FLT3 Ligand, CD40 Agonist Antibody, and Stereotactic Radiotherapy versus Standard Therapy for Advanced Non-small Cell Lung Cancer: A Phase I/II Randomized Trial

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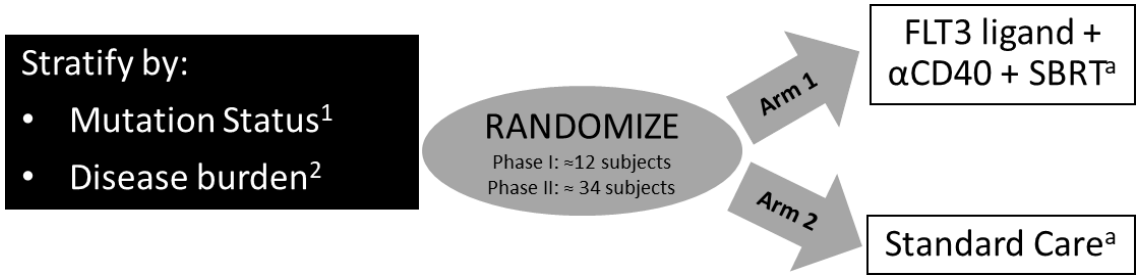
STUDY SCHEMA

Key Eligibility Criteria:

- Advanced/metastatic NSCLC, with at least one suitable SBRT target
- Previous treatment with at least 2 lines of standard systemic therapy
- ECOG performance status 0-2



[1]EGFR/ALK positive v. EGFR/ALK negative or unknown
[2]Limited Disease (suitable for comprehensive SBRT) v. Extensive Disease
[a]Including comprehensive SBRT for limited disease

- Safety assessment for Phase I will take place after 6 subjects are randomized to Arm 1 and followed for 8 weeks after treatment initiation
- If ≤ 1 subject out of 6 on Arm 1 experiences a DLT, the study will proceed to Phase II
- The total sample size (Phase I + Phase II) will be 46 subjects

Abbreviations

ADA	Anti-drug antibody
AE	Adverse event
AJCC	American Joint Committee on Cancer
ALT	Alanine transaminase (synonymous with SGPT)
ANC	Absolute neutrophil count
AST	Aspartate transaminase (synonymous with SGOT)
BMP	Basic Metabolic Panel
CBC	Complete Blood Count
CFR	Code of Federal Regulations
chemoRT	Chemoradiotherapy
CI	Confidence interval
CBR	Clinical benefit rate
CR	Complete response
CRF	Case report form
CT	Computed tomography
CT C/A/P	Computed tomography of the chest, abdomen, and pelvis
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	Cytotoxic T-lymphocyte antigen 4
CTV	Clinical Target Volume
DC	Dendritic cells
DFS	Disease-free survival
DLT	Dose-limiting toxicity
DSM	Data safety and monitoring
DSMC	Data safety and monitoring committee
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
FDG	Fludeoxyglucose
FLT3	Fms-like tyrosine kinase 3
GTV	Gross tumor volume
Gy	Gray
HIPAA	Health Insurance Portability and Accountability Act
i.v.	Intravenous
IB	Investigator's Brochure
ICOS	Inducible costimulator
iCPD	Immune confirmed progressive disease
iCR	Immune complete response
IgG2	Immunoglobulin G, Subclass 2
IMRT	Intensity modulated radiotherapy
iPR	Immune partial response
irAEs	Immune-related adverse events
IRB	Institutional review board
IRB/EC	Institutional Review Board/Ethics Committee
iRECIST	Response evaluation criteria in solid tumors for immune-based therapeutics
irRC	Immune-related response criteria
iSD	Immune stable disease
ITV	Internal target volume
iUCR	Immune unconfirmed complete response
iUPD	Immune unconfirmed progressive disease
iUPR	Immune unconfirmed partial response
IV	Intravenous
kg	Kilogram
LA-NSCLC	Locally advanced non-small cell lung carcinoma
LFTs	Liver function tests
mAb	Monoclonal antibody
mg	Milligram
mL	Milliliter
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NCCN	National Comprehensive Cancer Network
NSAID	Non-steroidal anti-inflammatory drug
NSCLC	Non-small cell lung cancer
ORR	Objective response rate
OS	Overall survival
PD-1	Program death receptor-1
PD-L1	Programmed death-ligand 1
PD-L2	Programmed death-ligand 2
PET	Positron emission tomography
PFS	Progression-free survival
PHI	Protected health information
PI	Principal investigator
PO	By mouth
PR	Partial response
PRO	Patient-reported outcome
PTV	Planning target volume
QoL	Quality-of-life
RECIST	Response Evaluation Criteria for Solid Tumors
RT	Radiotherapy
RTOG	Radiotherapy Oncology Group
SAE	Serious adverse event
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SBRT	Stereotactic body radiotherapy
SD	Standard deviation (or stable disease)
SUV	Standardized uptake value
ULN	Upper limit of normal
WBC	White blood cell

Contents

1.0 OBJECTIVES

1.1 Primary Objective

- Phase I: To establish the safety of combining FLT3 ligand, CD40 agonist antibody, and SBRT for advanced NSCLC that was previously treated with at least two lines of systemic therapy.
- Phase II: To assess the efficacy of combining FLT3 ligand, CD40 agonist antibody, and SBRT for advanced NSCLC that was previously treated with at least two lines of systemic therapy.

1.2 Secondary Objectives

- To evaluate potential surrogate outcomes for clinical efficacy of FLT3 ligand, CD40 agonist antibody, and SBRT for advanced NSCLC, including makers of immune activation.
- To characterize PROs for patients with advanced NSCLC who receive standard therapy or a combination of FLT3 ligand, CD40 agonist antibody, and SBRT.
- To explore the predictive value of physical activity metrics for patients with advanced NSCLC who receive standard therapy or a combination of FLT3 ligand, CD40 agonist antibody, and SBRT.

2.0 BACKGROUND

2.1 Non-small Cell Lung Cancer

Non-small cell lung cancer (NSCLC) is the leading cause of cancer mortality in the United States and worldwide, causing nearly one million deaths each year[1]. For the approximately 40% of NSCLC patients who present with stage IV disease and other patients who progress after presenting with localized disease, standard first-line therapy historically was combination chemotherapy, usually with a platinum doublet. Targeted therapy is now used as first-line therapy for patients with treatable driver mutations, such as activating EGFR mutations, ALK rearrangement, and ROS1 fusion[2]. For other patients, recent randomized trials have demonstrated that the addition of immunotherapy to first-line chemotherapy[3,4] or use of immunotherapy instead of chemotherapy for biomarker-selected patients[5] improves outcomes.

Optimal treatment strategies for advanced NSCLC patients who progress while receiving modern first-line systemic therapy are not well-defined. If not included in first-line therapy, platinum-based chemotherapy may be an option for fit patients. Other chemotherapy options include docetaxel (+/- ramucirumab), gemcitabine, albumin-bound paclitaxel, and pemetrexed. Response rates with these agents are approximately 10%, and disease progression or death typically occurs within a few months[6-8].

2.2 FLT3 Ligand

Fms-like tyrosine kinase 3 (FLT3) ligand is a potent hematopoietic growth factor that mobilizes stem cells and greatly increases the number of circulating dendritic cells (DCs) in blood and organs[9]. FLT3 ligand has been shown to evoke immune responses

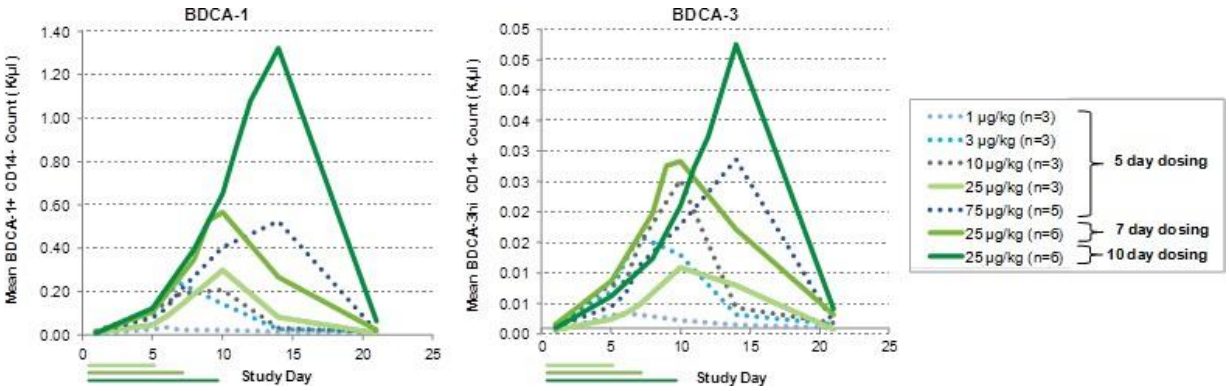
and cause tumor regression in animal models of fibrosarcoma, breast cancer, prostate cancer, and lung adenocarcinoma[10-13].

A key DC subset for cancer immunotherapy are those DCs expressing CD141 in humans and the corresponding subset expressing CD103 in mice. This subset is responsible for producing T cell attractant chemokines and for "cross-presenting" exogenous tumor antigens to CD8+ cytotoxic T cells[14]. Low levels or the absence of CD103+ DCs within the tumor microenvironment is associated with lack of response to immunotherapy in murine models[15,16]. Corresponding to the CD103+ DC subset in mice is the CD141+(BDCA3+) subset of human DCs that is similarly important in cross presentation of antigens[17]. Unfortunately, such DCs are often present in very low numbers or absent within the tumor microenvironment in humans and a high ratio of CD141+ DC associated transcripts within the tumor microenvironment correlates with better clinical outcomes[18]. Therefore, augmenting the presence of CD141+ DCs within the tumor microenvironment and providing the appropriate activation and maturation signals to the DCs may be an effective mechanism to enhance the anti-tumor and clinical activity of immunotherapy agents in patients with cancer.

Recombinant human FLT3 ligand, AMG949 originally developed by Immunex and subsequently acquired by Amgen, has been studied in numerous industry and investigator-initiated clinical trials. Collectively, approximately 150 healthy volunteers and 380 cancer patients were enrolled on those trials. Adverse events requiring cessation of treatment were rare (<5%). However, FLT3 ligand did not demonstrate definitive anti-tumor activity in patients with ovarian cancer, breast cancer, or non-Hodgkin's lymphoma. This may have been related to the mobilization of immature DCs by FLT3 ligand and the absence of appropriate DC maturation signals and/or limited DC access to tumor antigens.

More recently, recombinant human FLT3 ligand (CDX-301) developed by Celldex, which is identical in amino acid sequence to AMG949 but produced utilizing a serum-free manufacturing process, has been demonstrated to expand and mobilizes DCs into the peripheral blood and tissues, including into the tumor microenvironment. As of May 28, 2020, healthy volunteers (n=30), allogenic hematopoietic stem cell donors (n=4) or cancer patients (n=116), for a total of 150 subjects, have been treated with CDX-301 as monotherapy or in combination with other agents.

CDX-301 has been shown to expand CD141$^+$(BDCA3$^+$) conventional DCs (cDCs)[19] in healthy volunteers. In these same subjects, CDX-301 was also shown to expand precursor cDCs (pre-cDCs) that produce the two major cDC subsets, the CD141$^+$(BDCA3$^+$) and the CD1c$^+$ DCs[20]. DC expansion peaks approximately two weeks after initiation of FLT3 ligand therapy (see figure below). The intratumoral injection of CDX-301 also increases the number of CD141$^+$ DCs in blood and in the injected tumor[21]. CDX-301 also expanded CD141+ DCs and augmented the kinetics, magnitude, and frequency of cellular and immune responses to a NY-ESO-1 vaccine (CDX-1401) in patients with resected melanoma[22].



Expansion of two dendritic cell populations in healthy volunteers treated with CDX-301 peaks at 10-14 days after treatment initiation. (source: Celldex Therapeutics)

CDX-301 has been evaluated in two Celldex-sponsored clinical studies as monotherapy in healthy volunteers and donor/recipients (CDX301-02 and CDX301-03), in one study in combination with the antibody-drug conjugate glembatumumab vedotin in advanced melanoma (CDX011-05), and in one study in combination with CDX-1140 in advanced malignancies (CDX1140-01). CDX-301 has also been investigated in four investigator-initiated studies: in combination with radiotherapy for non-small cell lung cancer (CDX301-53, performed at Montefiore Medical Center), in combination with poly-ICLC and radiotherapy in lymphoma (CDX301-51), in combination with poly-ICLC, radiotherapy and pembrolizumab in non-Hodgkin's lymphoma, metastatic breast cancer and head and neck squamous cell carcinoma (CDX301-58), and in combination with poly-ICLC and the cancer vaccine CDX-1401 in malignant melanoma (CDX1401-54).

CDX-301 has been well tolerated. One SAE of grade 3 community-acquired pneumonia in the healthy volunteer study CDX301-02 was attributed to CDX-301. Across all trials, no additional SAEs, and no deaths, have been assessed as related to CDX-301. In general treatment-related AE following CDX-301 administration are infrequent and mild to moderate in severity. Across all trials through August 2019, the most common treatment-related AE has been lymphadenopathy in 6 subjects (13%). Additional treatment-related grade 1 and grade 2 AEs that have been reported ≥ 5% of all patients treated with CDX-301 include: injection site reaction (including erythema, rash, reaction or pain; 8%), chills (6%), dyspepsia (6%), fatigue (6%), and hot flush (6%). One case of chills (2%), two cases of fatigue (4%), and two cases of hot flush (4%) were also attributed to CDX-1140; a single case of fatigue (2%) was also attributed to glembatumumab vedotin. The CDX-301 Investigator Brochure provides additional details.

2.3 Stereotactic Body Radiotherapy (SBRT)

In recent years, hypofractionated stereotactic body radiotherapy (SBRT) has emerged as an effective treatment option for numerous malignancies. SBRT uses the principles of intracranial stereotactic radiosurgery (large daily doses delivered precisely to a small target volume over a short treatment course) to treat extracranial disease sites. For stage I NSCLC, SBRT yields excellent local control rates[23-25] and has now been proven to prolong survival compared to standard radiotherapy[26]. Serious adverse

events following SBRT for lung tumors are very rare[27], except when aggressive SBRT regimens are used to treat central lesions[28]. Importantly, highly-conformal delivery of SBRT allows relatively safe treatment of lung tumors in patients with poor pulmonary function[29]. Similarly, SBRT yields low toxicity rates when employed to treat extrathoracic disease sites, such as the liver, skeleton, and adrenal gland[30-33].

A wide variety of dosing and fractionation schedules has been used to deliver lung SBRT for NSCLC[24,25,34,35]. Although there is evidence of a dose-response relationship when relatively low-dose regimens (BED < 100 Gy) are used,[24,25] more intense regimens have yielded consistently excellent results and are considered to be ablative. These include 10-12.5 Gy x 4-5 fractions, 18-20 Gy x 3 fractions, and 25-34 Gy in a single fraction. The National Comprehensive Cancer Network (NCCN) provides guidelines for the selection of an SBRT (also known as SABR) schedule as well as relevant constraints for normal tissues, which will be adopted for this trial.

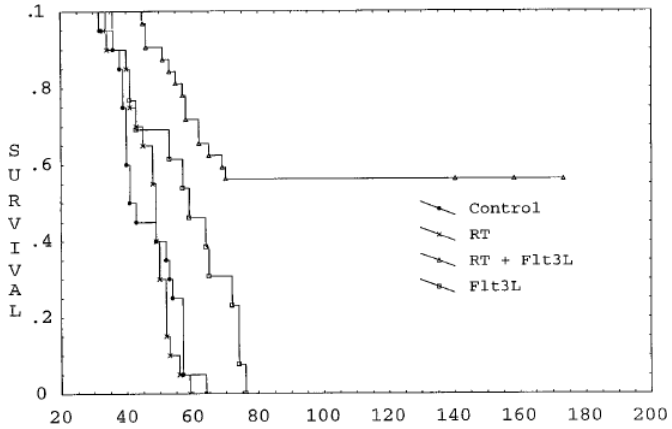
Several recent randomized trials suggest that, for patients with advanced NSCLC and limited ("oligometastatic") disease, radical treatment with SBRT to all active sites of disease could delay disease progression and prolong overall survival[32,36-38]. This principal is being tested for patients with oligometastatic NSCLC after first-line systemic therapy in NRG-LU002.

Our group and others have demonstrated that ionizing radiotherapy (RT) has the potential to enhance the effectiveness of cancer immunotherapy[39-45]. RT may serve to amplify the tumor-specific peptide repertoire and upregulate cell surface expression of MHC determinants and costimulatory molecules[46,47]. RT may also evoke changes in the tumor microenvironment that facilitate the host immune response[48,49]. These effects may be most pronounced when RT is delivered as SBRT, with short courses and high daily doses[50]. We believe that SBRT is a key to enhancing the potential of immunotherapy as an effective approach in the treatment of NSCLC.

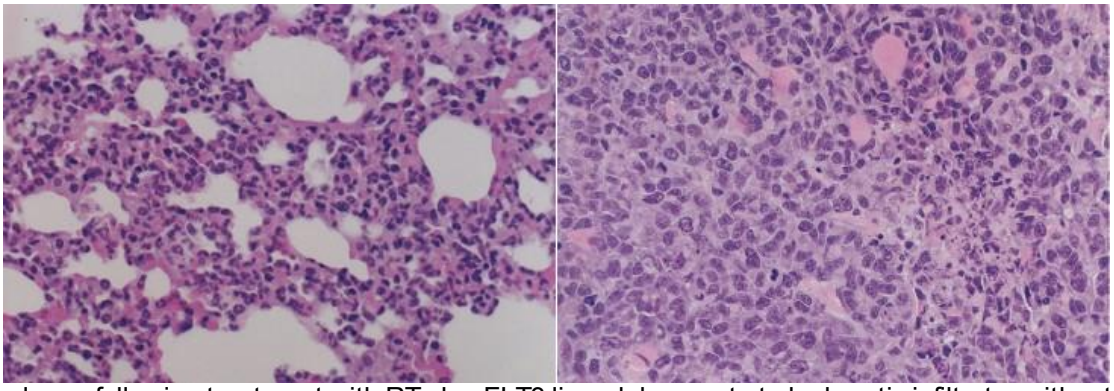
2.4 FLT3 Ligand and SBRT

2.4.1 Preclinical Data

Experiments performed in our department have demonstrated that pretreatment of a single lesion with high-dose RT increases the systemic efficacy of FLT3 ligand in a murine model of lung adenocarcinoma[10]. Mice with established, 3-week-old Lewis lung tumors were given a single dose of localized primary footpad tumor RT (60 Gy), with or without FLT3 ligand (administered daily for 10 days after RT). The combination of RT and FLT3 ligand significantly increased survival compared to either treatment alone and compared to controls (See Figure Below). Examination of animal lung tissue revealed that the lungs of mice treated with RT and FLT3 ligand showed no carcinoma cells but had infiltrates of neutrophils, lymphocytes, and mononuclear leukocytes. Lungs from other cohorts demonstrated massive tumor cell infiltration (See Figure Below).



