical Data Sheet (BD) for each antibody indicate reactivity, production, formulation and status of QC testing (with included example of QC data on stained human PBMC samples). Each lot also includes a Certificate of Analysis from the manufacturers indicating all QC criteria were met. All antibodies and lots were titrated in validation testing prior to use.

Validation information for cytometry mAbs can be found at:
BD Biosciences:
https://regdocs.bd.com/regdocs/qcinfo?_ga=2.203764563.2130108764.1598559655-2105922012.1596742586
Biolegend:
https://www.biolegend.com/en-us/concentration-expiration-lookup

Validation information for cytometry mAbs can be found at:
anti-NYESO-1: https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Datasheet/4/n2038dat.pdf
anti-human IFNɣ: 7-B6-1: mabtech-3420-6-250: https://www.mabtech.com/sites/default/files/datasheets/3420-6-250.pdf
anti-human IFNγ: 1-D1K: mabtech-3420-3-250: https://www.mabtech.com/sites/default/files/datasheets/3420-3-250.pdf
anti-biotin alkaline phosphatase: Vector-SP-3020: https://vectorlabs.com/productpdf/download/file/id/1281/name/Goat_Anti-Biotin%252C_Alkaline_Phosphatase_Conjugated.pdf/
Anti-human-Flt3L: 40416: R&D Systems-MAB608: https://www.rndsystems.com/products/human-flt-3-ligand-flt3l-antibody-40416_mab608

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Chinese Hamster Ovary cells for rhu-Flt3L (CDX-301): The host cell line was VeggieCHO, a serum-free medium adapted CHO-DXB11 cell line; Originally from Immunex.

Authentication

Identity testing was performed on the Master Cell Bank using an isoenzyme assay, confirming CHO. MCB was tested for Identity, Mycoplasma, as well as sterility at Microbiological Associates, Inc (Rockville, MD). Reference Master File #3493 of IND.

Mycoplasma contamination

Negative for Mycoplasma as the cell line was used for production of Clinical grade GMP material. Products were fully characterized using appropriate GMP assays.

Commonly misidentified lines
(See ICLAC register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Population characteristics are listed in Table I of the manuscript and are listed here: Variable Cohort 1 (n=30) Age Mean (SD) 54.6 (13.4) Median 53 Min-Max 20-76 Gender Male 20 (66.7%) Female 10 (33.3%) Race White 30 (100%) African American 0 (0%) Not Reported 0 (0%) Stage IIB 5 (16.7%) IIC 1 (3.3%) IIIA 9 (30%) IIIB 7 (23.3%) IIIC 7 (23.3%) IV 1 (3.3%) Variable Cohort 2 (n=30) Age Mean (SD) 51.6 (14.5) Median 55 Min-Max 20-78 Gender Male 21 (70%) Female 9 (30%) Race White 28 (93.3%) African American 1 (3.3%) Not Reported 1 (3.3%) Stage IIB 4 (13.3%) IIC 2 (6.7%) IIIA 11 (36.7%) IIIB 6 (20%) IIIC 5 (16.7%) IV 2 (6.7%)
Recruitment	N/A -- A recruitment strategy was not employed for this study. Each site that participated in this study has a large Melanoma Program with many eligible patients. Therefore it was deemed unnecessary to recruit patients actively. Also, given the patient population and the need for treatment options there was no need for a recruitment strategy. The initial study and subsequent protocol expansion enrolled 60 patients over a 12 month period of time.
Ethics oversight	The protocol was approved by the Fred Hutchinson Cancer Research Center's Institutional Review Board (IRB) and participating site IRBs. The following clinical sites involved in the study utilized Fred Hutch IRB as the central IRB: Mount Sinai and Providence Portland. The following clinical sites used their own IRBs: Cleveland Clinic, Duke, NYU, U Chicago, and U Wisconsin.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT02129075
Study protocol	The study protocol ("A Phase II Randomized Study of CDX-1401, a Dendritic Cell Targeting NY-ESO-1 Vaccine, in Patients with Malignant Melanoma Pre-Treated with Recombinant CDX-301, a Recombinant Human Flt3 Ligand"NCT02129075) is available upon request from authors. The study Protocol has also been provided to NatCancer editor.
Data collection	Sixty patients with resected melanoma and no evidence of disease were enrolled at 7 clinical sites between October 2014 and October 2015. Clinical data were collected at the following participating sites: Duke University Medical Center 450 Research Drive MSRBI, Office: Room 397 Durham, NC 27708 NYU Langone Medical Center Laura and Isaac Perlmutter Cancer Center 160 East 34th Street, 9th Floor New York, NY 10016 Providence Portland Medical Center

Providence Cancer Center
Oncology and Hematology Care Clinic – Eastside
4805 NE Glisan Street, 6N40
Portland, OR 97213

University of Wisconsin
K6/530 Clinical Science Center
600 Highland Avenue
Madison, WI 53792

Tisch Cancer Institute
Icahn School of Medicine at Mount Sinai
1470 Madison Avenue
New York, NY 10029

Cleveland Clinic Foundation
Hematology and Medical Oncology
9500 Euclid Avenue
Taussig Cancer Institute, Desk R35
Cleveland, OH 44195

University of Chicago
Melanoma and Developmental Therapeutics Clinics
5841 S. Maryland Ave. MC2115
Chicago, IL 60637

Outcomes

Outcome measures were determined by members of the CITN (Cancer Immunotherapy Trials Network) Protocol Working Group including the Protocol PI, Statisticians at the Fred Hutch Cancer Research Center, and discussions with NCI-CTEP Scientific Review Committee.

