PROTOCOL # 2015-174
2/4/16; Revised 8/11/16; Revised 11/14/18; Revised 4/11/19

WAYNE STATE UNIVERSITY SCHOOL OF MEDICINE
Karmanos Cancer Institute

2015-174: STUDY OF THE EFFECTS OF DEXAMETHASONE ON NON-SMALL CELL LUNG CANCER USING [F-18] FLT FOR IMAGING WITH POSITRON EMISSION TOMOGRAPHY (PET)

Principal Investigator:

Anthony F. Shields, M.D., Ph.D.
313-576-8735 voice, 313-576-8767 FAX
E-mail: shieldsa@karmanos.org
4100 John R Street, HW04HO
Detroit, MI 48201

Co-Participants:

Hirva Mamdani, M.D.
Manohar Ratnam, Ph.D.
Misako Nagasaka, M.D.
Ammar Sukari, M.D.
Ulka Vaishampayan, M.D.
Amy Weise, D.O.
Salman Fateh, D.O.
Frank Knechtl, D.O.
Paul Porter, M.D.
Rana Bilbeisi, M.D.
Seraphim Pallas, M.D.
Anteneh A. Tesfaye, M.D.

Larry Matherly, Ph.D.
Thomas Mangner, Ph.D.
Laurel Stroempl, P.A.-C
Wei Chen Ph.D., Biostatistician
Seong Ho Kim, Ph.D., Biostatistician
Courtney French, PA-C
Kimberly Belzer, P.A.
Mary Ann Schwartz, N.P.
Prahlad Parajuli, Ph.D., Tumor Immunologist
Rayna Rosati, Ph.D., Postdoctoral Fellow

FAST FACTS

Objectives

The purpose of this study is to assess the effect of the corticosteroid dexamethasone (Dex) in patients with non-squamous Non-Small Cell Lung Cancer (NSCLC). This will be conducted using 3'-fluorothymidine (FLT) positron emission tomography (PET) done under ongoing imaging protocol 2006-127. The principal objective will be to compare tumor maximum, mean and peak standardized uptake values (SUVmax, SUVmean and SUVpeak), at baseline and after Dex treatment using FLT-PET imaging under ongoing imaging protocol 2006-127.

Correlative Studies

1. Patients will undergo three FLT-PET scans:
    i. Scan 1: Before Dex treatment, for a baseline
    ii. Scan 2 either on day 3 or 13-14 of Dex: Image patients on Dex 4 mg bid to assess anti-proliferative effect. Patients will continue Dex for 14 days total to evaluate the effect on senescence and then stop Dex for 7 days.
    iii. Scan 3 after discontinuation of prolonged Dex treatment: Treat patients for 14 days with Dex and then stop for about 7 days (day 6-8) and reimage the patients determine if Dex induces irreversible senescence when given for 14 days.
2. Tumor Glucocorticoid Receptor α expression (GRα) will be assessed from tumor tissue, pre-treatment obtained for initial diagnosis and as part of routine care.
3. Blood samples will be collected at the time of each imaging to measure serum Dex concentration and T cell, NK cell and monocyte cell counts and activation status and, plasma senescence markers as described below.

Key Inclusion/Exclusion Criteria

1. Patients must have histologically or cytologically proven non-squamous NSCLC. Patients may have newly diagnosed, recurrent, progressive or refractory or non-refractory disease which may be localized or wide spread.
2. Measurable disease with at least one lesion ≥2 cm on CT/MR.
3. No chemotherapy for at least 4 weeks and no radiation to the index lesion or clear progression in previously radiated lesion (greater than 20% increase in longest diameter).
4. Must be able to undergo three FLT-PET scans and sign consent for this protocol and FLT-PET protocol 2006-127.

Treatment Plan

- Pre-study history, performance status, physical examination, and blood tests within 6 weeks period before the start of the study. A blood pregnancy test may be needed for females of childbearing potential. As described in the patient consent form.
- Scan 1: Baseline FLT-PET within 7 days prior to the start of Dex treatment.
- Oral Dex 4 mg twice daily

- Scan 2: Either on day 3 or 13-14 of Dex.
- Continue oral Dex 4 mg twice a day for a total of 14 days
- Scan 3: about 7 days (day 6-8) after the final dose of Dex.
- After Scan 3: Dex 4 mg twice daily (chronic Dex) may resume based on treating physician's discretion.

Prominent Adverse Effects

Dexamethasone: Headache, Nausea, Increased blood pressure, Mood changes, Increased susceptibility to infection.