Local RT acts synergistically with FLT3 ligand in a murine NSCLC model to improve overall survival.



Murine lungs following treatment with RT plus FLT3 ligand demonstrate leukocytic infiltrates without tumor cells (left), compared to tumor-laden lungs of mice from other cohorts (right).

2.4.2 Clinical Data

Based on the data summarized above, we recently completed a novel clinical trial combining FLT3 ligand and SBRT for the treatment of advanced NSCLC (ClinicalTrials.gov Identifier NCT02839265). The study design and results, which were presented in preliminary form at the 2018 AACR Annual Meeting[51] and at the 2020 ASCO Annual Meeting, are summarized below. Of note, the FLT3 ligand (CDX-301) dosing schedule from our recently completed trial remains unchanged in the present study.

Key Eligibility Criteria:

- AJCC stage 3 or 4 histologically proven NSCLC not amenable to curative therapy
- Prior treatment with at least one standard chemotherapy regimen or targeted agent prior to enrollment
- Measurable disease, including:
  - at least one tumor or metastasis ≥ 1 cm in greatest dimension that would be amenable to SBRT
  - at least one measurable lesion that would be outside of the SBRT treatment fields
- ECOG performance status 0-2

Study Procedures:

- Radiographic assessment with PET/CT at baseline and every 8 weeks

- One-week treatment course:
  - 5 daily subcutaneous injections of FLT3 ligand (75 µg/kg)
  - Stereotactic body radiotherapy (SBRT) to a single tumor or metastasis, delivered in 1, 3, or 5 fractions based on NCCN and NRG guidelines
  - No other cancer therapy administered until disease progression
  - Additional "cycles" of FLT3 ligand and SBRT allowed after 4 months

Study Endpoints:

- Primary endpoint: Progression-free survival 4 months after treatment initiation (PFS4)
  - Scored using Immune-related Response Criteria (irRC)[52]
  - H0: PFS4 ≤ 20% H1: PFS4 ≥ 40.5%
    Accept H1 if PFS4 is achieved in 10/29 subjects
- Secondary endpoints:
  - Adverse events / Dose-limiting toxicities
  - Overall Survival
  - Radiographic responses in lesions not treated with SBRT
    - CT: evaluated using irRC[52]
    - PET: evaluated using PERCIST[53]
      - Total Glycolytic Activity (TGA): volumetric sum of activity in all hypermetabolic lesions
      - Partial Metabolic Response (PMR): Decrease in TGA of at least 45%

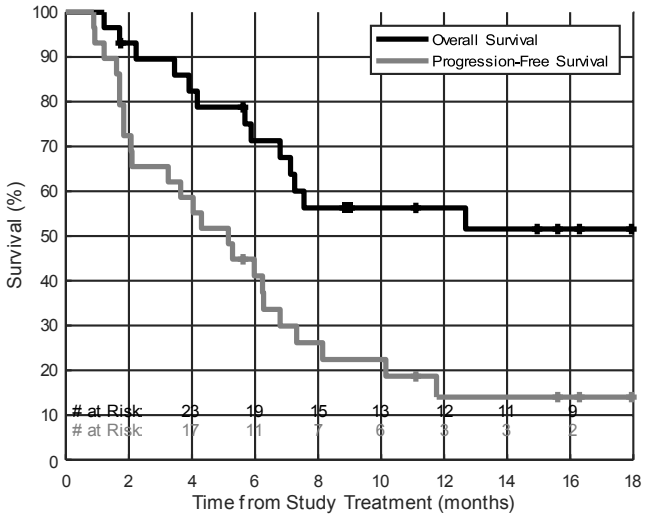
Results

- 29 subjects (described in table below) were enrolled from October 2016 to January 2020. The median follow-up duration for living subjects is 16 months
- No grade 3 or higher adverse events attributed to study therapy have been observed.
- PFS4 was achieved in 17/29 patients (59%), meeting our pre-specified efficacy objective (PFS and OS Kaplan-Meier curves below).
- Partial responses ("abscopal effects") were observed in 9 subjects (31%) using PET criteria and in 4 subjects (14%) using CT criteria (patient example below).
- Seven subjects (24%) received a second course of SBRT and CDX-301 after initial study therapy.
- Only six subjects (21%) have received additional chemotherapy or immunotherapy after study treatment.

Study Conclusions

- The combination of FLT3 ligand and SBRT for advanced and previously treated NSCLC is well-tolerated.
- The combination of FLT3 ligand and SBRT demonstrated activity in advanced NSCLC patients, most of whom were previously treated with immunotherapy (checkpoint inhibitors targeting PD1/PD-L1), with survival outcomes far superior to published series of NSCLC patients who receive salvage chemotherapy after immune checkpoint inhibitor therapy[7,54].
- Disease extent/multifocality may be a critical prognostic factor.

Gender, n (%)	
Male	18 (62%)
Female	11 (38%)
Age, mean (range)	68 (47-81)
ECOG Performance Status, n (%)	
0	4 (14%)
1	13 (45%)
2	12 (41%)
Histology, n (%)	
Adenocarcinoma	21 (72%)
Squamous cell carcinoma	4 (14%)
Other/Unknown	4 (14%)
Actionable Mutation, n (%)	
EGFR	2 (7%)
None	27 (93%)
PD-L1 expression, n (%)	
0%	10 (34%)
1 to 49%	7 (24%)
50 to 100%	3 (10%)
Unknown	9 (31%)
Previous lines of systemic therapy, n (%)	
1	3 (10%)
2	11 (38%)
3	12 (41%)
4 or more	3 (10%)
Prior anti-PD-(L)1 therapy, n (%)	26 (90%)
Initial SBRT target, n (%)	
Lung/Mediastinum	24 (83%)
Extrathoracic lymph node	3 (10%)
Other	2 (7%)
SBRT schedule, n (%)	
1 fraction	3 (10%)
3 fractions	8 (28%)
5 fractions	18 (62%)



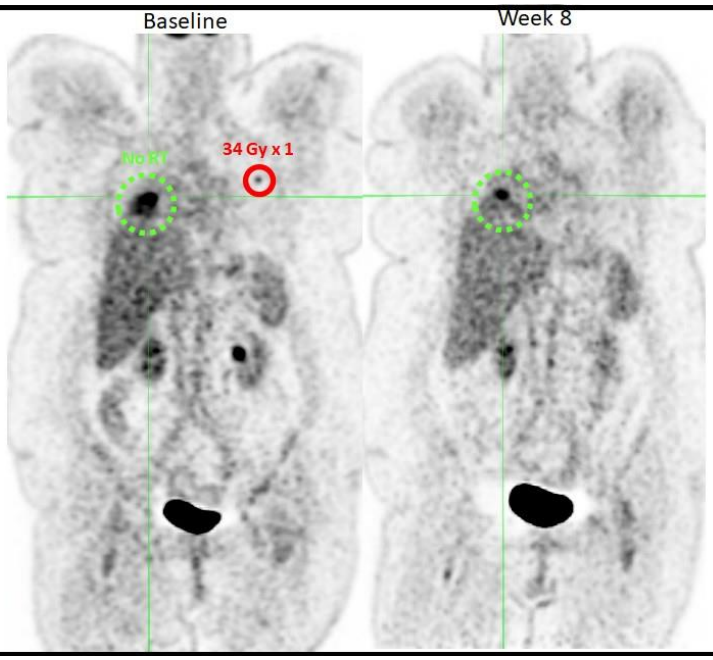
Top Left: Patient characteristics

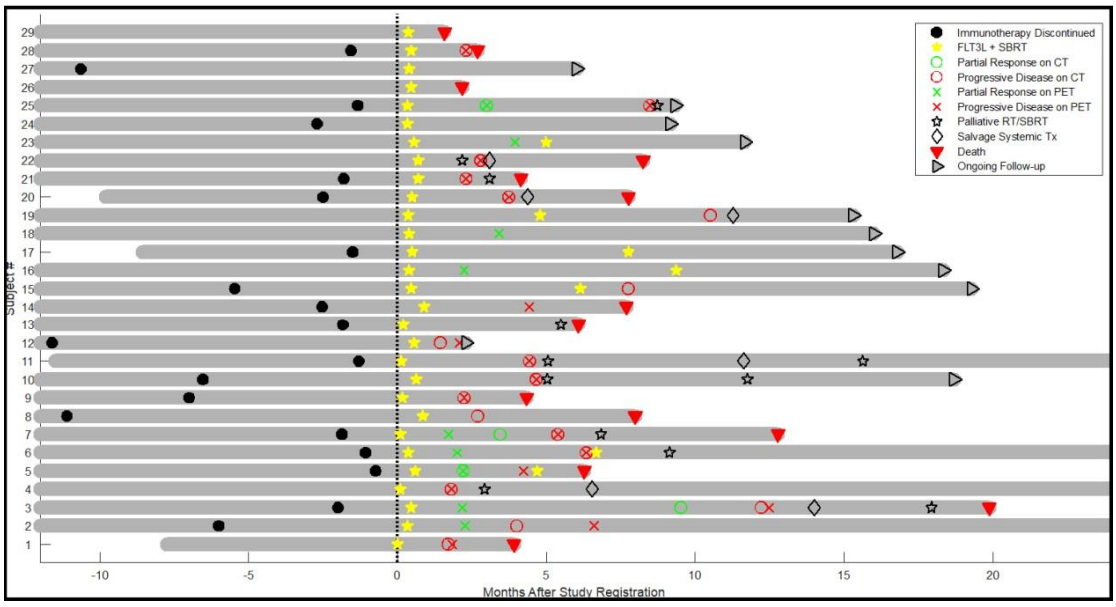
Top Right: Kaplan-Meier PFS and OS curves

Bottom: Sample patient who demonstrated an abscopal response after receiving SBRT directed at a small nodule contralateral to the larger primary tumor.

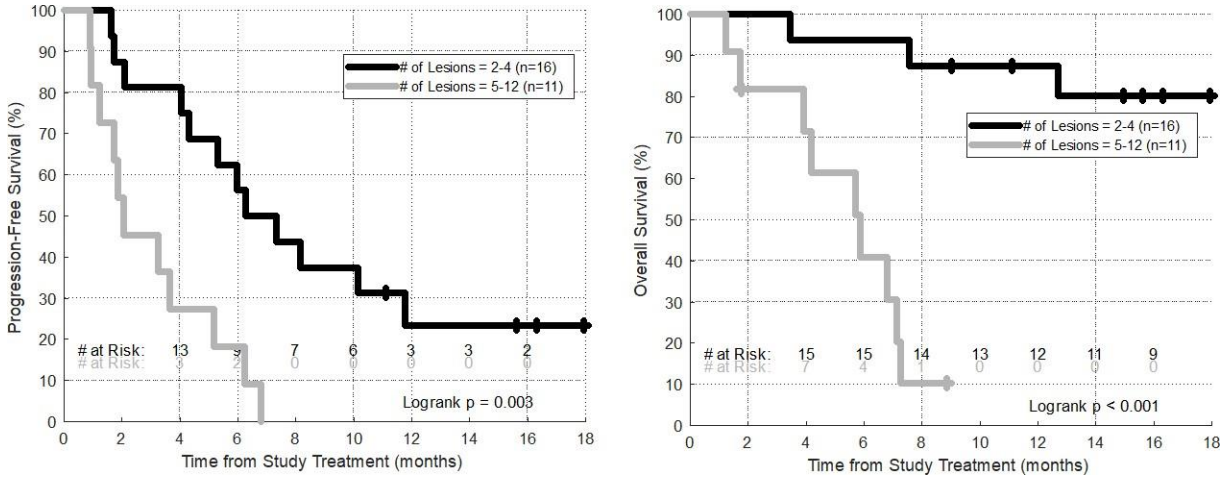
Patient 2

- 55 year-old female with right lung adenocarcinoma, left lung nodules
  - 1st line: carboplatin, pemetrexed (PD)
  - 2nd line: nivolumab (arthralgias), discontinued June 2016
  - Nov 2016: SBRT + CDX-301





Swimmer plot summarizing subjects' treatment courses after study enrollment



Exploratory analyses revealing that disease extent at the time of study enrollment was a powerful predictor of progression-free survival and overall survival. 8 out of 16 subjects with ≤4 lesions at the time of study entry received a second "cycle" of study therapy. 3 of those subjects (#6, #17, and #23 [see swimmer plot above]) are alive and *without any evidence of active disease* on recent scans.

2.5 Agonist Anti-CD40 Antibody

CD40 is a key molecule in the regulation of immune responses whose activity can be modulated using antibodies. CD40 is a tumor necrosis factor receptor superfamily member expressed on antigen presenting cells, including dendritic cells[55], as well as a host of other cell types, including a wide range of tumor cells[56]. The ligand for CD40, CD40L (CD154), is predominately expressed on activated CD4+ helper T cells[57,58]. On dendritic cells, an important function of the interaction of CD40 with CD40L expressing CD4+ T-helper cells is the activation and "licensing" of dendritic cells to

prime CD8+ effector T cells. Furthermore, CD40-activated macrophages can be tumoricidal and, in some cases, deplete tumor stroma[59]. Thus, CD40 on antigen presenting cells plays a critical role in the induction of effective innate and adaptive immune responses. The ligation of CD40 on certain malignant cells using CD40 ligand or anti-CD40 antibodies may also inhibit proliferation or trigger apoptotic cell death[60-62]. Thus, two independent mechanisms provide opportunities for the use of agonist anti-CD40 monoclonal antibodies in cancer therapy: the enhancement of anti-tumor immunity and the direct growth inhibition or killing of tumors.

Anti-CD40 monoclonal antibodies have been tested in several early-phase clinical trials[63-67]. These studies have demonstrated a similar toxicity profile, with infusion reactions and transient changes in liver function tests and hematologic parameters being most common. CDX-1140 (Celldex) is a fully human IgG2κ monoclonal antibody that binds to human CD40 with high affinity and promotes CD40 signaling[68,69].

The safety, biological activity, and preliminary clinical activity of CDX-1140 as monotherapy and in combination with CDX-301 (FLT3 ligand) is being established in an ongoing phase I dose escalation and expansion trial (CDX1140-01; NCT03329950) whose interim results were presented at the 2019 AACR Annual Meeting[70]. CDX-1140 is administered intravenously monthly, and CDX-301 is administered 75 μg/kg subcutaneously daily for 5 days the week prior to CDX-1140 during cycles 1 and 2. As of May 28th, 2020, a total of 89 patients have been administered CDX-1140, with 55 administered CDX-1140 monotherapy, 31 administered CDX-1140 in combination with CDX-301, and 3 patients administered CDX-1140 in combination with pembrolizumab. The Maximum Tolerated Dose (MTD) of CDX-1140 was determined to be 1.5 mg/kg, which will be used in the present study. To date in the CDX1140-01 trial, 23 patients have been administered CDX-1140 monotherapy at 1.5 mg/kg, and 10 have been administered CDX-1140 at 1.5 mg/kg in combination with CDX-301.

Although not the primary objective of the study, evidence of anti-tumor activity has been observed, with an unconfirmed iRECIST partial response in a patient with gastroesophageal adenocarcinoma, treated with CDX-1140 + CDX-301. In addition, 2 patients with head and neck squamous cell carcinoma who received CDX-1140 monotherapy have had early evidence of an anti-tumor effect with, cavitation and/or necrosis of lesions on CT scan.

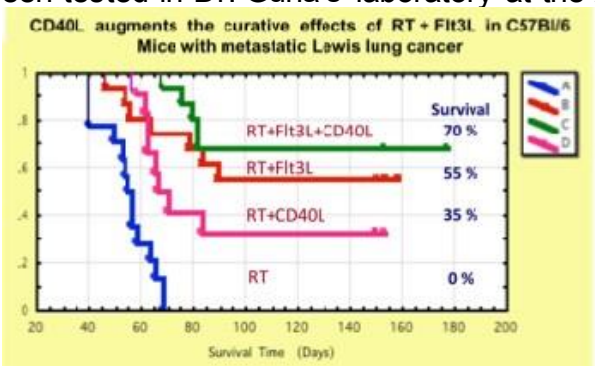
CDX-1140 is biologically active, with increases in serum cytokine and chemokines observed in the study, as well as increased activation markers on dendritic cells and B-cells. Preliminary data indicates that CDX-301 increased the level of serum IL-12p40.

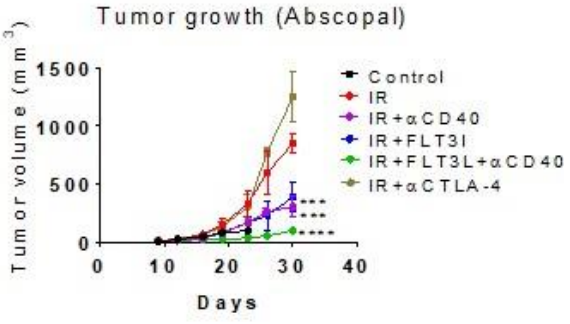
Most side effects associated with CDX-1140 have occurred in the 24-48 hours following the CDX-1140 infusion, consisted of infusion reactions/cytokine release syndromes, have been mild to moderate in severity, and have resolved with or without medications such as acetaminophen, NSAIDS, and/or anti-nausea medication. In a few cases, the side effects have been severe and have required hospitalization and treatment with corticosteroids or other immunosuppressive medications. Treatment related adverse events that have occurred in > 10% of patients are as follows: fatigue, joint pain, nausea, chills, fever, diarrhea, muscle pain, vomiting. Transient (usually low-grade) increases in liver transaminases have been seen in some patients, but no patient

has had to discontinue therapy because of increased liver function tests. Cytokine release syndrome (including symptoms of fever, hypotension, hypoxia, fatigue, headache, and nausea) and pneumonitis have been the most common SAEs reported. Thus far, CDX-301 does not seem to add to the side effect profile of CDX-1140. The CDX-1140 Investigator Brochure provides additional details.