Primary Outcome Measures:

Immune T-cell response to NY-ESO-1 [Time Frame: At 4 weeks after final vaccination]
Will be compared between cohorts 3 and 4. One-sided binomial exact test with significance level of 0.1 will be performed, assuming the proportion of responders of 50% as the null hypothesis. One-sided 90% confidence interval estimate will be calculated for the proportion of responders.

Immune T-cell response to NY-ESO-1 [Time Frame: At 12 weeks after final vaccination]
Response rates will be analyzed by tabulating the frequency of positive response for each assay by antigen and treatment arm at each time point for which an assessment is performed. Response rates will be presented with their corresponding 95% confidence interval estimates calculated using the score test method. Fisher's exact tests will be used to compare cohort 1 and cohort 2 at the peak time point, with a significant difference declared if the 1-sided P value is =< 0.10.

Secondary Outcome Measures:

T cell responses to other ongoing and nascent antitumor response antigens associated with melanoma (e.g. PRAME, MAGE-A3, p53, and gp1000) as well as memory and chronic viral responses (CMV, EBV) [Time Frame: Up to 12 weeks after final vaccination]
Will be considered between each pair of randomized cohorts.

Frequency and phenotypic character of PBMC subsets including DCs, monocyte populations, T cells, and NK cells [Time Frame: Up to 12 weeks after final vaccination]
Graphical and tabular summaries of the assay data will be made. Linear mixed effects model and possibly weighted generalized estimating equation methods will be considered for a supportive analysis of the longitudinal data over time.

Incidence of adverse events, graded and reported using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 [Time Frame: Up to 12 weeks after final vaccination]
Will be presented in tabular form.

Dose limiting toxicities graded and reported using the NCI CTCAE version 4.0 [Time Frame: Up to 12 weeks after final vaccination]
Will be presented in tabular form.

Time to tumor recurrence [Time Frame: Up to 12 weeks after final vaccination]
Summarized by treatment arms using Kaplan-Meier estimates.

Overall survival [Time Frame: Up to 1 year after patient's 12 week visit]
Summarized by treatment arms using Kaplan-Meier estimates.

Change in T cell receptor sequencing in blood (Cohorts 3 and 4) [Time Frame: Baseline up to 12 weeks after final vaccination]

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Serial samples of whole blood were obtained by veni-puncture and shipped (ambient) overnight for: i) immediate Whole Blood Flow cytometry studies; and ii) processing to PBMC via ficol purification and cryo-preservation for cryo-PBMC Flow Cytometry studies, as described in the methods.
Instrument	Becton Dickinson LSR II and BD Fortessa
Software	BD FACSDiva_v8
Cell population abundance	No sorting performed
Gating strategy	DC Subsets Gating steps: Lineage- Blue+ HLA-DR+ CD45RA- CD123- CD11c+ (CD11c+), gating on CD11c+ --> CD141+ Clec9a+ (DC1), gating on CD11c+ --> CD141-Clec9a- CD1c+ (DC2), gating on CD11c+ --> CD141-Clec9a- CD1c- CD16+ (DC4), gating on Lineage- Blue+ HLA-DR+ --> CD123+ CD45RA+ (DC6), gating on Lineage- Blue+ HLA-DR+ CD123+ CD45RA+ CD33+ AXL- (pre-DC) PBMC Subset Gating steps (measured populations): 1. Side scatter-A (SSC-A) x Forward scatter-A (FSC-A) to confirm normal cell distribution 2. SSC-A x CD122-APC(uncompensated) to gate on Trucount Beads 3. Singlets gate (FSC-H x FSC-A) 4. CD45+ leukocytes (excluding most neutrophils) 5. SSC-A x CD14 V450 (CD14- cells (not monocytes); CD14+ Monocytes) 6. Dendritic Cell gating (sequencial from CD14-): a. CD3-CD19- (not T cells, not B cells) b. CD56-CD15- (not NK cells) c. HLADR+ d. CD123+ (pDC), CD11c+ (cDC) e. HLADR expression in cDC: activation of DCs 7. From CD14- (not monocytes): SSC-A x FSC-A Lymphocyte gate (cleanup) 8. CD3 x CD19: 3 gates: CD3+, CD19+, CD3-CD19- 9. CD3+ T cells a. CD4+ T cells b. CD8+ T cells c. For both: CD3 x HLADR gated on HLADR++ for activation state of T cells 10. CD19+ ? HLADR x CD19: gated on HLADR+ ? B cells 11. NK cells: CD3-CD19- a. CD56 x CD16 ?NK cell subsets i. CD56+ & CD16+ NK cells ii. CD56dim CD16+ iii. CD56dim CD16- iv. CD56bright To determine different DC subsets, gating was performed as follows: 1. Lineage- Blue+ HLA-DR+ CD45RA- CD123- CD11c+ (CD11c+), gating on CD11c+ --> 2. CD141+ Clec9a+ (DC1), gating on CD11c+ --> 3. CD141-Clec9a- CD1c+ (DC2), gating on CD11c+ --> 4. CD141-Clec9a- CD1c- CD16+ (DC4), gating on Lineage- Blue+ HLA-DR+ --> 5. CD123+ CD45RA+ (DC6), gating on Lineage- Blue+ HLA-DR+ CD123+ CD45RA+ CD33+ AXL- (pre-DC).

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.