Correlative Markers:

- GRα expression in tumor tissue will be measured from the most recent tumor sample available using either one or both of 1) optimized TaqMan probes to quantify GRα mRNA; and 2) semi-quantitative immunohistochemistry to measure GR protein.
- Blood (5 ml) drawn at the time of each scan will be used to prepare serum which will be frozen for analysis of Dex levels.
- Additional blood samples (15 ml) drawn at each scan will be used for the following analyses:
- Measurement of T cell, monocyte and NK cell numbers and activation status by multi-parametric flow cytometry
- Measurement of various plasma cytokines and chemokines which will include, CCL2 (MCP-1), CCL4(MIP-1b), CXCL1 (GROa) and CXCL2 (GROb) using a multiplex cytokine array.

- In summary, blood will be obtained at baseline, at every scan and 7-15 days after patients are started on chronic Dex treatment in patients wishing to continue on therapy.

I. OBJECTIVES

1. **Primary Objective:** The primary objective of this pilot study is to evaluate the effect of dexamethasone (Dex) treatment in patients with non-squamous Non-Small Cell Lung Cancer (NSCLC) using 3'-fluorothymidine (FLT) positron emission tomography (PET) as measured by changes in tumor maximum standardized uptake value (SUVmax), SUVmean and SUVpeak.
2. **Secondary Objectives:**
   - **i.** Measure tumor Glucocorticoid Receptor α expression (GRα) from tumor tissue, when available.
   - **ii.** Analyze blood samples obtained during imaging to determine:
     - **a.** serum Dex concentration
     - **b.** counts and activation status of peripheral blood mononuclear cells
     - **c.** plasma levels of cytokines and chemokines associated with tumor senescence

II. BACKGROUND

1. Non-Small Cell Lung Cancer

Lung cancer is the leading cause of cancer-related mortality in the United States, accounting for approximately 27% of cancer deaths (1). Over 80% of lung cancer patients have non-small cell lung cancer (NSCLC), the majority of whom present with advanced disease. NSCLC patients face an overall 5-year survival rate of only 18%, which falls to 4% in patients with metastatic disease. Current first-line treatment decisions for advanced non-squamous NSCLC are based on the presence of targetable genetic aberrations, such as sensitizing mutations of *EGFR*, *BRAF*, and *MET*, or translocations of *ALK* and *ROS1* genes. However, these oncogenic drivers are present in only up to 20% of patients, and for rest of the patients, treatment options include the PD-1 inhibitor pembrolizumab either alone or in combination with platinum-pemetrexed chemotherapy (2, 3). The advent of immune checkpoint inhibitors has brought a paradigm shift in the management of advanced non-small cell lung cancer (NSCLC) with more than 2-fold improvement in median overall survival (mOS) in a subset of patients. However, inevitable development of resistance to these agents with eventual disease progression is a challenge. Therefore, novel treatment options are urgently needed.

2. Dexamethasone

As standard practice, patients undergoing pemetrexed-based chemotherapy are administered 4 mg of Dex twice daily starting the day before therapy and continuing until the day after treatment (4). The purpose of Dex in this context is to reduce the possibility of severe (grade 3 and 4) skin rash associated with pemetrexed therapy. In addition, Dex is frequently used as an anti-emetic for patients receiving combination therapy with cisplatin or carboplatin (5). The pharmacological actions of Dex are mediated by the glucocorticoid receptor (GR), which functions principally as a transcription factor (6). Regulation of gene expression by glucocorticoids produces a myriad of profound, tissue-specific cellular events (7). Glucocorticoid signaling controls cell proliferation and apoptosis, as well as inflammation and the inflammatory response (8, 9). The nature of the physiological response, as well as sensitivity to synthetic glucocorticoids such as Dex, is variable and tissue-dependent (10).

In cancer cells, glucocorticoids can modulate cell growth, cell survival and cellular senescence in a cell type-specific fashion (11-13). Several studies have indicated that, unfortunately, Dex may attenuate the anti-neoplastic effects of the chemotherapy whose side effects it is being used to alleviate (13-16). This effect has been shown to occur by distinct mechanisms, depending on the type of tumor. In triple-negative breast cancer cells, for example, Dex strongly activates pro-survival signaling (14-16). Recent work in NSCLC cells has reported that Dex produces reversible arrest of tumor cells in the early G1 phase of the cell cycle, rendering the cells resistant to pemetrexed (13).

Moreover, Dex treatment decreases expression of the reduced folate carrier and proton-coupled folate transporter, the major transporters of pemetrexed into the cell. Our research in clinically relevant NSCLC cell lines including loss- and gain-of-function studies has demonstrated that GRα expression is the principal determinant of the ability of Dex to protect NSCLC cells against pemetrexed (13). An analysis of GRα in advanced NSCLC tumors found that there is an approximately equal distribution of tumors with high and low GR expression (17).