2.6 FLT3 Ligand, Anti-CD40 Antibody, and Stereotactic Radiotherapy

The dendritic cell subset expressing CD141 in humans (CD103 in mice) is responsible for producing T cell attractant chemokines and for "cross-presenting" exogenous tumor antigens to CD8+ cytotoxic T cells[14]. The absence of such cells is associated with lack of response to immunotherapy in murine models[15,16] and poor clinical outcomes in a variety of cancers[18]. CDX-301 (FLT3 ligand) has been shown to expand CD141+ dendritic cells[71]. The key rationale for combining CDX-1140 and CDX-301 is to increase the number of dendritic cells (especially CD141+ dendritic cells) that are available to be activated by CDX-1140. Stereotactic radiotherapy to a single tumor or metastasis in this setting is expected to release tumor neoantigens into the circulation, augmenting the potential for an effective *in situ* vaccine. This principle has been tested in Dr. Guha's laboratory at the Albert Einstein College of Medicine[72]:





2.7 SBRT for NSCLC Patients with Limited Disease Burden

Several phase II randomized trials have recently demonstrated that, for patients with "oligometastatic" and non-progressive NSCLC after first-line systemic therapy, comprehensive local therapy may delay disease progression and death[36-38]. This hypothesis is now being tested in a large multi-institutional trial (NCT03137771). Radical local therapy has also shown promise in other settings of limited metastatic disease[32,73,74]. For advanced/metastatic NSCLC, current NCCN guidelines indicate that "definitive local therapy to isolated or limited metastatic sites… is an appropriate option in such cases if it can be delivered safely to the involved sites[75]."

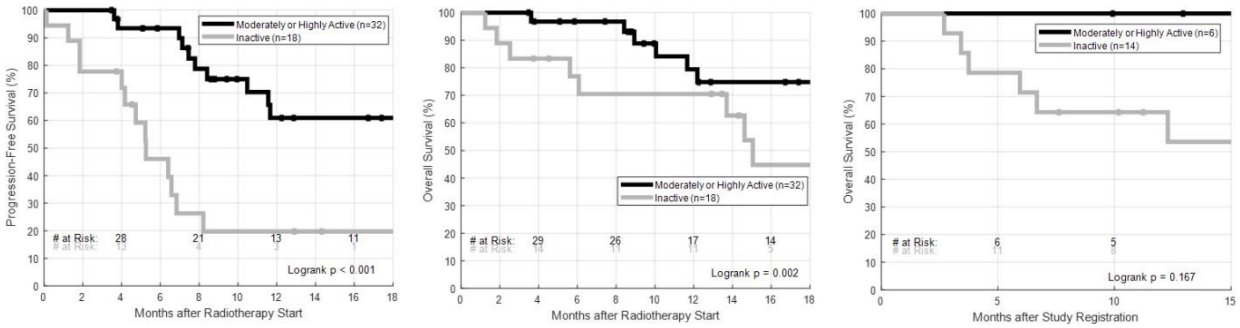
For patients in our practice who have limited (typically less than 3-5) sites of active disease after several lines of systemic therapy (leaving few appealing systemic treatment options), we routinely offer "comprehensive" SBRT as standard of care. As a result, *all subjects enrolled on this study for whom the treating physicians deem comprehensive SBRT to be safe and feasible will receive comprehensive SBRT*.

2.8 Quality-of-Life Assessments in NSCLC

Quality-of-life (QoL) is a critical endpoint in clinical trials for advanced lung cancer. QoL can be affected dramatically by both the disease and its treatment. In this study, we will assess QoL using the EORTC QLQ-C30 tool. The QLQ-C30 is the most widely used cancer-specific HRQoL instrument. It contains 30 items and measures 5 functional dimensions (physical, role, emotional, cognitive, and social), 3 symptom items (fatigue, nausea/vomiting, and pain), 6 single items (dyspnea, sleep disturbance, appetite loss, constipation, diarrhea, and financial impact), and a global health and quality of life (QoL) scale. We will also utilize the EORTC QLQ-LC13, which is a disease-specific questionnaire developed and validated to address measurements specific to lung cancer[76,77].

2.9 Physical Activity Monitoring

Wearable devices that can monitor steps taken, stairs climbed, energy expenditure, heart rate, and sleep metrics are now marketed as fitness trackers. Data from these user-friendly and inexpensive devices may provide important information to clinicians caring for cancer patients[78,79]. In a series of trials we have performed at Montefiore/Einstein, we have discovered that a simple measure – daily step count – can provide valuable prognostic information regarding both short-term hospitalization risk[80-82] and long-term clinical outcomes[83].



Baseline activity level (based on step count average and age) can predict clinical outcomes for patients treated with chemoradiotherapy for locally advanced NSCLC (left, middle)[83] or patients receiving palliative systemic therapy for advanced solid tumors (right, preliminary data from an ongoing trial)

Subjects on this trial may opt to wear a commercial fitness tracker (provided by the study team) as part of this study. We will assess baseline activity level as a prognostic factor for study subjects and also compare rates of activity change for subjects who receive study therapy versus standard care, with the expectation that study therapy will preserve physical function compared to standard therapy, such as cytotoxic chemotherapy.

3.0 PATIENT ELIGIBILITY

3.1 Inclusion Criteria

- Histologically proven NSCLC, not classically deemed to be amenable to curative therapy based on disease extent at the time of diagnosis or disease progression after the initial diagnosis

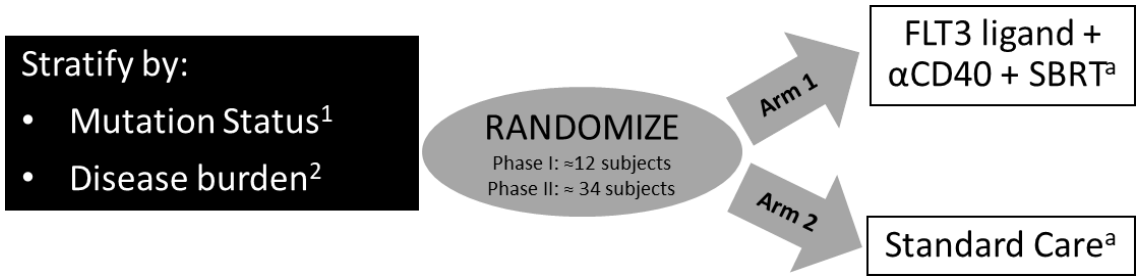
- Age ≥ 18 years
- Prior treatment with at least two lines of systemic therapy for advanced NSCLC, including one line of platinum-based combination chemotherapy
    - Treatment with concurrent chemotherapy and immunotherapy can count as two lines of therapy for the purposes of study eligibility.
- Radiological assessments, including CT within 21 days prior to study entry and PET/CT within 42 days prior to study entry, demonstrating measurable disease that includes at least one pulmonary or extrapulmonary lesion ≥ 1 cm in greatest dimension that would be amenable to SBRT and at least one measurable lesion that would be outside of the SBRT treatment fields
- ECOG performance status 0-2
- Both men and women enrolled in this trial must agree to use adequate birth control measures during the trial and for at least 90 days after last receipt of study therapy. Patients and/or partners who are surgically sterile or postmenopausal are exempt from this requirement.
- The following laboratory results, within 21 days prior to study registration:
    - Hemoglobin ≥ 9.0 g/dL
    - Absolute neutrophil count ≥ 1.5 x 10^9/L
    - Platelet count ≥ 100 x 10^9/L
    - Serum creatinine ≤ 1.5 x ULN OR creatinine clearance (by Cockcroft-Gault formula) > 60 mL/min
    - AST and ALT ≤ 2.5 x ULN
    - Total bilirubin ≤ 2.0 x ULN (except patients with Gilbert's syndrome, who must have a total bilirubin ≤ 3.0 mg/dL)
    - Negative SARS-CoV-2 (COVID-19) PCR test
        - COVID-19 testing may be repeated periodically based on institutional policies and must be repeated for any subject with unexplained signs (e.g., imaging findings, fever) or symptoms (e.g., anosmia) concerning for COVID-19 infection or with recent known exposure to COVID-19.

3.2 Exclusion Criteria

- Prior therapy with any anti-CD40 antibody or FLT3 ligand
- Less than 21 days between planned study treatment start and the last receipt of chemotherapy, targeted cancer therapy, immunotherapy, radiotherapy (excluding palliative radiotherapy), or major surgery.
- Untreated central nervous system metastases. Patients with a history of brain metastases must have had no CNS-directed therapy within the past 60 days and radiological assessment within 30 days of study entry demonstrating a lack of progressive CNS disease. Patients without a history of brain metastases and without symptoms suggestive of brain metastasis do not require staging imaging of the brain prior to study enrollment.
- Known mutation/amplification in FLT3
- Ongoing use of high dose oral corticosteroids (≥ 2 mg of dexamethasone daily or equivalent) or inhaled corticosteroids. Intranasal and/or intraarticular corticosteroid use is permitted.

- History of non-infectious pneumonitis or any ongoing pneumonitis
- History of allogeneic organ transplant or active autoimmune disease
- Other active malignancy for which systemic therapy (excluding hormonal therapy for breast or prostate cancer) is indicated.
- History of myocardial infarction, cerebral vascular accident, thrombosis, or pulmonary embolus within 12 months prior to study registration.
- Uncontrolled intercurrent illness including, but not limited to, symptomatic congestive heart failure, unstable angina pectoris, uncontrolled cardiac arrhythmia, or psychiatric illness/social situation that would limit compliance with study requirements as judged by the treating physicians
- Active infection requiring systemic therapy, known HIV infection, or positive test for hepatitis B surface antigen or hepatitis C (antibody screen and if positive confirmed by RNA analysis). If positive results are not indicative of a true active or chronic infection, the patient can be enrolled after discussion with, and agreement by, the Principal Investigator.
- Receipt of a live vaccine within 30 days prior to the first dose of study drug. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and are not allowed.
- *Subjects who are not ambulatory or ambulate using a walker are not eligible for the objective activity monitoring component of this study.*

4.0 STUDY DESIGN



[1]EGFR/ALK positive v. EGFR/ALK negative or unknown
[2]Limited Disease (suitable for comprehensive SBRT) v. Extensive Disease
[a]Including comprehensive SBRT for limited disease

- Safety assessment for Phase I will take place after 6 subjects are randomized to Arm 1 and followed for 8 weeks after treatment initiation
- If ≤ 1 subject out of 6 on Arm 1 experiences a DLT, the study will proceed to Phase II
- The total sample size (Phase I + Phase II) will be 46 subjects

4.1 General Design

This is a randomized, open-label phase I/II study. Subjects will be randomized in a 1:1 ratio to receive FLT3 ligand, anti-CD40 antibody, and SBRT (Arm 1) versus standard care (Arm 2). Subjects on either study arm with limited disease will receive SBRT to all evident sites of active disease. Subjects on Arm 1 with extensive disease will initially receive SBRT to a single site of disease but may receive additional "cycles" of FLT3 ligand, anti-CD40 antibody, and SBRT at later time points. Subjects on Arm 2 with extensive disease are expected to receive some form of standard systemic therapy (e.g., docetaxel). Subjects on Arm 2 with limited disease may also receive standard systemic therapy following completion of SBRT to all sites of evident disease, at the discretion of the treating physicians. All subjects will be followed closely for treatment-related toxicities. Whole-body PET/CT and CT imaging will be performed prior to study entry, and restaging CT will be performed every 8 weeks thereafter.

In the phase I component of this study, subjects will be randomized between study arms until 6 subjects have been randomized to Arm 1. Study accrual will then be halted until 8 weeks after the last study subject begins treatment.

If the pre-specified safety criteria are achieved in the Phase I portion of this study, the study will proceed to phase II. The total sample size (Phase I and Phase II) will be 46 subjects. All 46 subjects will be included in efficacy analyses.

If the pre-specified safety criteria are not achieved in the Phase I portion of this study, the investigators and Celldex will review safety data from this trial as well as other trials using CDX-301 and CDX-1140 and determine if the experimental treatment regimen should be altered (e.g., by reducing the CDX-1140 dose). The study protocol would be amended accordingly, and Phase I component with the new treatment regimen would be added.

4.2 Study Calendars

4.2.1 Pre-Registration and Pre-treatment Assessments

	Timing
CBC, BMP, LFTs	Within 21 days prior to study entry
Amylase, Lipase	Pre-treatment
Negative SARS-CoV-2 PCR	Within 21 days prior to study entry
PET/CT	Within 42 days prior to study entry
CT C/A/P	Within 21 days prior to study entry
QoL Assessments	Pre-treatment
Correlative Study Blood Draw	Pre-treatment[1]
CDX-301 ADA and CDX-1140 PK	Pretreatment (Arm 1 only)[1]

1. Must be obtained at least 21 days after last receipt of chemotherapy, targeted cancer therapy, immunotherapy, radiotherapy (excluding palliative radiotherapy), or major surgery.

4.2.2 Arm 1 (experimental study arm)

ARM 1	Week 1	Week 2	Week 4	Week 6	Weeks 9[1], 17[2], 25[2], 33[2], 41[2], 49[2]
History and Physical Examination	X	X[5]X[6]	X	X[5]X[6]	X
CBC, BMP, LFTs, Amylase, Lipase	X	X[5]X[6]	X	X[5]X[6]	X
Activity Data Download[3]	X	X	X	X	X
QoL Assessments		X	X	X	X
Correlative Study Blood Draw		X[7]		X[7]	X
CDX-301 ADA and CDX-1140 PK		X[7]X[8]X[6]		X[7]X[6]	
CT C/A/P					X
Stereotactic Radiotherapy (SBRT)[4]	XXX				
FLT3 Ligand (CDX-301)	XXXXX				
CD40 Agonist Antibody (CDX-1140)		X		X	

1. +/- 1 week
2. +/- 2 weeks
3. For subjects who opt to participate in this part of the study
4. SBRT should be delivered in 1, 3, or 5 fractions per target and may continue beyond Week 1 when treating multiple sites.
5. On the day of CDX-1140 infusion
6. One day after CDX-1140 infusion
7. Immediately before CDX-1140 infusion
8. 2 hours after CDX-1140 infusion

Cycles of SBRT(to an untreated lesion)+CDX-301+CDX-1140 may be repeated.

- *At least 8 weeks must separate the start of each treatment cycle.*
- *CDX-1140 may be omitted from retreatment cycles for subjects with prior DLT or intolerable toxicity attributed to CDX-1140.*
- *The schedule of assessments will reset when a new cycle is initiated.*

4.2.3 Arm 2 (control arm)

ARM 2	Week 1	Week 2	Week 4	Week 6	Weeks 9[1], 17[2], 25[2], 33[2], 41[2], 49[2]
History and Physical Examination	X	X	X	X	X
CBC, BMP, LFTs, Amylase, Lipase	X	X	X	X	X
Activity Data Download[3]	X	X	X	X	X
QoL Assessments		X	X	X	X
Correlative Study Blood Draw		X		X	X
CT C/A/P					X
Stereotactic Radiotherapy (SBRT)[4]	X X X				

1. +/- 1 week
2. +/- 2 weeks
3. For subjects who opt to participate in this part of the study
4. SBRT is only required for subjects with limited disease burden. SBRT should be delivered in 1, 3, or 5 fractions per target and may continue beyond Week 1 when treating multiple sites.

Standard systemic therapy may be administered at any time for subjects with extensive disease burden and any time after completion of all planned SBRT for subjects with limited disease.

4.3 Primary Endpoints

- Phase I: Dose-limiting toxicity (DLT), as defined in Section 7.2.
- Phase II: Progression-free survival (PFS) duration, defined as time from study registration until disease progression (scored using iRECIST[84]) or death, whichever comes first.

4.4 Secondary Endpoints

- Overall survival (OS) duration, defined as time from study registration until death.
- Radiographic responses, scored using iRECIST and RECIST.
- Patient-reported outcomes (PROs), assessed using the EORTC QLQ-C30 and QLQ-LC13
- Physical activity metrics, including daily step counts, obtained using wearable devices
- Correlative studies, including measures of DC expansion and DC maturation, anti-drug antibodies (ADA), and CDX-1140 pharmacokinetics

5.0 STUDY THERAPY – ARM 1

5.1 Stereotactic Body Radiotherapy

5.1.1 Patient Selection – Limited v. Extensive Disease

Stereotactic body radiotherapy (SBRT) will be administered to all subjects on Arm 1 and to subjects with limited disease burden on Arm 2. For the purposes of this trial, "limited disease" is defined as disease that, in the estimation of the treating physician, can reasonably and safely be addressed in a comprehensive fashion with SBRT. This definition will generally align with recent consensus definitions of oligometastatic disease, such as having 5 or fewer active lesions[85-87]. Occasionally, a

study subject with 5 or fewer active lesions may be deemed to be ineligible for comprehensive SBRT due to large target size (above **7 cm for lung tumors**[88] or above **5 cm for other lesions**[89,90]), target location adjacent to a radiosensitive normal structure, or prior radiotherapy exposure. Rarely, comprehensive SBRT a subject with 6 or more active lesions may be deemed feasible and safe (e.g., two small lung tumors, two small liver metastases, and two spine metastases). Any subject with more than 10 active lesions will be deemed to have extensive disease[91].

5.1.2 SBRT Timing

For subjects on Arm 1, SBRT will be delivered concurrently with FLT3 ligand during Week 1 of study therapy. If only one lesion is being treated, SBRT should be completed during week 1 (e.g., with daily treatments for a 5-fraction course of treatments every other day for a 3-fraction course). In cases where multiple lesions are being treated with SBRT (Arm 1 or Arm 2) or if treatment is interrupted due to technical issues or intercurrent illness, SBRT may extend beyond Week 1.

5.1.3 Immobilization, Simulation, and Localization

Patients will be positioned in a stable position capable of allowing accurate reproducibility of the target position from treatment to treatment. A variety of immobilization systems may be used, including stereotactic frames that surround the patient on three sides and large rigid pillows (conforming to patients' external contours) with reference to the stereotactic coordinate system. Patient immobilization must be reliable enough to ensure that the gross tumor volume (GTV) does not deviate beyond the confines of the planning treatment volume (PTV) with any significant probability (i.e., < 5%).

For thoracic and upper abdominal radiotherapy targets, special considerations will be made to account for the effect of internal organ motion (e.g., breathing) on target positioning and reproducibility. Acceptable maneuvers include reliable abdominal compression, accelerator beam gating with the respiratory cycle, active breath-holding techniques, and use of 4D simulation CT to generate internal target volumes (ITVs). Internal organ inhibition maneuvers must be reliable enough to ensure that the GTV does not deviate beyond the confines of the PTV with any significant probability (i.e., < 5%).

Computed tomography will be the primary image platform for targeting and treatment planning. Axial acquisitions with gantry 0 degrees and spacing ≤ 3.0 mm between scans in the region of the tumor are required. Images will be transferred to the treatment planning system for radiotherapy planning.

Verification CT scans should be obtained immediately before each treatment to ensure proper alignment of the geometric center (isocenter) of the simulated fields. Verification CT scans and/or portal films during or following each treatment may be acquired at the discretion of the treating physician.

5.1.4 Target Volumes

Parenchymal lung tumors greater than 7 cm in maximal diameter and other lesions greater than 5 cm in maximal diameter cannot serve as SBRT target lesions for this trial. Study subjects with lesions exceeding these size criteria are considered to have "Extensive" disease and will not receive SBRT to all active lesions. A smaller lesion must be chosen as the SBRT target for such patients.

Gross tumor volumes (GTVs) for target lesions will be drawn on simulation CT imaging, using appropriate window levels. Other imaging modalities (e.g., PET, MRI) imaging may be fused to simulation CT scans to aid with localization of target lesions.

Respiratory motion management for thoracic and upper abdominal tumors is required. A deep inspiration breath hold (DIBH) technique may be utilized to eliminate respiratory motion. In other cases, 4D CT simulation will be employed. If 4D CT simulation is used, GTVs should be generated on each CT phase that will be used for treatment (typically 4/10 phases if respiratory gating is employed or 10/10 phases if the patient will be treated while breathing freely). These GTVs will be combined to form internal target volumes (ITVs).

No clinical target volumes (CTVs) will be employed in this protocol.

GTVs or ITVs will be expanded to form planning target volumes (PTVs), using expansions of 4-5 mm in all directions. Expansion may be increased by up to 5 additional mm in the craniocaudal directions if there is concern for unmeasured respiratory motion (e.g., in cases of limited 4D CT quality for thoracic or upper abdominal targets).

5.1.5 SBRT Dosing

SBRT doses for this study follow NCCN guidelines[75] and cooperative group recommendations[33].

Lung tumors:

Schedule	Criteria
34 Gy x 1	Peripheral tumor measuring ≤ 2 cm in greatest dimension and > 1 cm from the chest wall
18 Gy x 3	Peripheral tumor measuring ≤ 5 cm and not eligible for 34 Gy x 1 fraction
10 Gy x 5	Central or peripheral tumor not eligible for 34 Gy x 1 fraction or 18 Gy x 3 fractions

A "central" lung tumor will be defined based on the eligibility criteria for RTOG 0813[34] ("Seamless Phase I/II Study of Stereotactic Lung Radiotherapy (SBRT) for Early Stage, Centrally Located, Non-Small Cell Lung Cancer (NSCLC) in Medically Inoperable Patients"): Tumor within or touching the zone of the proximal bronchial tree, defined as a volume 2 cm in all directions around the proximal bronchial tree (carina, right and left main bronchi, right and left upper lobe bronchi, intermedius bronchus, right middle lobe bronchus, lingular bronchus right and left lower lobe bronchi). [See figure below] Tumors that are immediately adjacent to mediastinal or pericardial pleura (PTV touching the pleura) also are considered central tumors. Tumors that are not "central" are considered to be "peripheral."

Other target lesions:

Schedule	Target Lesion Location
10 Gy x 3	Spinal or Paraspinal
10 Gy x 3	Osseous
10 Gy x 5	Mediastinal or Cervical Lymph Node
15 Gy x 3	Liver
15 Gy x 3	Abdominal or Pelvic (lymph node or adrenal gland)

5.1.6 Target Coverage

At least 95% of each PTV should receive the full prescription dose. If this cannot be achieved while respecting normal tissue constraints, the prescription dose may be lowered by up to 30%. Alternatively, the portion of the PTV abutting/overlapping a radiosensitive structure may be excluded from the target coverage evaluation structure ("PTV_eval").

5.1.7 Critical Structures

Normal tissue structures visualized on the simulation CT will be delineated using contouring guidelines established by RTOG:
https://www.rtog.org/CoreLab/ContouringAtlases.aspx

The following normal tissue constraints, adopted from 2020 NCCN guidelines[75] and RTOG 0813[34], will be used for planning SBRT to lung tumors:

	1 Fraction	3 Fractions	5 Fractions
Brachial plexus	max dose < 17.5 Gy	max dose < 24 Gy	max dose < 32 Gy
Esophagus	max dose < 15.4 Gy	max dose < 30 Gy	max dose < 52.5 Gy
Great vessels	max dose < 37 Gy	max dose < 39 Gy	max dose < 52.5 Gy
Heart/pericardium	max dose < 22 Gy	max dose < 30 Gy	max dose < 52.5 Gy
Lungs	At least 1500 cc receiving < 12.5 Gy	At least 1500 cc receiving < 12.5 Gy	At least 1500 cc receiving < 12.5 Gy
Skin	max dose < 26 Gy	max dose < 24 Gy	max dose < 32 Gy
Spinal cord	max dose < 14 Gy	max dose < 18 Gy	max dose < 30 Gy
Stomach	max dose < 12.4 Gy	max dose < 27 Gy	max dose < 35 Gy
Trachea and proximal bronchi	max dose < 20.2 Gy	max dose < 30 Gy	max dose < 52.5 Gy

The following normal tissue constraints, adopted from NRG-BR001[33,92], will be used for planning SBRT to other target lesions:

OAR Dose Limits for 3 fraction SBRT

Serial Organ	Volume	Volume Dose (Gy)	Avoidance Endpoint (Reference)
Spinal Cord	<0.03 cc	22.5	Myelitis (Timmerman)
	<1.2 cc	13	Myelitis (Timmerman)
Ipsilateral Brachial Plexus	< 0.03 cc	26	Brachial Plexopathy (Timmerman)
	<3 cc	22	Brachial Plexopathy (Timmerman)
Cauda Equina	<0.03 cc	25.5	Neuritis (Timmerman)
	<5 cc	21.9	Neuritis (AAPM TG-101)
Sacral Plexus	<0.03 cc	24	Neuropathy (AAPM TG-101)
	<5 cc	22.5	Neuropathy (AAPM TG-101)
Trachea and Ipsilateral Bronchus*	<0.03 cc	30	Stenosis/Fistula (Z4099)
	<5cc	25.8	Stenosis/Fistula (Timmerman)
Esophagus*	<0.03 cc	27	Stenosis/Fistula (Timmerman 2006 /RTOG 0618)
	<5cc	17.7	Stenosis/Fistula (Z4099)
Heart/Pericardium	<0.03cc	30	Pericarditis (Z4099)
	<15 cc	24	Pericarditis (Z4099)
Great vessels*	<0.03cc	45	Aneurysm (Z4099)
	<10 cc	39	Aneurysm (Z4099)
Skin	<0.03cc	33	Ulceration (Z4099)
	<10cc	31	Ulceration (Timmerman)
Stomach	<0.03cc	30	Ulceration/Fistula (Timmerman)
	<10cc	22.5	Ulceration/Fistula (Timmerman)
Duodenum*	<0.03cc	24	Ulceration (Timmerman 2006)
	<10cc	15	Ulceration (Timmerman 2006)
Bowel*	<0.03 cc	34.5	Ulceration (Timmerman)
	<20cc	24	Colitis/Fistula (Z4099)
Rectum*	<0.03 cc	49.5	Ulceration (Timmerman)
	<3.5 cc	45	Proctitis/Fistula (Timmerman)
	< 20 cc	27.5	Proctitis/Fistula (Timmerman)
Bladder	0.03cc	33	Cystitis/Fistula (Timmerman)
	<15 cc	16.8	Cystitis/Fistula (AAPM TG-101)
Ureter	<0.03 cc	40	Stenosis (Timmerman)
Penile bulb	< 3cc	25	Impotence (Timmerman)
Femoral heads	<10 cc	24	Necrosis (Timmerman)
Bile duct	< 0.03 cc	36	Stenosis (Timmerman)
Renal hilum/vascular trunk	<15 cc	19.5	Malignant Hypertension (Timmerman)
Rib	< 0.03 cc	50	Pain or Fracture (Timmerman
	<5 cc	40	Pain or Fracture (Timmerman
Parallel Organ	**Volume**	**Volume Dose (Gy)**	**Avoidance Endpoint (Reference)**
Lung (total)	<15% lung volume	20	Pneumonitis/Lung Function (RTOG 0618)
	< 37% lung volume	11	Pneumonitis (Timmerman)
	1500 cc	10.5	Basic Lung Function (Z4099)
	1000 cc	11.4	Pneumonitis (Z4099)
Ipsilateral kidney	<130 cc	12.3	Nephritis (Timmerman 2006)
Total Kidney	<200cc	15	Basic Renal Function (Timmerman)
Liver	<700 cc	17.1	Liver function (Timmerman 2006/Z4099)

*NOTE: Avoid circumferential irradiation.

OAR Dose Limits for 5 fraction SBRT

Serial Organ	Volume	Volume Dose (Gy)	Avoidance Endpoint
Spinal Cord	<0.03 cc	28	Myelitis (Timmerman)
	<0.35 cc	22	Myelitis (Timmerman)
	<1.2 cc	15.6	Myelitis (Timmerman)
Ipsilateral Brachial Plexus	< 0.03 cc	32	Brachial Plexopathy (RTOG 0813)
	<3 cc	30	Brachial Plexopathy (RTOG 0813)
Cauda Equina	<0.03 cc	32	Neuritis (AAPM TG-101)
	<5 cc	30	Neuritis (AAPM TG-101)
Sacral Plexus	<0.03 cc	32	Neuropathy (AAPM TG-101)
	<5 cc	30	Neuropathy (AAPM TG-101)
Trachea and Ipsilateral Bronchus*	<0.03cc	40	Stenosis/Fistula (Timmerman)
	<5cc	32	Stenosis/Fistula (RTOG 0813)
Esophagus*	<0.03cc	35	Stenosis/Fistula (Timmerman)
	<5 cc	27.5	Stenosis/Fistula (RTOG 0813)
Heart/Pericardium	<0.03 cc	38	Pericarditis (Timmerman)
	<15 cc	32	Pericarditis (RTOG 0813)
Great vessels*	<0.03 cc	53	Aneurysm (Timmerman)
	<10 cc	47	Aneurysm (RTOG 0813)
Skin	< 0.03cc	38.5	Ulceration (Timmerman)
	< 10cc	36.5	Ulceration (Timmerman)
Stomach	< 0.5cc	35	Ulceration/Fistula (Timmerman)
	< 5cc	26.5	Ulceration/Fistula (Timmerman)
Duodenum*	< 0.5 cc	30	Ulceration (RTOG 1112)
	< 5 cc	18.3	Ulceration (Timmerman 2006)
Bowel*	< 0.03 cc	40	Ulceration (Timmerman)
	<20 cc	28.5	Colitis/Fistula (Timmerman)
Rectum*	<0.03 cc	55	Ulceration (Timmerman)
	<3.5 cc	50	Proctitis/Fistula (Timmerman)
	<20 cc	32.5	Proctitis/Fistula (Timmerman
Bladder	< 0.03	38	Cystitis/Fistula (Timmerman)
	<15 cc	20	Cystitis/Fistula (Timmerman)
Ureter	< 0.03 cc	45	Stenosis (Timmerman)
Penile Bulb	<3 cc	30	Impotence (Timmerman)
Femoral head	<10 cc	30	Necrosis (Timmerman)
Bile Duct	<0.03 cc	41	Stenosis (Timmerman)
Renal hilum/Vascular Trunk	<15 cc	23	Malignant Hypertension (Timmerman)
Rib	<0.03 cc	57	Pain or Fracture (Timmerman)
	<5 cc	45	Pain or Fracture (Timmerman)
Parallel Organ	**Volume**	**Volume Dose (Gy)**	**Avoidance Endpoint**
Lung (total)	< 37% lung volume	13.5	Pneumonitis (Timmerman)
	< 1500 cc	12.5	Basic Lung Function (RTOG 0813)
	< 1000 cc	13.5	Pneumonitis (RTOG 0813)
Ipsilateral Kidney	< 130 cc	14.5	Basic Renal Function (Timmerman)
Total Kidney	< 200cc	18	Basic Renal Function (Timmerman)
Liver	<700 cc	21	Liver Function (Timmerman)

*NOTE: Avoid circumferential irradiation.

5.2 CDX-301 and CDX-1140

5.2.1 Dosing and Administration

CDX-301

Each 2 mL clear borosilicate glass vial contains 2.5 mg/mL CDX-301 glycoprotein in a 1.0 mL (with an overfill to 1.2 mL) volume of buffered solution composed of 10 mM sodium phosphate, 140 mM NaCl, pH 7.0. CDX-301 Drug Product is formulated as a sterile solution intended for single-use subcutaneous administration.

An initial study drug shipment will be provided following receipt of all required documents. Additional supplies should be ordered with at least one week advance notice using a form supplied by Celldex.

All study supplies must be stored in a secured area with limited, approved access. CDX-301 temperature must be maintained between 2° to 8°C (36 to 46°F) and monitored daily. Deviations in storage conditions must be reported to IIR@celldex.com within 24 hours of awareness.

The individual dose is calculated using the actual baseline body weight of the subject according to the calculations below. All subjects will receive 75 µg/kg of CDX-301 at each administration.

Body Weight (kg) x Dose Level (75µg/kg) = Desired Dose (µg)
Desired Dose (µg) ÷ 2500 µg/mL = Volume of CDX-301 (mL)

For example, for an 80 kg subject, the total dose required to deliver the 75 µg/kg dose would be 6000 µg. The calculated volume required from the 2500 µg/ml CDX-301 stock solution to obtain 6000 µg of CDX-301 would be 2.4 ml. Three vials would be needed to administer this dose.

CDX-301 liquid should be withdrawn from the vial gently, avoiding foaming and excess shearing. As the maximum volume of any individual injection will be 2.0 ml, the dose may be split into up to 3 separate syringes/subcutaneous injections as needed to administer the required volume.

CDX-301 will be administered as a daily subcutaneous injection. The injection site may be rotated between the extremities. CDX-301 injections should not be given to areas of skin with conditions such as scarring, tattoos or persistent injection site reactions that will not allow easy access for study drug administration or evaluation of localized adverse events. CDX-301 administration will be performed within the clinical setting by trained staff personnel.

CDX-1140

Each vial to be used for intravenous administration contains a nominal 3.0 mg/mL CDX-1140 protein in a 10.0 mL volume of buffered solution composed of sodium phosphate, potassium phosphate, potassium chloride, sodium chloride, and polysorbate 80, with a pH of 7.0.

CDX-1140 must be stored at ≤ -65°C (-85°F) until use. CDX-1140 should be protected from light. However, sufficient light protection is provided by the secondary

container (carton); no specific light protection is needed during preparation of the dosing solution and infusion.

Upon removal from storage at ≤ -65°C, vials should be thawed at ambient temperature for approximately 1.5 hours. After equilibration to room temperature and before use, the vial should be gently swirled to ensure uniform mixing of the contents. Do not invert or shake vials. Solution should be gently withdrawn (avoiding foaming and excess shearing).

CDX-1140 should be administered as a 90-minute infusion using a volumetric pump with a 0.22 micron pore size, low-protein binding polyethersulfone (PES) membrane in-line filter.

The CDX-1140 dose will be calculated using the actual baseline body weight of the patient. The following formula should be used to calculate the volume of CDX-1140 required for each administration:

Body Weight (kg) x Desired Dose (mg/kg) ÷ 3 mg/mL = Volume of CDX-1140 (mL)

For example, for an 80 kg subject, the total dose required to deliver the 1.5 mg/kg dose would be 120 mg. the calculated volume required from the 3 mg/mL CDX-1140 stock solution to obtain 120 mg would be 40 mL. As each vial of CDX-1140 contains a withdrawable volume of 10 mL, administration of the 1.5 mg/kg dose would require 4 vials of CDX-1140.

5.2.2 Treatment Schedule

CDX-301 and CDX-1140 will only be administered to subjects on Arm 1. Subjects on Arm 2 will not “crossover” if disease progression is observed. The Study Calendar (Section 4.2) describes the timing of study drug administrations.

Some subjects on Arm 1 may receive more than one “cycle” of CDX-301, CDX-1140, and SBRT (targeting a lesion not previously treated with SBRT). The decision to administer treatment cycles beyond cycle 1 must be agreed upon by the treating physicians, the principal investigators, and the study subject. Additional treatment cycles will not be offered to subjects who develop disease progression within four months of receiving a previous cycle of study therapy or to subjects who have no evidence of active disease. Subjects who previously experienced DLT or intolerable toxicity related to CDX-1140 may be treated with CDX-301 and SBRT and without CDX-1140 in subsequent treatment cycles.

For subjects who receive more than one cycle of study therapy, the schedule of events will reset at the beginning of each cycle. At least 8 weeks must separate the start of each treatment cycle.

CDX-301

Five injections of CDX-301 should be administered daily during Week 1 (Monday-Friday) of a treatment cycle. If that is not possible due to department closure for a national holiday, inclement weather, or another reason that is not treatment-related toxicity, CDX-301 treatment may extend to Week 2 (though not beyond 10 days after the first CDX-301 dose).

CDX-1140

The first CDX-1140 will take place 4 days (+/- 1 day) after the last dose of CDX-301. As CDX-301 will typically be given from Monday-Friday, CDX-1140 will usually be given on a Tuesday. The second dose of CDX-1140 will be given 4 weeks (+/- 3 days) after the first CDX-1140 dose. CDX-1140 will not be administered on a Friday, as a clinic visit the day after each CDX-1140 dose is required.

5.2.3 Medications to be given before/after study drug administration

CDX-301

No premedication is required for CDX-301.
No medication is required after CDX-301 injections.