In contrast to the above reversible effects of administering Dex for relatively short durations (24 h – 72 h), recent studies have demonstrated that in cells with glucocorticoid receptor-α (GR) expression corresponding to higher clinical tumor levels, extended (2 weeks) treatment with Dex induced a senescence phenotype (18). Under these conditions, the initial Dex-induced growth arrest was followed by marked cell expansion, beta-galactosidase expression and Ki67 negativity, regardless of p53 and K-RAS status. Dex induced a transient early surge in $p21^{Cip1}$. However, a progressive, irreversible loss of clonogenic growth, whose time of onset was dependent on GR level and Dex dose, was independent of $p21^{Cip1}$and caused by gradual accumulation of $p27^{Kip1}$ due to transcriptional activation of $p27^{Kip1}$ by Dex. This effect was independent of canonical pathways of senescence or $p27^{Kip1}$ regulation. The in vitro observations were reflected by growth suppression and P27Kip1 induction in GR-overexpressing tumor xenografts compared with isogenic low-GR tumors.

Further unpublished studies have shown that induction of senescence by Dex was accompanied in high GR expressing lung adenocarcinoma cells by the release of select chemokines, including CCL2 (MCP-1), CCL4(MIP-1b), CXCL1 (GROa) and CXCL2 (GROb). The production of these cytokines continued even after withdrawal of Dex. Paracrine effects of the Dex treated cancer cells induced migration of monocytes, NK cells and T cells, expansion and activation of NK cells, tumor cell cytolytic activity in NK cells, a modest reduction in MHC Class I expression, increase in MIC-A/B expression and PBL cytotoxicity.

Even in patients who received Dex prior to potentially curative surgery may benefit from Dex treatment, as shown in a recently published study(19). 588 patients with a median follow-up duration of 5.2 years, were found to have improved survival based on more complete resection, smaller tumor size, and highly differentiated tumors. Furthermore, perioperative use of dexamethasone was associated with long survival (HR, 0.70; 95% CI, 0.54–0.90; P = .006).

In summary, extended Dex treatment induces irreversible cell cycle blockade and a senescence phenotype through chronic activation of the p27Kip1 gene in GR overexpressing lung tumor cell populations. The Dex responsive tumor cells secrete chemokines that attract immune cells and activate a response against the tumor cells. Hence, extended Dex treatment of high GR expressing tumors could improve outcome of surgery/pemetrexed chemotherapy and sensitize tumors to immunotherapy.

Pharmacokinetic Data

The pharmacokinetics of Dex has been well characterized in humans as well as animal models. Dex has been shown to have an oral bioavailability of 70-80% (20). Although the plasma half-life of Dex is 3.5-4.5 hours, the pharmacological effects of Dex significantly outlast its plasma concentration due to significant (up to 77%) plasma protein binding. The biological half-life has been found to be 36-54 hours, which has made Dex suitable for medical conditions requiring continuous glucocorticoid action. There is a high uptake of Dex by the liver, kidney, and adrenal glands. Metabolism in the liver is slow via CYP3A4 and excretion is principally renal, as unconjugated steroids.

Safety Information

Dexamethasone is a steroid drug that is indicated for a variety of medical uses including inflammatory conditions, cancer, and hormonal disorders. Dex has been approved for use in

humans and a thorough understanding of its side effects has been established. Dosages of up to 20 mg are routinely used in cancer patients undergoing chemotherapy to prevent nausea and vomiting, allergic reactions and rashes. Dex is also given long term in patients with brain metastases to decrease inflammation. Finally, in a randomized trial, Dex (4 mg twice daily) was shown improve quality of life and cancer related fatigue in patients with advanced cancer who were no longer receiving chemotherapy (21).

Side effects of dexamethasone could include: irritation or stomach ulcers; increased blood sugar; hypertension; increased susceptibility to infection; and acne. More uncommon side effects that have been reported include: skin rash, itching or hives; swollen face, lower legs, or ankles. (See Section VI: Adverse Events: List and Reporting Requirements for more information).

3. Status of Clinical PET Imaging in Lung Cancer and Studies of FLT-PET

PET is now routinely used with fluorodeoxyglucose (FDG) for the diagnosis and staging of lung cancer, but monitoring treatment response by PET is still not part of regular assessment (22). Multi-center trials, including ACRIN 6678 and MK-0646-008 are working to determine the sensitivity of FDG-PET early in the course of lung cancer therapy, and have recently published reproducibility of FDG-PET in NSCLC (23). FLT-PET has been shown to provide reproducible measurement of tumor proliferation in patients with NSCLC (24). Further, several small trials have shown FLT-PET to be useful in evaluating treatment response in lung cancer. One study found early FLT to be predictive of progression-free survival (HR, 0.31; 95% CI, 0.10 to 0.95; $P < 0.05$) but not overall survival in patients treated with erlotinib (25, 26).