CDX-1140

Prophylactic premedication is required, at least 30 minutes prior to administration of CDX-1140:

- diphenhydramine 25/50 mg (or equivalent antihistamine)
- 500 to 750 mg paracetamol (acetaminophen)
- naproxen sodium 250 mg (or equivalent NSAID), at least 30 minutes prior to CDX-1140 administrations.
- Ranitidine 150 mg (or equivalent) and/or an anti-emetic (ondansetron 8 mg, or equivalent) may also be incorporated into the premedication regimen.
- Prophylactic premedication with corticosteroids is to be avoided because of the potential to attenuate the anti-tumor activity of CDX-1140.

Post-infusion medication is required over the 24-48 hours after each CDX-1140 infusion:

- Alternating oral acetaminophen (1000 mg) and non-steroidal anti-inflammatory (e.g., ibuprofen 200 mg) q6h.
- An antihistamine and anti-emetic may also be utilized if clinically indicated.
- Patients should be evaluated to determine if continuation of post-infusion medication is needed when they are seen on the Cycle 1 Day 2 visit.
- The potential for toxicity with these drugs should be recognized, and appropriate precaution should be utilized in patients with risk factors for toxicity, such as patients with peptic ulcers, increased LFTs, or increased creatinine. Also, care should be taken to medicate for no longer than is necessary, to avoid adverse events.

5.2.4 Potential Toxicities and Toxicity Management

Potential toxicity and guidance for the management of toxicity is summarized below. However, the CDX-1140 and CDX-301 Investigator Brochures (IB) are the Single Reference Safety Documents that provide complete and relevant information about the known safety profile of CDX-1140 and CDX-301.

CDX-301

CDX-301 was generally well-tolerated in all prior studies, including our prior study of CDX-301 and SBRT for advanced NSCLC, which utilized the same CDX-301 dose/schedule as the present trial. The most frequently observed toxicities related to CDX-301 have been asymptomatic lymphadenopathy and mild injection site reactions.

Injection site reactions have been infrequent and mild with CDX-301. No injection site reactions were observed in our recent trial combining CDX-301 and SBRT (29 subjects, 180 injections). Generally mild to moderate injection site reactions were reported in studies using AMG949. Pre-medication with diphenhydramine has been reported to be effective in the prevention of pruritic and erythemic reactions[93] and may be considered for subjects who experience local reactions after treatment with CDX-301. Injection site reactions may also be treated with analgesics (i.e., Tylenol). If there is injection site pain, a numbing cream such as Emla cream can be used prior to administration of CDX-301. Patients will be monitored for 30 minutes following each CDX-301 injection.

Leukocytosis, especially monocytosis, is expected during administration of CDX-301. Hematological parameters, including WBC and differential, should be monitored in subjects receiving CDX-301. CDX-301 should not be administered if marked leukocytosis, e.g. WBCs greater than 50,000 cells/mm^3, is observed.

CDX-1140

The CDX1140-01 study was the first clinical trial with CDX-1140, and therefore there is limited clinical experience to define expected toxicities. Management of drug-related adverse events and serious adverse events should be in accordance with the institution's standard of care and guidelines for managing immune related toxicities[94].

CDX-1140 infusion should take place in a monitored setting that has ready access to an intensive care unit in case of a severe infusion reaction. All subjects should be monitored for 2 hours after each administration of CDX-1140; patients who experience any treatment-related adverse events during the observation period should be further monitored as clinically appropriate. CDX-1140 will be administered once every four weeks. CDX-1140 will be administered intravenously over 90 minutes using a 0.22 micron in-line filter. The dose of study treatments will be calculated based on actual weight at screen and may remain constant throughout the study, unless greater than 10% change in weight is observed. The infusion duration can be increased by 30-minute intervals up to 180 minutes if warranted due to prior infusion reactions.

In the phase I trial testing CDX-1140, infusions were generally well-tolerated. Drug-related adverse events have been observed in the post-infusion period, typically within 24-48 hours after completing the infusion. Symptoms have included fever, fatigue, nausea, chills, and arthralgias. As of May 28th, 2020, there were 4 grade 2 and 2 grade 3 cytokine release syndrome adverse events. These adverse events were treated with corticosteroids, and in one case, tocilizumab. It is therefore recommended that medication to treat infusion reactions, including severe infusion reactions/cytokine release syndrome, be readily available at the time of the study drug infusion. All patients should be monitored for 2 hours after each administration of CDX-1140; patients who experience any treatment-related adverse events during the observation period should be further monitored as clinically appropriate.

Recommendations for the management of peri-infusional reactions are provided below and may be modified based on local treatment standards and guidelines, as appropriate. Infusion reactions should be graded according to NCI-CTCAE (Version 5.0) guidelines, http://ctep.cancer.gov. Serum should be obtained at the time of an infusion reaction/cytokine release syndrome. Patients who experience a grade 4 infusion reaction or a grade 3 infusion reaction lasting > 6 hours will permanently discontinue CDX-1140, and the adverse event will be considered a DLT.

- For Grade 1 symptoms (Mild transient reaction; infusion interruption not indicated; intervention not indicated):
  - Remain at bedside and monitor patient until recovery from symptoms.
- For Grade 2 symptoms (infusion interruption indicated but responds promptly to symptomatic treatment [e.g., antihistamines, nonsteroidal anti-inflammatory drugs, narcotics, corticosteroids, i.v. fluids]; prophylactic medications indicated for ≤ 24 hours):
  - Stop the CDX-1140 infusion, begin an i.v. infusion of normal saline, and treat the patient with diphenhydramine 50 mg i.v. (or equivalent) and/or 500 to 1000 mg paracetamol/acetaminophen; remain at bedside and monitor patient until resolution of symptoms. Corticosteroid therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor patient closely. If symptoms recur, then no further CDX-1140 will be administered at that visit. The amount of study drug infused must be recorded on the case report form (CRF). Patients who experience an adverse event, including an infusion reaction of Grade 2, during the 4 to 6 hour observation period that does not resolve during this time should be observed for 24 hours or until the adverse event resolves with vital sign measurements every 4 hours and additional evaluations as medically indicated for the management of the adverse event.
- For Grade 3 or Grade 4 symptoms (Grade 3: prolonged [i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [e.g., renal impairment, pulmonary infiltrates]. Grade 4: life-threatening consequences; urgent intervention indicated):
  - Immediately discontinue infusion of CDX-1140. Begin an i.v. infusion of normal saline, and treat the patient as follows: Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for i.v. administration, and/or diphenhydramine 50 mg i.v. with methylprednisolone 100 mg i.v. (or equivalent), as needed. Tocilizumab should be considered for patients with severe or prolonged infusion reactions/cytokine release syndrome who are not responding to appropriate therapy.

Subjects with peri-infusional reactions should be monitored until the Investigator is comfortable that the symptoms will not recur. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Patients who experience an

infusion reaction of Grade ≥ 3, regardless of resolution, will be observed for 24 additional hours or until the adverse event resolves with vital sign measurements every 4 hours and additional evaluations as medically indicated for the management of the adverse event. In the case of late-occurring hypersensitivity symptoms (e.g., appearance of a localized or generalized pruritus within 1 week after treatment), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids). CDX-1140 will be permanently discontinued for patients with Grade 4 infusion reactions or Grade 3 infusion reactions lasting more than 6 hours.

5.2.5 Dose Modifications

CDX-301

There will be no CDX-301 dose modifications. If discontinued, CDX-301 will not be reinstituted. CDX-301 should not be administered if marked leukocytosis, e.g., WBCs greater than 50,000 cells/mm^3, is observed. Any ≥ Grade 3 injection site reactions thought related to CDX-301 warrant discontinuation of CDX-301 treatment and will be considered a DLT. If CDX-301 is discontinued due to a DLT, treatment with CDX-1140 may continue in the absence of a CDX-1140-related DLT.

CDX-1140

CDX-1140 dose modifications are not allowed. The second CDX-1140 infusion should only be administered if the subject is receiving ≤ 10 mg/day prednisone or equivalent for treatment of drug related toxicity, and all toxicity related to prior treatment (including laboratory abnormalities) has resolved to ≤ grade 1, with the following exceptions:

- Subjects may receive treatment in the presence of grade 2 fatigue
- Subjects who have not experienced a grade 3 drug-related skin AE may receive treatment in the presence of grade ≤ 2 skin toxicity
- Subjects with baseline grade 1 AST/ALT or total bilirubin elevation may receive treatment in the presence of grade 2 AST/ALT or total bilirubin elevation
- Grade 2 drug-related pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is received
- Patients with drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may receive treatment
- Asymptomatic lymphopenia
- Clinically asymptomatic increased amylase or lipase. The medical monitor should be consulted in the event of grade 4 elevations in amylase or lipase.

5.2.6 Additional Warnings and Precautions

CDX-301

Drug-drug Interactions

Interactions between CDX-301 and other drugs have not been fully evaluated.

Allergic Reaction

CDX-301 is a fully human protein, and it is unlikely that it will induce a hypersensitivity reaction. No such reactions have been observed in the CDX-301 programs to date; nor were any such reactions reported in the AMG949 clinical development program. If such reactions were to be observed, possible manifestations could include fever, chills, rash, rigors, pruritus, or other symptoms.

Immunogenicity

None of the 30 healthy volunteers treated in the Phase I trial with CDX-301 (5 to 10-day regimen) developed anti-rhuFlt3L antibody. In the Celldex-sponsored pilot study, CDX301-03, none of the four donors administered CDX-301 had detectable anti-rhuFLT3L antibodies at any collection time point.

The immunogenicity of CDX-301 has also been investigated in two investigator-sponsored studies. In CDX301-51, the effects of intratumoral injection of CDX-301 and poly-ICLC were studied in low grade B-cell lymphoma. Samples were collected from 15 patients for anti-CDX-301 immunogenicity testing. One patient has been found positive for anti-CDX-301 immunoreactivity. To date, no neutralizing responses have been observed. Immunogenicity samples were collected from 30 patients in CITN-07 (CDX1401-54). Of the 144 total samples collected, none were found to exhibit a specific anti-CDX-301 response.

Approximately 16% of subjects treated with AMG949 in Immunex sponsored studies and tested for immune response were noted to develop anti-rhuFlt3L antibody. Anti-rhuFlt3L antibodies appeared to develop more frequently in studies with greater exposure to AMG949. However, none of the anti-rhuFlt3L antibodies were neutralizing.

In theory, neutralizing antibodies could develop following administration of CDX-301, with potential consequences of neutralizing subsequent CDX-301 effects, neutralization of endogenous Flt3L activity that could result in bone marrow suppression, inhibition or enhancement of immune responses, and/or result in immune complex mediated disease. Although this scenario is considered extremely unlikely to occur with CDX-301, patients will continue to be monitored for anti-CDX-301 antibodies and whether these antibodies are neutralizing.

Coagulation

No thrombotic events have been reported as potentially related to CDX-301 in the Celldex clinical development program to date. Thrombotic events (hypercoagulability, IV line thrombosis, and venous thrombosis) were observed rarely in oncology patients (n = 6) receiving AMG949 and was more common when combined with GM-CSF or G-CSF. Low grade thrombocytopenia has also been observed following AMG949 administration. Evaluation of coagulation parameters and clotting factors in several of these patients did not reveal any abnormalities.

Autoimmune Disease

Subjects with a known history of autoimmune disorders should not be given CDX-301, until further studies have determined the safety profile of CDX-301 in this patient population. Pre-clinical studies have demonstrated that rhuFlt3L may have a complicated role in regulating autoimmune diseases and can either exacerbate or ameliorate autoimmune disease manifestations. Therefore, CDX-301 may have the potential to exacerbate autoimmune disorders by its immunomodulatory effects.

Subjects administered CDX-301 will be monitored for the development of potential autoimmune phenomena, such as hyper- or hypothyroidism.

Development of Immune Tolerance

CDX-301-mediated increases in immature dendritic cells and/or Treg cells could potentially be tolerogenic. Therefore, CDX-301 could potentially have deleterious effects in an infectious disease setting or other pre-existing or developing disease. AMG949 worsened the outcome in a mouse Streptococcus pneumoniae pneumonia challenge model[95], indicating that rhuFlt3L treatment could pose a risk for increased lung injury associated with S pneumoniae pneumonia. Study subjects should be monitored for the development of infections conditions.

rhuFlt3L may also be associated with a differential response to vaccines. In another murine model, intramuscular injection of rhuFlt3L in combination with a hepatitis B vaccine suppressed the antibody response to the vaccine in a dose-dependent manner, although in situ delivery by plasmid increased the antibody response[96]. In a clinical study in which healthy volunteers were administered hepatitis B vaccine and either placebo or AMG949, there was no inter-group difference in hepatitis B antibody responses, demonstrating that AMG949 neither augmented or tolerized humoral responses to the vaccine.

Leukemia

There are data showing that prophylactic treatment with rhuFlt3L can prevent or abrogate leukemic activity[97]. There also are data showing the expression of Flt3 and the Flt3L receptor, on some leukemias, and extensive data that support an association between an increase in proliferation or suppression of apoptosis in clinical leukemias that bear constitutively activated Flt3 mutations. These mutations do not appear to be causal for AML[98-101]. It is not known whether the addition of rhuFlt3L to patients with leukemia expressing Flt3 will promote leukemic proliferation, or whether the ligand can promote the development of leukemia in people without pre-existing leukemia. Since the effect of administered rhuFlt3L is transient, it would seem unlikely to have any persistent effects and no cases of leukemia were reported after rhuFlt3L administration in previous studies.

Pregnancy, Carcinogenesis, and Teratogenicity

The effects of CDX-301 on fertility, pregnancy, or the unborn fetus have not been determined. It is not known if CDX-301 is excreted in milk or can cross the placenta. For this reason, pregnant and nursing women should not receive CDX-301. Women of childbearing potential treated with CDX-301 must take adequate contraceptive measures.

CDX-1140

Most side effects associated with CDX-1140 to date have occurred in the 24-48 hours following the CDX-1140 infusion, been mild to moderate, and have resolved with or without medications such as acetaminophen, NSAIDS, and/or anti-nausea medication. In a few cases, the side effects have been severe and have required hospitalization and treatment with corticosteroids or other immunosuppressive medications. Treatment related adverse events that have occurred in > 10% of patients include fatigue, arthralgia, nausea, chills, fever, diarrhea, myalgia, vomiting, AST increase, and ALT increase.

Cytokine Release Syndrome

2 patients who received CDX-1140 monotherapy and 4 patients who received CDX-1140 in combination with CDX-301 have experienced cytokine release syndrome. Symptoms included fever, hypotension, hypoxia, fatigue, headache, and nausea.

Pneumonitis

4 patients who received CDX-1140 monotherapy experienced severe treatment-related pneumonitis, with 3 of the 4 developing pneumonitis during the first week of treatment. These patients developed shortness of breath, and imaging of the lungs demonstrated new inflammatory changes. Two patients required intubation. All four patients were treated with high dose corticosteroids, and two of the patients subsequently died, due to a documented infection in one patient and presumed infection in the other. Two patients demonstrated clinical improvement temporally associated with the administration of tocilizumab. Of note, tocilizumab has not been incorporated into the treatment guidelines for management of pneumonitis associated with immune checkpoint blockade therapy,[102] but there is a case series suggesting that tocilizumab is effective in the management of corticosteroid-refractory pneumonitis irAEs[103]. Based on the rapid response temporally associated with the administration of tocilizumab in the cases discussed above, and the case series suggesting that tocilizumab may be beneficial in treating checkpoint-related pneumonitis, tocilizumab should be considered as a therapeutic option in those patients with CDX-1140 related, steroid-refractory pneumonitis, as well as for other high grade steroid-refractory adverse events.

Thrombosis

CD40 is functionally expressed on platelets and endothelial cells, and CD40 signaling could theoretically promote a pro-thrombotic state. In the CDX1140-01 study, 1 patient developed a deep vein thrombosis and pulmonary embolism that was considered related to CDX-1140. Administration of CP-870,893, another agonist anti-CD40 mAb, was associated with transient increases in D-dimer and thrombin-antithrombin 3 complex formation levels at the 2 highest doses tested (0.2 and 0.3 mg/kg) in the Phase 1 study[63]. Standard coagulation parameters were not affected by CP-870,893. One patient with a prior history of deep vein thrombosis had a drug-related thromboembolic dose-limiting toxicity.

Elevated liver enzymes

Increases in liver function tests in the 24-48 hours following the CDX-1140 are an expected pharmacodynamic effect of the drug. The increases are usually low-grade, but can be grade 3, and liver function tests typically return to approach baseline within a week. Treatment of these transient changes is not indicated. More significant changes in laboratory tests may be followed more closely, if clinically warranted, and may require intervention per irAE guidelines[102,104] if the changes are associated with clinical symptoms or persistent

Decreased lymphocyte and monocyte counts

Decreases in lymphocyte and monocyte counts are a pharmacodynamic effect of CDX-1140, occurring within 24-48 hours of a dose and typically recovering by 1 week. Decreases in B cells, as determined by flow cytometry, predominates.

Other Adverse Events

Based on a review of the relevant literature and the proposed mechanism of action of CDX-1140 in binding to CD40 expressing cells and enhancing immune reactions, the following additional adverse effects may be encountered:

- Infusion reactions/cytokine release syndrome/hypersensitivity reactions
  - As mentioned above, infusion reactions are likely to occur and may include chills, rigors, fever, rash, nausea, vomiting, myalgias, headache, and hypotension. Obtain serum at the time of an infusion reaction/cytokine release syndrome.
  - As discussed above, patients are to be pre-medicated with antihistamine, acetaminophen, and/or NSAID at least 30 minutes prior to CDX-1140 administrations. Prophylactic premedication with corticosteroids is to be avoided with CDX-1140 because of the potential to attenuate antitumor activity. In the 24 hours following the CDX-1140 infusion, alternate oral acetaminophen (1000 mg) and non-steroidal anti-inflammatory (e.g., ibuprofen 200 mg) q6h. An antihistamine and anti-emetic may also be utilized if clinically indicated.
- Immune-related adverse events (irAEs)
  - An irAE is defined as a clinically significant adverse event of any organ that is associated with study drug exposure, of unknown etiology, and is consistent with an immune-mediated mechanism. Serologic, immunologic, and histologic (biopsy) data should be used to support an irAE diagnosis. Appropriate efforts should be made to rule out neoplastic, infectious, metabolic, toxin, or other etiologic causes of the adverse event. Most commonly irAEs involve skin, gastrointestinal (GI) tract, endocrine organs and liver, but can involve any organ system. irAEs typically clinically manifest several weeks to a few months following initiation of immune enhancing anti-tumor therapy. Possible clinical manifestations include:
    - Constitutional: fever, fatigue
    - GI: diarrhea, colitis, constipation, hematochezia, melena, abdominal pain, pancreatitis
    - Skin: rash, pruritus, alopecia, desquamation
    - Liver: elevated liver enzymes, hepatitis

- Endocrine: hypothyroidism, hyperthyroidism, adrenal insufficiency, hypophysitis, hypogonadism, diabetes
- Ocular: conjunctivitis, iritis, uveitis
- Others: neuropathy, including Guillain-Barre syndrome, hemolytic anemia, thrombocytopenia, pneumonitis

Guidelines have been published for the management of irAEs associated with immunotherapy,[102,104,105] and investigators should be familiar with the workup and treatment of irAEs. Obtain serum at the time of any ≥ Grade 3 suspected drug related irAE.

5.2.7 Concomitant Therapy

Subjects may continue to use any ongoing medications not prohibited by the inclusion/exclusion criteria. However, efforts should be made to maintain stable doses of concomitant medications during study treatment. Subjects must be instructed not to take any medications, including over-the-counter products, without first consulting with the investigator.

Investigators may prescribe concomitant medications or treatments deemed necessary to provide adequate supportive care. However, while on study, when clinically appropriate, subjects should strictly follow the study-prescribed treatment regimen in accordance with the following guidance:

- Concurrent administration of any anticancer therapies and/or other investigational agents are prohibited throughout the treatment period.
  - Following treatment, but prior to documented progression of disease, additional anticancer therapies should be avoided if clinically feasible. If additional anticancer therapies are needed, it should be discussed and agreed upon with the Principal Investigator prior to administration.
  - Following progression of disease, subjects may receive any appropriate alternate therapies.
- During study treatment, subjects may receive supportive care to include bisphosphonates, hematologic, and anti-infectious support and pain management.
- Thoracentesis or paracentesis may be performed if needed for comfort. If surgical intervention or localized radiation become indicated (either for palliation or down-staging of previously non-resectable tumor), these interventions are permitted, but should be avoided if clinically feasible until after the second response assessment, following consultation with the Principal Investigator. A tumor response assessment should be conducted prior to any intervention, in order to document progression and/or confirm an objective response. Subjects who undergo surgical resection or radiation in the absence of progression may continue to receive study treatment until remaining lesions meet criteria for progression of disease. Platelet counts should be checked immediately before any invasive intervention, as CDX-1140 may a decrease in platelet count in the days following each infusion.
- Immunosuppressive agents are prohibited during the study, with the following exceptions:

- Immunosuppressive agents and the use of systemic corticosteroids are permitted in the context of treating AEs. Subjects receiving corticosteroids for treatment of drug-related AEs must be at ≤ 10 mg/day prednisone or equivalent prior to re- initiation of study therapy.
- Subjects are permitted the use of topical, ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption).

- Vaccinations:
  - Non-study vaccinations are prohibited until at least 28 days after the last dose of CDX-301. Otherwise, any vaccination containing live, attenuated, or inactivated virus may be permitted if clinically indicated. However, this must be discussed with the Principal Investigator prior to administration and may require a study drug washout period prior to and after administration of the vaccine. Inactivated influenza vaccination is permitted on study without restriction.
  - Some of the systemic adverse events attributed SARS-CoV2 vaccines, such as fever, fatigue, arthralgias, and myalgias, overlap with the adverse events associated with CDX-1140. Administration of the SARS-CoV2 vaccine in close temporal association with CDX-1140 may confound causality assessments for any accompanying adverse events. Therefore, administration of the SARS-CoV2 vaccination and CDX-1140 should be separated by at least 1 week, if possible. For CDX-301, if possible, the vaccine should be administered either at least 1 week prior to the initiation of CDX-301 or at least 3 weeks after the first dose in a cycle, and if eligible for another cycle of treatment, at least 1 week prior to the next cycle. The recommendation is based on the biological activity of CDX-301, which induces a transient increase in hematopoietic cells, including dendritic cell, that peaks at approximately 2 weeks from the initiation of treatment and then rapidly declines. No data is available on the potential impact of CDX-1140 or CDX-301 on the immunogenicity or adverse event profile of the SARS-CoV2 vaccines, therefore decisions to vaccinate patients while participating in this study will be individualized and based on the judgement of the treating investigator.

- Prophylactic premedication is required prior to CDX-1140 administration: diphenhydramine 25/50 mg (or equivalent anti-histamine), 500 to 750 mg paracetamol (acetaminophen), and naproxen sodium 250 mg (or equivalent NSAID) at least 30 minutes prior to CDX-1140 administrations. Ranitidine 150 mg (or equivalent) may also be incorporated into the premedication regimen. Prophylactic premedication with corticosteroids is to be avoided because of the potential to attenuate the anti-tumor activity of CDX-1140.

- Post-infusion medication is required following CDX-1140 administration: In the 24-48 hours following the CDX-1140 infusion, alternate oral acetaminophen (1000 mg) and non-steroidal anti-inflammatory (e.g., ibuprofen 200 mg) q6H. An anti-histamine and anti-emetic may also be utilized if clinically indicated. Patients should be evaluated to determine if continuation of post-infusion medication is needed when they are seen on the Cycle 1 Day 2 visit. Note, the potential for toxicity with these drugs and appropriate precaution should be utilized in patients with risk factors for toxicity, e.g. patients with peptic ulcers, increased LFTs, or increased creatinine. Also, care should be taken to medicate for no longer than is necessary in order to avoid

potential confounding factors in determining adverse event causality (e.g. increased LFTs).

- The effect of CDX-1140 on the absorption, metabolism, or excretion of other drugs has not been studied. As CDX-1140 is a human monoclonal antibody, inhibition or induction of cytochrome P450 (CYP) enzymes or other typical drug metabolizing enzymes is unexpected, and thus, interaction with other medications metabolized through these pathways is unlikely.
- For patients in Part 2, concomitant administration of CDX-301 with growth factors such as G-CSF should be done with caution and the CBC followed closely.

6.0 STUDY THERAPY – ARM 2

Stereotactic Body Radiotherapy for Limited Disease

Subjects on Arm 2 with "limited disease", as defined in Section 5.1.1, will be treated with SBRT to all sites of known active disease. SBRT details are provided in Section 5.1. SBRT will be delivered in the same fashion for both study arms.

Other radiotherapy

Subjects on Arm 2 with disease that is not amenable to comprehensive SBRT may be treated with any form of palliative radiotherapy, including SBRT, to address symptoms or prevent symptom progression. Treatment details will be left to the discretion of the managing physicians but are expected to align with institutional standards and established guidelines[75,106].

Systemic therapy

Subjects on Arm 2 with extensive disease burden are expected to receive a standard treatment regimen. Approved options at this time include docetaxel (+/- ramucirumab), pemetrexed, and gemcitabine[75]. Selection of the systemic treatment regimen will be left to the discretion of the treating physician. Assessments for subjects receiving standard systemic therapy should follow the Study Calendar (Section 4.2).

Subjects on Arm 2 with limited disease may receive systemic therapy after completion of SBRT or may undergo surveillance without active therapy. Systemic therapy should not be initiated until all planned SBRT is completed.

7.0 SUBJECT ASSESSMENTS

7.1 Clinical Assessments

Clinical assessments will take place according to the schedules depicted in the Study Calendars (Section 4.2) and will include complete histories and physical examinations. History and physicals may be obtained remotely. Additional visits may take place, and events that are noted at those visits(e.g., treatment-related toxicity) will be included in data analyses.

Adverse events will be scored at each clinic visit using CTCAE version 5.0. Any new or worsening toxicities will be classified as "unrelated" or "related" to study treatment (See Section 11.1). Adverse events will be documented in this manner for subjects on both study arms, so that adverse event rates with study therapy in Arm 1 can be compared to adverse event rates with standard therapy in Arm 2.

7.2 DLT Definition

The definition of a dose-limiting toxicity (DLT) will be nearly identical to the definition used in the recent study combining CDX-301 and CDX-1140[70]. A DLT will be defined as any of the following outcomes, attributed to study therapy (including SBRT) and occurring within 8 weeks after initiation of initial study therapy:

- Death
- Any ≥ Grade 3 non-hematological toxicity, with the following exceptions:
  - Grade 3 alopecia, vitiligo, or endocrinopathies controlled by hormone replacement therapy
  - Grade 3 nausea that resolves to ≤ grade 2 with or without treatment within 72 hours
  - Grade 3 vomiting and diarrhea that resolves to ≤ grade 2 with or without treatment within 72 hours
  - Grade 3 fatigue that resolves to ≤ grade 2 within 5 days
  - Grade 3 hypertension in the absence of maximal medical therapy
  - Grade 3 adverse event of tumor flare (defined as local pain, irritation, or rash localized at sites of known or suspected tumor) of ≤ 7 days in duration
  - Grade 3 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis. It is recommended to consult with the Principal Investigator for grade 4 amylase or lipase abnormalities
  - Grade 3 clinically significant laboratory abnormalities that are asymptomatic and can be reversed within 72 hours, however:
  - Any Grade 4 ALT or AST elevation will be a DLT
  - Any Grade 3 ALT or AST elevation that lasts longer than 7 days will be a DLT
  - ALT or AST > 8 X ULN regardless of duration will be considered a DLT
  - ALT or AST > 5 X ULN that lasts longer than 14 days will be considered a DLT
  - ALT or AST of > 3 X ULN with a concurrent total bilirubin > 2 X ULN will be considered a DLT in the absence of another cause (other than study drug) of these elevations.
- Any of the following hematologic toxicities:
  - Grade 4 anemia (regardless of duration)
  - Grade 4 neutropenia persisting > 5 days
  - Grade 3 thrombocytopenia with hemorrhage
  - Grade 4 thrombocytopenia
  - Any other ≥ Grade 3 hematologic adverse event not returning to baseline or ≤ grade 1 prior to the next planned study drug dose, excluding asymptomatic lymphopenia which will not be considered a DLT
- ≥ Grade 3 infusion reaction
  - Excluding grade 3 infusion reactions resolving within 6 hours with or without treatment
- ≥ Grade 3 related injection site reaction will be considered a DLT and no further treatment with CDX-301 will be permitted
- Any ≥ grade 2 eye pain or reduction of visual acuity that does not improve to ≤ grade 1 severity within 2 weeks of the initiation of topical therapy or requires systemic treatment
- Treatment delay of > 2 weeks due to drug-related toxicity that is not improving will be considered a DLT.

7.3 Radiographic Assessments

Pre-registration imaging will be obtained prior to study entry and will consist of a whole-body PET/CT (within 42 days) and a diagnostic CT of the chest, abdomen, pelvis (within 21 days). The surveillance imaging schedule is detailed in the Study Calendars (Section 4.2) and includes CT of the chest, abdomen, and pelvis every 8 weeks for one year. CT imaging of the pelvis may be omitted for patients with no history of metastatic lesions in the anatomical area. Intravenous and oral contrast will be used, unless a contraindication to contrast administration exists. Additional imaging studies (e.g., FDG-PET, MRI) may be added at any time to assess disease that is not well-visualized on CT and/or if there is concern for disease progression. The surveillance imaging schedule beyond year 1 will be left to the discretion of the treating physicians but should follow current guidelines[75].

Subjects with a history of brain metastases will undergo surveillance for the development of new/progressive brain metastases. Imaging timing and details will be left to the discretion of the treating physicians but will generally follow current guidelines[107] and most often include high-resolution MRI of the brain with i.v. contrast every 2-3 months. Prompt MRI of the brain is recommended for any study subject who develops symptoms suggestive of new/worsening brain metastasis.

Tumor response and progression-free survival (PFS) will be assessed using iRECIST criteria (Appendix) and secondarily using RECIST criteria.

7.4 Quality-of-Life Assessments

Quality-of-life (QoL) will be assessed using the EORTC QLQ-C30 and EORTC QLQ-LC13. Of note, these tools are available in multiple languages. In the rare case where a study subject is not fluent in any language in which the QLQ-C30 and QLQ-LC13 are available, QoL assessments will be omitted. In cases where QoL assessments are due but cannot be completed in clinic for logistical reasons, QoL surveys may be administered over the telephone.

The EORTC QLQ-C30 and EORTC QLQ-LC13 are described in Section 2.9, and the assessment schedule is displayed in the Study Calendars (Section 4.2). The complete surveys are included in the Appendix. The statistical plans for QoL analysis are described in Section 8.3.

7.5 Physical Activity Monitoring

A commercially available fitness tracker (e.g., Garmin Vivofit, shown below on left) will be provided at no cost to the patient and placed on the patient's wrist at the time of study registration. The device will be activated and synced with a computer or mobile device, and the device will be set to show the time. Patients will be shown how to remove the device (like removing a wristwatch), in case they wish to remove it temporarily or permanently. However, patients will be asked to keep the device on continuously throughout the course of the study. The device is waterproof, so patients will not need to remove it when bathing. The device has a battery life of over one year, so patients will not need to charge it. Data will be synced automatically every time the patient enters one of the Medical Oncology or Radiation Oncology clinics using a wireless access client (shown below on right). In cases where the data is not synced automatically, a study team member will sync the data using a mobile device.

Wearable activity monitor (left) and wireless access client (right)

7.6 Correlative Studies

We will collect blood samples at regular intervals (See Section 4.2) to facilitate monitoring immune responses, such as DC expansion and maturation. We will also follow T-cell inducible costimulator (ICOS) expression. ICOS is a member of the immunoglobulin superfamily. Responses to immunotherapy have been associated with increased ICOS expression on CD8+ T-cells, particularly following CTLA-4 blockade[108-111]. We will follow serum levels of high-mobility group box 1 (HMGB1), whose release has been linked to antitumor immune responses[112,113]. Flow cytometry will be performed to track measures of DC maturation such as CD83 and CD86 expression[114]. Other correlative studies will be performed in collaboration with Dr. Guha's laboratory and the study sponsor. Details regarding blood processing for peripheral blood mononuclear cell (PBMC)isolation are shown in the Appendix.

Due to potential transient lymphocytic effects of the SARS-CoV2 vaccine, correlative blood should not be drawn within a 5-day period after administration.

Testing for anti-drug antibody (ADA) and CDX-1140 pharmacokinetics will be performed at baseline and prior to, two hours after, and one day after each CDX-1140 infusion. These tests will be performed at Celldex Lab in Fall River, MA. Approximately 10 mL of blood will be collected in serum-separating tubes (SST). After clotting at room temperature for 30 minutes, the samples will be spun at 3000 RPM at 4°C for 15 minutes. Plasma will be aliquoted into 3-4 cryovials and frozen -80°C. Study samples will be shipped to Celldex at convenient intervals or at Celldex's request.

8.0 STATISTICAL CONSIDERATIONS

8.1 Randomization Procedure

At the time of study registration, subjects will be stratified by EGFR/ALK mutation status and disease burden and then will be randomized 1:1 between the two study arms using a modified permuted block treatment allocation scheme[115]. Block sizes of 2 (65%) and 4 (35%) will be considered. Study subjects and study personnel will NOT be blinded with respect to the randomization result.

8.2 Phase I Primary Endpoint – Dose-Limiting Toxicity

A stopping rule for safety will be implemented as follows: After 6 subjects are randomized to Arm 1 (which will take place after approximately 12 total subjects are enrolled), accrual will be temporarily suspended for DLT monitoring. 8 weeks after the 6th subject on Arm 1 starts treatment, the study will proceed to Phase II if no more than one out of six subjects on Arm 1 has experienced a DLT (defined in Section 7.2).

If the pre-specified safety criteria are not achieved in the Phase I portion of this study, the investigators and Celldex will review safety data from this trial as well as other trials using CDX-301 and CDX-1140 and determine if the experimental treatment

regimen should be altered (e.g., by reducing the CDX-1140 dose). The study protocol would be amended accordingly, and Phase I component with the new treatment regimen would be added.

At the conclusion of the trial, study therapy will be considered safe and worthy of further study if no more than 4 out of 23 subjects on Arm 1 experience a DLT. The target Grade 3 toxicity rate is no more than 12.5%, and a toxicity rate greater than 35% is considered clinically unacceptable. This design has the following operating characteristics: The probability of accepting the treatment for further study if the true toxicity rate is unacceptably high (>35%) is at most 5%. In contrast, there is at least an 85% probability of accepting the treatment for further study if the true toxicity rate is less than 12.5%.

The proportion of patients on Arm 1 who develop a DLT will be computed, along with Clopper-Pearson 95% exact confidence intervals. Frequencies of specific treatment-related adverse events (including delayed adverse events) and event grades will be characterized using counts and percentages.

8.3 Phase II Primary Endpoint – Progression-free Survival

The primary study hypothesis is that treatment with FLT3 ligand, CD40 agonist antibody, and SBRT (Arm 1) will improve progression-free survival (PFS) compared to standard therapy (Arm 2). Based on published data for patients with advanced NSCLC who require treatment after several lines of standard therapy, we expect that the median PFS duration for subjects on Arm 2 will be 5 months. Based on our recent study using FLT3 ligand and SBRT and the expectation that CD40 agonist antibody will enhance treatment efficacy, we hypothesize that the median PFS duration for subjects on Arm 1 will be 10 months. This improvement is approximately equivalent to a hazard reduction of 50%. We are employing a *randomized phase II screening design*, using $\alpha = 0.20$ (wherein a p-value <0.20 indicates a positive trial). We anticipate that study accrual will take place over 24 months, and we will analyze treatment efficacy 12 months after accrual is completed. We do not expect any subjects to be lost to follow-up before the primary endpoint occurs. Based on these assumptions, our total sample size of 46 subjects yields study power of 81% using a two-sided log-rank test.

We will compare the distributions of PFS between treatment arms using a two-sided log-rank test. The rates at various timepoints (e.g., every 6 months after randomization) and median PFS durations for each arm will be estimated using the Kaplan-Meier method. Associated 95% confidence intervals (CI) will be calculated using Greenwood's formula. Cox proportional hazards models will be used to estimate the impact of study therapy on PFS duration.

8.4 Secondary Endpoints and Exploratory Analyses

Overall survival (OS) rates and median OS durations will be estimated using the Kaplan-Meier method. Exploratory comparisons of OS distributions between study arms will be performed using a two-sided log-rank test and Cox proportional hazards models.

Radiographic response rates will be characterized using descriptive statistics. The clinical benefit rate (CBR) will be defined as the percentage of subjects who achieve best response of confirmed CR or PR, or stable disease (SD) for at least four months. CBR in Arm 1 and Arm 2 will be compared using chi-square testing or Fisher

exact test, as appropriate. Objective response rate (ORR) will be defined as the percentage of subjects who do not receive comprehensive SBRT who achieve best response of confirmed CR or PR. ORR in Arm 1 and Arm 2 will be compared using chi-square testing or Fisher exact test, as appropriate.

Quality-of life (QoL) will be assessed using the EORTC QLQ-C30 and EORTC QLQ-LC13. Summary statistics (mean, standard deviation, median, 25th and 75th percentiles, and range) and the mean change from baseline of linear-transformed scores will be reported for all the items and subscales of the EORTC QLQ-C30 questionnaire and the QLQ-LC13, according to the EORTC scoring manual guidelines and the. Completion and compliance rates will be summarized at each timepoint by treatment arm. Only patients with a non-missing baseline assessment and at least one non-missing post-baseline assessment will be included in the analyses.

Daily step count averages at baseline and at various timepoints after study registration will be characterized using descriptive statistics. To test the hypothesis that study therapy preserves physical function compared to standard therapy, a linear mixed effects model with random coefficients using a restricted maximum likelihood procedure will be used to model daily step count as a function of study arm, using adjustment variables such as age and gender.

Exploratory multivariable and subgroup analyses of PFS and OS predictors will be performed. Adjustment/grouping variables will include baseline disease burden, performance status, NSCLC subtype, and prior response to immunotherapy.

9.0 REGULATORY CONSIDERATIONS

9.1 Protection of Human Subjects

The Investigator must ensure that patients or their legally acceptable representatives are clearly and fully informed about the purpose, potential risks andother critical issues regarding clinical trials in which they volunteer to participate. Preparation of the consent form is the responsibility of the Investigator and must include all elements required by CFR 21 Part 50.25 and the local IRB.

9.2 Compliance with the Protocol and Protocol Revisions

The study must be conducted as described in this approved protocol. All revisions to the protocol must be provided to the PI. The PI should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB/IEC of an Amendment, except where necessary to eliminate an immediate hazard(s) to study patients. The investigator must ensure that patients or their legally acceptable representatives are clearly and fully informed about the purpose, potential risks and other critical issues regarding clinical trials in which they volunteer to participate. Preparation of the consent form is the responsibility of the PI.

10.0 DATA HANDLING AND RECORD KEEPING

10.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI. In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

10.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

10.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

10.4 Data Safety and Monitoring Board

The Albert Einstein College of Medicine/Albert Einstein Cancer Center Data Safety Monitoring Committee (DSMC) has the responsibility for ensuring data and

safety monitoring along with the PI who is ultimately responsible for the ongoing monitoring and safety of clinical protocols. The primary functions of the AECC DSMC are as follows:

1. To review and ensure protocol compliance with dose escalation in phase I trials
2. To review/assure protocol compliance for all trials that have two-stage phase II designs,
3. Reviewing all internal and external serious adverse reports, investigator alerts, action letters, and other safety reports for trials being performed at AECC-affiliated institutions and;

   To implement and to determine the adequacy of DSM plans of all approved protocols.

The DSMC is an independent committee and meets on a bimonthly basis. During its bimonthly meeting, the DSMC will review serious (grade 3 or higher) adverse events from this study. In the event that the DSMC decides that a protocol revision is warranted, the committee will immediately notify the principal investigator of this study. The DSMC has the authority to close trials to patient accrual should the risk to patients be excessive or outweigh the potential benefits of the study. All study suspensions and closures will be forwarded to the IRB/CCI and study sponsor from the DSMC. The DSMC will review all safety data from Phase I before the study proceeds to Phase II.

11.0 ADVERSE EVENTS

An adverse event (AE) is any untoward medical occurrence in a subject administered a study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study treatment, whether or not related to the study treatment. All observed or volunteered AEs regardless of suspected causal relationship to the study treatment will be reported as described in the following sections. For the purposes of this current study, “study treatment” is defined as CDX-1140, CDX 301, and radiotherapy. AEs for subjects on arm 2 who receive standard therapy (e.g., chemotherapy) will also be reported.

11.1 Adverse Event Definitions

Adverse Event (AE): any new, undesirable medical experience or change of an existing condition that occurs during or after treatment, whether or not considered product-related.

Serious Adverse Event (SAE): An AE occurring at any dose that results in any of the following outcomes (CFR 312.32)

- Death
- Life-threatening adverse experience
- Inpatient hospitalization or prolongation of existing hospitalization excluding those for study therapy administration, transfusional support, disease staging/re-staging procedures, thoracentesis / paracentesis, or placement of an indwelling catheter, unless associated with other serious events
- Persistent or significant disability or incapacity
- Congenital anomaly / birth defect.

The definition of SAE also includes important medical event. Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse. A new diagnosis of cancer during the course of treatment should be considered an important medical event.

Causality: the relationship of each AE to study drug will be defined as “unrelated” or “related” to study treatment using the following definitions:

- Unrelated: There is little or no possibility that the study treatment(s) caused the reported AE; and other factor(s) including concurrent illnesses, progression and expression of the disease state, concurrent medications, or a reaction to concurrent medications appear to explain the AE.
- Related: There exists at least a reasonable possibility that the study treatment(s) caused or contributed to the AE; an inability to identify an alternate etiology for an AE should not, by itself, justify a “related” attribution.

Unexpected Adverse Event: An AE that is not mentioned in the Investigator's Brochure or package insert or the specificity or severity of which is not consistent with the investigator's brochure or package insert.

Life-threatening: Any adverse experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred. It does not include a reaction that, had it occurred in a more severe form, might have caused death.

AEs will use the descriptions and grading scales found in the revised Common Terminology Criteria for Adverse Events (CTCAE) version 5.0

11.2 Adverse Event Reporting

Study site personnel must notify the PI and the sponsor immediately of any SAE experienced by a patient. In general, SAEs assessed as clearly being due to disease progression, and not due to study drug(s), should be excluded from AE reporting. Study-specific clinical outcomes of death because of disease progression are exempt from SAE reporting, unless the investigator deems them related to use of the study drug. Hospitalization for study drug administration is not an SAE.

The following steps will be taken to report promptly and document accurately any SAE, even if it may not appear to be related to the study treatment:

- Report the SAE to the PI and the treating physician by email, telephone or fax within 24 hours of becoming aware that a patient has experienced an SAE.
- Record the SAE accurately on the AE page of the patient’s CRF.
- Using the standard IRB-SAE report form, submit all known patient information within 24 hours of SAE occurrence to the clinical trial office to submit to IRB and

DSMC. Date and sign each report before submission. Include the following information (or as much as possible to obtain and still report the event within 24 hours):

- Study protocol number and indication
- Study site and investigator's identification
- Patient's ID (patient number and initials), age or date of birth, and sex
- Date of enrollment
- Description of SAE, including date of onset and duration, severity, and outcome
- Date of first and most recent (last) dose administered
- Action taken regarding study treatment
- Relationship of SAE to study treatment
- Concomitant medications, including regimen and indication
- Intervention, including concomitant medications used to treat SAE
- Pertinent laboratory data/diagnostic tests conducted and date
- Pertinent medical history of patient
- Date of hospital admission/discharge
- Date of death (if applicable)

Within 10 days of initial IRB notification, the PI is required to submit a completed Adverse Event Report to the IRB. The treating physicians should perform appropriate diagnostic tests and therapeutic measures and submit all follow-up substantiating data, such as diagnostic test reports and autopsy report to the PI, IRB, and DSMB.

Reporting to Celldex is required in addition to reporting to the FDA and does not replace the requirement to notify the FDA if required. The PI will inform Celldex in writing using an SAE form or MEDWATCH 3500A form of any SAE within 24 hours of being aware of the event. The written report must be completed and supplied to Celldex by facsimile or email within 24 hours/1 business day. The initial report must be as complete as possible, including an assessment of the causal relationship between the event and the investigational product(s), if available. Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up report.

Completed SAE reports are to be submitted to:

Celldex Therapeutics, Inc.
Pharmacovigilance
Facsimile : 781-644-6434
Email: SAE@celldex.com (note - check for removal of HIPAA identifiers prior to sending information.
For questions regarding SAE reporting: 908-323-2233 (SAE Hotline)

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13.0 APPENDICES

13.1 Appendix A - ECOG Performance Status

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
5	Dead

Adapted from Oken 1982[116].

13.2 Appendix B – Common Terminology Criteria for Adverse Events Version 5.0

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

13.3 Appendix C – iRECIST Criteria

Tumors may respond differently to immunotherapies compared with chemotherapeutic drugs, raising questions about the assessment of changes in tumor burden—a mainstay of evaluation of cancer therapeutics that provides key information about objective response and disease progression. Immunotherapeutics may result in infiltration of immune cells leading to transient increase in the size in malignant lesions, or undetectable lesions becoming detectable. A consensus guideline—iRECIST—was developed by the RECIST working group for the use of modified Response Evaluation Criteria in Solid Tumors (RECIST version 1.1) in cancer immunotherapy trials, to ensure consistent design and data collection, facilitate the ongoing collection of trial data, and ultimate validation of the guideline[84]. This guideline describes a standard approach to solid tumor measurements and definitions for objective change in tumor size for use in trials in which an immunotherapy is used. The criteria are identical to those of RECIST 1.1 in many respects but have been adapted to account for instances where an increase in tumor burden, or the appearance of new lesions, does not reflect true tumor progression.

To assess objective response or future progression, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for subsequent measurements. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment. Assessments should be identified on a calendar schedule and should not be affected by delays in therapy. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination, unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam. (See “Methods of Lesion Measurement” for further guidance.)

Measurable Disease:

Measurable tumor lesions (nodal, subcutaneous, lung parenchyma, solid organ metastases) are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with chest x-ray and as ≥ 10 mm with CT scan or clinical examination.

- *Malignant lymph nodes* must be ≥15 mm in the short axis to be considered measurable; only the short axis will be measured and followed. All other pathological nodes (those with short axis ≥ 10 mm but < 15 mm) should be considered non-target lesions. Nodes that have a short axis < 10 mm are considered non-pathological and should not be recorded or followed.
- *Bone lesions:* Bone scan, PET scan, or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions. Bone lesions are considered measurable only if assessed by CT scan and have an identifiable soft tissue component that meets these requirements (soft tissue component ≥ 10 mm by CT scan). Blastic bone lesions are non-measurable.
- *Cystic lesions:* Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts. 'Cystic lesions' thought to

represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if noncystic lesions are present in the same patient, these are preferred for selection as target lesions.

- Previously irradiated lesions are not considered measurable unless progression has been documented in the lesion.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Non-measurable Disease:

All other lesions (or sites of disease), including small lesions are considered non-measurable disease. Bone lesions without a measurable soft tissue component, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, lymphangitic involvement of lung or skin and abdominal masses followed by clinical examination are all non-measurable. Lesions in previously irradiated areas are non-measurable, unless progression has been demonstrated.

Target Lesions:

- When more than one measurable tumor lesion is present at baseline all lesions up to a maximum of 5 lesions total (and a maximum of 2 lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline.
- Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements.
- At baseline, the sum of the target lesions (longest diameter of tumor lesions plus short axis of lymph nodes: overall maximum of 5) is to be recorded.

Special notes on the assessment of target lesions:

- *Target lesions that become 'too small to measure':* After baseline, a value should be provided on the CRF for all identified target lesions for each assessment, even if very small. If extremely small and faint lesions cannot be accurately measured but are deemed to be present, a default value of 5 mm may be used. If lesions are too small to measure and indeed are believed to be absent, a default value of 0 mm may be used.
- *Lymph nodes* identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to below 10 mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of < 10mm. For PR, SD and PD, the actual short axis measurement of the nodes is to be included in the sum of target lesions.
- *Lesions that split or coalesce on treatment:* When non-nodal lesions 'fragment', the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each

individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'coalesced lesion'.

Non-Target Lesions:

- All other measurable/non-measurable lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline.
- Measurements are not required but these lesions should be noted at baseline and should be followed as “present” or “absent”.

New lesions:

New lesions should be assessed and categorized as measurable or non-measurable using RECIST 1.1 principles. Five lesions (no more than two per organ) should be measured and recorded as New Lesions-Target (NLT) and New Lesion-Non-Target (NLNT) but should not be included in the sum of measures of the original target lesions identified at baseline.

Methods of lesion measurement:

- Clinical exam: Clinical lesions will only be considered measurable when they are superficial and ≥ 10 mm as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is recommended. If feasible, imaging is preferred.
- Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions ≥ 20 mm on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.
- CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). Other specialized imaging or other techniques may also be appropriate for individual case. For example, while PET scans are not considered adequate to measure lesions, PET-CT scans may be used providing that the measures are obtained from the CT scan and the CT scan is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast).
- Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. If new lesions are identified by ultrasound in the course of the study, confirmation by CT is advised.
- Endoscopy, laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

- Tumor markers: Tumor markers alone cannot be used to assess objective tumor response. If markers are initially above the upper normal limit, however, they must normalize for a patient to be considered in complete response.
- Cytology, histology: These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). When effusions are known to be a potential adverse effect of treatment (e.g. with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is advised to differentiate between response or stable disease and progressive disease.

Response Definitions:

- Immune Complete Response (iCR): disappearance of target and non-target lesions and normalization of tumor markers. Pathological lymph nodes must have short axis measures <10 mm (Note: continue to record the measurement even if < 10 mm and considered CR). Residual lesions (other than nodes < 10 mm) thought to be non-malignant should be further investigated (by cytology specialized imaging or other techniques as appropriate for individual cases4 before CR can be accepted. Confirmatory scans should be performed at least 4 weeks after iCR.
- Immune Partial Response (iPR): at least a 30% decrease in the sum of measures (longest diameter for tumor lesions and short axis measure for nodes) of target lesions, taking as reference the baseline sum of diameters. Non target lesions must be non-PD. Confirmatory scans should be performed at least 4 weeks after iPR.
- Immune Stable Disease (iSD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as reference the smallest sum of diameters on study.
- Immune Unconfirmed Progressive Disease (iUPD): at least a 20% increase in the sum of diameters of measured lesions taking as references the smallest sum of diameters recorded on study (including baseline) AND an absolute increase of ≥ 5 mm. Appearance of new lesions will also constitute iUPD (including lesions in previously unassessed areas). In exceptional circumstances, unequivocal progression of non-target disease may be accepted as evidence of iUPD, where the overall tumor burden has increased sufficiently to merit discontinuation of treatment or where the tumor burden appears to have increased by at least 73% in volume. Modest increases in the size of one or more non-target lesions are NOT considered unequivocal progression. Confirmatory scans should be performed at least 4 weeks, but no longer than 8 weeks, after iUPD.
- Immune Confirmed Progressive Disease (iCPD): iCPD is confirmed if further increase in tumor burden, compared to the last assessment, is seen as evidenced by one or more of the following:
    - Continued increase in tumor burden (from iUPD) in the lesion category (target, non-target disease or new lesions) where RECIST 1.1 definitions of progression had previously been met
        - Continued progression in target disease with an increase of at least 5 mm in the absolute value of the sum of measures of target disease

- Continued unequivocal progression in non-target disease with an increase in tumor burden
- Increase in size of previously identified new lesion (s) (an increase of at least 5 mm in the absolute value of the sum of those considered to be target new lesions) or additional new lesions.

  - RECIST 1.1 criteria are met in lesions types (target or non-target or new lesions) where progression was not previously identified, including the appearance of additional new lesions.

Notes on confirmation of progression:

- Treatment beyond initial RECIST 1.1-defined progression (ie, iUPD) is permitted only for patients who are clinically stable. Such patients may continue treatment until the next assessment (≥ 4 weeks but no longer than 8 weeks later). An assignment of clinical stability requires that no worsening of performance status has occurred, that no clinically relevant increases in disease-related symptoms such as pain or dyspnea occur that are thought to be associated with disease progression (these symptoms are generally understood to mean a requirement for increased palliative intervention), and that no requirement for intensified management of disease-related symptoms exists, including increased analgesia, radiotherapy, or other palliative care. The imaging findings and the recommendation to continue with treatment despite iUPD should be discussed with the patient before a decision is made about whether or not to continue therapy. All decisions regarding continuation or discontinuation of therapy should be made by the patient and their health-care provider.
  - Patients who have iUPD and are not clinically stable should be designated as not clinically stable in the case report form to allow the best overall response to be calculated and the date of iUPD to be used in estimates of progression-free survival.
- If iUPD is not confirmed at the next assessment, but instead tumor shrinkage occurs (compared with baseline) which meets the criteria of iCR, iPR, or iSD, then the bar is reset so that iUPD needs to occur again (compared with nadir values) and then be confirmed (by further growth) at the next assessment for iCPD to be assigned.
- If no change in tumor size or extent from iUPD occurs, then the timepoint response would again be iUPD.
- iUPD can be assigned multiple times as long as iCPD is not confirmed at the next assessment.
- The prior documentation of iUPD does not preclude assigning iCR, iPR, or iSD in subsequent time-point assessments or as best overall response (BOR) providing that iCPD is not documented at the next assessment after iUPD.
- The date of disease progression should be the first date at which progression criteria are met (ie, the date of iUPD) provided that iCPD is confirmed at the next assessment. If iUPD occurs, but is disregarded because of later iSD, iPR, or iCR, that iUPD date should not be used as the progression event date.

Integration of Target, Non-Target and New Lesions into Time-point iResponse

Target Lesions	Non-Target Lesions	New Lesions (NLs)	Time Point (TP) Response	
			No prior iUPD*	Prior iUPD*; **
iCR	iCR	No	iCR	iCR
iCR	Non-iCR/Non-iUPD	No	iPR	iPR
iPR	Non-iCR/Non-iUPD	No	iPR	iPR
iSD	Non-iCR/Non-iUPD	No	iSD	iSD
iUPD with no change OR decrease from last TP	iUPD with no change OR decrease from last TP	Yes	NA	NLs confirms iCPD if NLs were previously identified and increase in size (≥ 5 mm in sum of measurements for New Lesion-Target or any increase for New Lesion – Non-target) or number. If no change in NLs (size or number) from last TP, remains iUPD
iSD	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based in further increase in size of non-target disease (need not meet RECIST 1.1 criteria for unequivocal PD)
iUPD	Non-iCR/Non-iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on: o further increase in sum of measurements of at least 5 mm, otherwise remains iUPD
iUPD	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on further increase in: o previously identified target lesion iUPD sum of measurements ≥5 mm and / or o non-target lesion iUPD (prior assessment - need not be unequivocal PD)
iUPD	iUPD	Yes	iUPD	Remains iUPD unless iCPD confirmed based on further increase in: o previously identified target lesion iUPD ≥ 5 mm and / or o previously identified non-target lesion iUPD (need not be unequivocal) and /or o size or number of new lesions previously identified
Non-iUPD/PD	Non-iUPD/PD	Yes	iUPD	Remains iUPD unless iCPD confirmed based on o increase in size or number of new lesions previously identified
* in any lesion category. ** previously identified in assessment immediately prior to this TP.				

Adapted from Seymour 2017[84]

13.4 Appendix D – RECIST 1.1 Criteria

To assess objective response or future progression, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for subsequent measurements. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination, unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam. (See "Methods of Lesion Measurement" for further guidance.)

At baseline, lesions should be identified as either "Target" or "Non-Target" as follows:

Target Lesions:

- Up to a maximum of five measurable target lesions total (with a maximum of two target lesions per organ) should be identified as target lesions and will be recorded and measured at baseline. (This means in instances where patients have only one or two organ sites involved a maximum of two and four lesions respectively will be recorded.)
- Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, and should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected.
- All target lesion measurements should be recorded in metric notation, using calipers if clinically assessed.
- A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease. (See "Tumor response evaluation".)

Non-Target Lesions:

- All other measurable/non-measurable lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. It is acceptable to record multiple non-target lesions involving the same organ as a single item on the case report form (e.g. 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').
- Non-target lesions should be followed as 'present', 'absent', or in rare cases 'unequivocal progression'. (See "Tumor response evaluation".) While some non-target lesions may actually be measurable, they need not be measured and instead should be assessed only qualitatively.

MEASURABILITY OF TUMOR AT BASELINE

- Measurable: Tumor lesions must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:
    - 10 mm by CT scan (CT scan slice thickness no greater than 5 mm).
    - 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
    - 20 mm by chest X-ray.

  Note: To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15mm in short axis when assessed by CT scan (for lymph nodes, only the short axis is measured and followed).
- Non-measurable: All other lesions, including small lesions (longest diameter < 10mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, and abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

Special considerations regarding lesion measurability:

- Malignant lymph nodes: Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumour. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane; for MRI the plane of acquisition may be axial, saggital or coronal). The smaller of these measures is the short axis. At baseline and in follow-up, only the short axis will be measured and followed. To be considered pathologically enlarged and measurable, a lymph node must be ≥15mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). All other pathological nodes (those with short axis ≥10mm but <15 mm) should be considered non-target lesions. Nodes that have a short axis <10mm are considered non-pathological and should not be recorded or followed.
- Bone lesions: Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions. Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above. Blastic bone lesions are non-measurable.
- Cystic lesions: Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts. 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if noncystic lesions are present in the same patient, these are preferred for selection as target lesions.

- Lesions with prior local treatment: Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion.

METHODS OF LESION MEASUREMENT

- Clinical exam: Clinical lesions will only be considered measurable when they are superficial and ≥ 10mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.
- Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.
- CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.
- FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression. (See “Tumor Response Evaluation”.)
- Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised.
- Endoscopy, laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.
- Tumor markers: Tumor markers alone cannot be used to assess objective tumor response. If markers are initially above the upper normal limit, however, they must normalize for a patient to be considered in complete response.
- Cytology, histology: These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). When effusions are known to be a potential adverse effect of treatment (e.g. with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the

neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumor has met criteria for response or stable disease in order to differentiate between response (or stable disease) and progressive disease.

TUMOR RESPONSE EVALUATION

Evaluation of target lesions:

Target lesions will be assigned an overall response assessment at each evaluation time point according to the following definitions:

- *Complete Response (CR):* Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10mm.
- *Partial Response (PR):* At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
- *Progressive Disease (PD):* At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).
- *Stable Disease (SD):* Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Special notes on the assessment of target lesions

- Lymph nodes: Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical planeas the baseline examination), even if the nodes regress to below 10mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of < 10mm. For PR, SD and PD, the actual short axis measurement of the nodes is to be included in the sum of target lesions.
- Target lesions that become 'too small to measure': While on study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g. 2 mm). However, sometimes lesions or lymph nodes which are recorded as target lesions atbaseline become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measure and may report them as being 'too small to measure'. When this occurs it is important that a value be recorded on the case report form. If itis the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0mm. If the lesion is believed to be presentand is faintly seen but too small to measure, a default value of 5mm should be assigned. (Note: It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fatsuch as in the retroperitoneum; however, if a lymph node is believed to be present

and is faintly seen but too small to measure, a default value of 5mm should be assigned in this circumstance as well). This default value is derived from the 5mm CT slice thickness (but should not be changed with varying CT slice thickness). The measurement of these lesions is potentially non-reproducible, therefore providing this default value will prevent false responses or progressions based upon measurement error. To reiterate, however, if the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5mm.

- Lesions that split or coalesce on treatment. When non-nodal lesions 'fragment', the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'coalesced lesion'.

Evaluation of non-target lesions:

Non-target lesions will be assigned an overall response assessment at each evaluation time point according to the following definitions:

- *Complete Response (CR):* Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10mm short axis).
- *Non-CR/Non-PD:* Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
- *Progressive Disease (PD):* Unequivocal progression (see comments below) of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

Special notes on assessment of progression of non-target disease:

- When the patient also has measurable disease: In this setting, to achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy (see further details below). A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to quality for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.
- When the patient has only non-measurable disease: The same general concepts apply here as noted above, however, in this instance there is no measurable disease assessment to factor into the interpretation of an increase in non-measurable disease burden. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are truly non-measurable) a useful test that canbe applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e., an increase in tumor burden representing an additional

73% increase in 'volume' (which is equivalent to a 20% increase diameter in a measurable lesion). Examples include an increase in a pleural effusion from 'trace' to 'large', an increase in lymphangitic disease from localized to widespread, or may be described in protocols as 'sufficient to require a change in therapy'. If 'unequivocal progression' is seen, the patient should be considered to have had overall PD at that point. While it would be ideal to have objective criteria to apply to non-measurable disease, the very nature of that disease makes it impossible to do so, therefore the increase must be substantial.

New lesions:

The appearance of new malignant lesions denotes disease progression; therefore, some comments on detection of new lesions are important.

- There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal: i.e. not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumour (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the patient's baseline lesions show partial or complete response. For example, necrosis of a liver lesion may be reported on a CT scan report as a 'new' cystic lesion, which it is not.
- A lesion identified on a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression. An example of this is the patient who has visceral disease at baseline and while on study has a CT or MRI brain ordered which reveals metastases. The patient's brain metastases are considered to be evidence of PD even if he/she did not have brain imaging at baseline.
- If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.
- While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:
  a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion. (A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.)
  b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up

corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

Evaluation of overall response:

It is assumed that at each protocol specified time point, an overall response assessment occurs. The patient's overall response assignment will depend on the findings of both target and non-target disease and will also take into consideration the appearance of new lesions.

Table 1 provides a summary of the overall response status calculation at each time point for patients who have measurable disease at baseline. When patients have non-measurable (therefore non-target) disease only, Table 2 is to be used.

Special notes on evaluation of overall response:

- Missing assessments and inevaluable designation:
  - When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point.
  - If only a subset of lesion measurements are made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD. For example, if a patient had a baseline sum of 50mm with three measured lesions and at follow-up only two lesions were assessed, but those gave a sum of 80 mm, the patient will have achieved PD status, regardless of the contribution of the missing lesion.
- 'Symptomatic deterioration': Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as 'symptomatic deterioration'. Every effort should be made to document objective progression even after discontinuation of treatment. Symptomatic deterioration is not a descriptor of an objective response: it is a reason for stopping study therapy. The objective response status of such patients is to be determined by evaluation of target and non-target disease as shown in Table 1 and Table 2.
- In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before assigning a status of complete response. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring.
- For equivocal findings of progression (e.g. very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.
- Confirmation of response: In the event of complete or partial responses, efforts should be made to obtain a confirmatory scan (no sooner than 28 days later).

Table 1: Overall response: patients with target +/–non-target disease.

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

Table 2: Overall response: patients with non-target disease only.

Non-target lesions	New lesions	Overall response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD [a]
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

CR = complete response, PD = progressive disease, and NE = inevaluable.

[a] 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

FREQUENTLY ASKED QUESTIONS

What should be done if several unique lesions at baseline become confluent at a follow-up evaluation?

Measure the longest diameter of the confluent mass and record to add into the sum of the longest diameters.

How large does a new lesion have to be to count as progression? Does any small sub-centimeter lesion qualify, or should the lesion be at least measurable?

New lesions do not need to meet 'measurability criteria' to be considered valid. If it is clear on previous images (with the same technique) that a lesion was absent then its definitive appearance implies progression. If there is any doubt (because of the techniques or conditions) then it is suggested that treatment continue until next scheduled assessment when, generally, all should be clear. Either it gets bigger and the date of progression is the date of the first suspicion, or it disappears and one may then consider it an artifact with the support of the radiologists.

How should one lesion be measured if on subsequent exams it is split into two?

Measure the longest diameter of each lesion and add this into the sum.

Does the definition of progression depend on the status of all target lesions or only one?

As per the RECIST 1.1 guideline, progression requires a 20% increase in the sum of diameters of all target lesions AND a minimum absolute increase of 5 mm in the sum.

What is the criterion for a measurable lesion if the CT slice thickness is > 5 mm?

RECIST 1.1 recommends that CT scans have a maximum slice thickness of 5 mm and the minimum size for a measurable lesion is twice that: 10 mm (even if slice thickness is < 5 mm). If scanners with slice thickness > 5 mm are used, the minimum lesion size must have a longest diameter twice the actual slice thickness.

What should we record when target lesions become so small they are below the 10 mm 'measurable' size?

Target lesion measurability is defined at baseline. Thereafter, actual measurements, even if < 10 mm, should be recorded. If lesions become very small, some radiologists indicate they are 'too small to measure'. This guideline advises that when this occurs, if the lesion is actually still present, a default measurement of 5 mm should be applied. If in fact the radiologist believes the lesion has gone, a default measurement of 0 mm should be recorded

If a patient has several lesions which have decreased in size to meet PR criteria and one has actually disappeared, does that patient have PD if the 'disappeared' lesion reappears?

Unless the sum meets the PD criteria, the reappearance of a lesion in the setting of PR (or SD) is not PD. The lesion should simply be added into the sum. If the patients had had a CR, clearly reappearance of an absent lesion would qualify for PD.

When measuring the longest diameter of target lesions in response to treatment, is the same axis that was used initially used subsequently, even if there is a shape change to the lesion that may have produced a new longest diameter?

The longest diameter of the lesion should always be measured even if the actual axis is different from the one used to measure the lesion initially (or at different time point during follow-up). The only exception to this is lymph nodes: as per RECIST 1.1 the

short axis should always be followed and as in the case of target lesions, the vector of the short axis may change on follow-up

Target lesions have been selected at baseline and followed but then one of these target lesions then becomes non-evaluable (i.e. different technique used). What is the effect this has on the other target lesions and the overall response?

What may be done in such cases is one of the following:

(a) If the patient is still being treated, call the centre to be sure that future evaluations are done with the baseline technique so at least SOME courses are fully evaluable

(b) If that is not possible, check if there IS a baseline exam by the same technique which was used to follow patients in which case if you retrieve the baseline measures from that technique you retrieve the lesion evaluability

(c) If neither (a) nor (b) is possible then it is a judgement call about whether you delete the lesion from all forms or consider the impact of the lesion overall is so important that its being non-evaluable makes the overall response interpretation inevaluable without it. Such a decision should be discussed in a review panel. It is NOT recommended that the lesion be included in baseline sums and then excluded from follow-up sums since this biases in favour of a response.

What if a single non-target lesion cannot be reviewed, for whatever reason; does this negate the overall assessment?

Sometimes the major contribution of a single non-target lesion may be in the setting of CR having otherwise been achieved: failure to examine one non-target in that setting will leave you unable to claim CR. It is also possible that the non-target lesion has undergone such substantial progression that it would override the target disease and render patient PD. However, this is very unlikely, especially if the rest of the measurable disease is stable or responding.

A patient has a 32% decrease in sum cycle 2, a 28% decrease cycle 4 and a 33% decrease cycle 6. Does confirmation of PR have to take place in sequential scans or is a case like this confirmed PR?

It is not infrequent that tumour shrinkage hovers around the 30% mark. In this case, most would consider PR to have been confirmed looking at this overall case. Had there been two or three non-PR observations between the two time point PR responses, the most conservative approach would be to consider this case SD.

A patient has a lesion measurable by clinical exam and by CT scan. Which should be followed?

CT scan. Always follow by imaging if that option exists since it can be reviewed and verified

A lesion which was solid at baseline has become necrotic in the centre. How should this be measured?

The longest diameter of the entire lesion should be followed. Eventually, necrotic lesions which are responding to treatment decrease in size. In reporting the results of trials, you may wish to report on this phenomenon if it is seen frequently since some agents (e.g. angiogenesis inhibitors) may produce this effect.

If I am going to use MRI to follow disease, what is minimum size for measurability?

MRI may be substituted for contrast enhanced CT for some sites, but not lung. The minimum size for measurability is the same as for CT (10 mm) as long as the scans are

performed with slice thickness of 5mm and no gap. In the event the MRI is performed with thicker slices, the size of a measurable lesion at baseline should be two times the slice thickness. In the event there are inter-slice gaps, this also needs to be considered in determining the size of measurable lesions at baseline.

Can PET–CT be used with RECIST?

At present, the low dose or attenuation correction CT portion of a combined PET–CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if your site has documented that the CT performed as part of a PET–CT is of the same diagnostic quality as a diagnostic CT (with IV and oral contrast) then the PET–CT can be used for RECIST measurements. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Adapted from Eisenhauer 2009[117].

13.5 Appendix E – EORTC QLQ-C30 and LC13

ENGLISH

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: _____
Your birthdate (Day, Month, Year): __ __ / __ __ / __ __ __ __
Today's date (Day, Month, Year): 31 / __ __ / __ __ __ __

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

Please go on to the next page

ENGLISH

During the past week:	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

EORTC QLQ - LC13

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week. Please answer by circling the number that best applies to you.

During the past week :		Not at All	A Little	Quite a Bit	Very Much
31.	How much did you cough?	1	2	3	4
32.	Did you cough up blood?	1	2	3	4
33.	Were you short of breath when you rested?	1	2	3	4
34.	Were you short of breath when you walked?	1	2	3	4
35.	Were you short of breath when you climbed stairs?	1	2	3	4
36.	Have you had a sore mouth or tongue?	1	2	3	4
37.	Have you had trouble swallowing?	1	2	3	4
38.	Have you had tingling hands or feet?	1	2	3	4
39.	Have you had hair loss?	1	2	3	4
40.	Have you had pain in your chest?	1	2	3	4
41.	Have you had pain in your arm or shoulder?	1	2	3	4
42.	Have you had pain in other parts of your body?	1	2	3	4
	If yes, where _____				
43.	Did you take any medicine for pain?				
	1 No 2 Yes				
	If yes, how much did it help?	1	2	3	4

13.6 Appendix F – Correlative Blood Sample Processing

Purpose	To freeze and store PBMC from patients.	
Methods	Human peripheral blood mononuclear cells (PBMC) from whole blood will be isolated using a density gradient centrifugation (Ficoll-Paque) and prepared to be ready to use or stored in a cryoprotective media. This method has shown an acceptable % cell recovery, viability and yield for further flow cytometry analysis.	
Time	90 minutes	
Reagents	1. 14-20 ml whole blood in heparinized tubes (sodium heparin green lid) 2. RPMI-1640 medium with 2mM L- glutamine and 5% FBS 3. Ficoll Histopaque 1.077 (Sigma) 4. Phosphate-buffered saline (dPBS) 5. 2ml Cryovials 6. 50 ml tubes 7. Sterile pipets 8. Centrifuge eppendorf 5810R	
Procedure	1. Mix all blood tubes in a 50 ml tube. Take note of the approximate blood volume. 2. Centrifuge collection tubes at 200 x g for 10 min at RT. 3. Carefully remove plasma and collect 3ml. Centrifuge the isolated plasma at 1,000 x g for 10 minutes. Aliquot the plasma supernatant in 1ml x3 cryovials and store at -80C. 4. Replace the plasma removed for an equal volume of RPMI 5% FBS. Add the same volume of RPMI 5% FBS (no more than 35 ml final volume). 5. Gently transfer the diluted blood in a 50 ml tube containing 15 ml of Ficoll-Paque with the help of a 25ml sterile pipet. 6. Centrifuge 30 min at 1800 rpm (or 400 x g), room temperature (acceleration: 4, brake: 0). 7. Transfer the lymphocyte-containing band (buffy coat) into a new 50ml tube using a 1 ml micropipet. 8. Wash the total lymphocyte population with 3V of RPMI 1640 containing 5% FBS two times. Between washes, pellet down the cells, centrifuge 10 min at 1800 rpm, 4C (acceleration: 9 brake: 9). 9. Count number of cells before the last centrifugation (final volume= 10ml). 10. If the cells will be cryopreserved then resuspend cells in the corresponding volume of cRPMI (or ExVivo media) to have at least 2-3x10e7 cells/ml and continue with cryopreservation protocol. 11. If the cells will be directly used for FACS then resuspend cells in 3ml of cRPMI and incubate at 37C for up to 2h for the cells to recover. 12. Collect the cells by centrifuging and resuspend with PBS to its corresponding volume (1x10e6 cells/100 ul) and continue with FACS protocol.	**Notes** 15ml of ficoll is sufficient for isolate pbmcs from 35ml blood solution (blood + RPMI) Label should include study number, sample identification, the date, and "PBMC". It is important to avoid the blood to get mixed with the ficoll. Cc = $\frac{\#\text{ cells x 10e4 x VF}}{\#\text{ square x dil}}$ = cells/ml

Reference: **Standard Operating Protocol - HIMC**
Title: Isolation of Plasma – Undiluted, Pre-Ficoll Revised: 8/12/2013 – Version 1.2
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