An ECOG-ACRIN multi-center trial of FLT-PET to assess response to neoadjuvant chemotherapy was conducted in breast cancer in which 51 patients underwent FLT-PET imaging at baseline, and following one cycle of treatment. The percent change in tumor maximum standardized uptake value (ΔSUVmax) between scans was calculated. Chemotherapy led to a significant ΔSUVmax (mean, 36%; 95% CI, 28-45%) and an area under the curve for ΔSUVmax in the prediction of pathologic complete response (pCR) = 0.68. The best ΔSUVmax cut-off for predicting pCR was 51% (sensitivity 56%; specificity 79%). The difference in ΔSUVmax between patients with and without pCR was significant (20%; one-sided p=0.045).

4. Imaging Dexamethasone Activity with FLT-PET in NSCLC

An ongoing pilot study at the Karmanos Cancer Institute is currently examining the effect of Dex in patients with NSCLC using FLT-PET. Thus far, 2/4 patients scanned have shown marked reductions in tumor FLT retention after Dex treatment (-64.7% and -54.3%). Of interest, patients had variable changes in FLT retention between lesions, indicating that FLT-PET may be able to detect the effect of Dex in lung cancer and assess differences in GR expression across metastases. In this protocol, we will seek to further investigate this phenomenon, and to characterize its reversibility following the withdrawal of Dex treatment.

5. Experimental Rationale

Despite the emergence of newer treatment strategies including immune checkpoint inhibitors and targeted agents, the 5-year overall survival for advanced NSCLC patients remains poor. Only a small fraction of patients achieve prolonged responses to immune checkpoint inhibitors (27). Furthermore, development of resistance to currently available treatment is an enormous challenge. Therefore, it is imperative that novel treatment strategies and therapeutic targets can be identified to improve outcomes of NSCLC.

Recent data has demonstrated that Dex, in a GRα-dependent manner, causes reversible G1 arrest as well down-regulation of the major transporters and targets of pemetrexed in a subset of NSCLC cell models (13). Critically, these effects are associated with a profound protective effect from the cytotoxic effect of pemetrexed, particularly when tumor cells are pre-treated for 24 hours with Dex, a schedule similar to that which is used clinically. Furthermore, data from a pilot in patients with

advanced NSCLC indicate that the effects of Dex on cell cycle progression within NSCLC tumors may be monitored by FLT-PET imaging. Relative expression of GRα has been found to be the primary determinant of variability in the Dex response among NSCLC cells, and analyses of GRα expression in primary lung tumors have reported tumor levels of GRα to be highly variable (17).

Despite the rapid systemic clearance of pemetrexed, cellular retention of pemetrexed as a result of its metabolism to polyglutamates confers a special advantage to pemetrexed-based chemotherapy (28). Both reduced polyglutamylation of pemetrexed and increased degradation of polyglutamate chains have been associated with pemetrexed resistance (29). In the clinical setting, clearance of Dex is relatively slow, with pharmacologically effective concentrations remaining in circulation for several days following administration (30). These and additional considerations including the fact that patients also receive Dex the day after pemetrexed; it takes a further 12-24 hours for the tumor cells to re-enter S-phase after Dex has been cleared; decreased pemetrexed uptake by RFC and PCFT occurs after Dex treatment, suggest that Dex-sensitive NSCLC tumors will be less responsive to pemetrexed when given with a standard Dex regimen prior to chemotherapy.

Stratifying patients on the basis of Dex sensitivity (or pemetrexed resistance) by FLT-PET imaging and/or by evaluating tumor GRα status may not only help to avoid subjecting predicted non-responders to futile treatment cycles, but could allow the clinical oncologist to switch to a potentially more useful regimen. Indeed, patients with high GRα tumors may respond better to alternative chemotherapies that require limited Dex treatment or no Dex at all (e.g. nab-paclitaxel instead of paclitaxel). Given the high degree of patient variability to response to pemetrexed, strategic treatment decisions would be expected to improve the treatment of tens-of-thousands of patients per year.

More recent studies have demonstrated that Dex-induced tumor cell senescence may be utilized as a potential treatment strategy, either by itself or in combination with other treatment modalities, for GRα expressing NSCLC. Thus in lung adenocarcinoma tumors expressing relatively high levels of GR (about a third to a half of all lung adenocarcinoma lesions), extended (2 weeks) Dex treatment may be expected to cause irreversible cell cycle blockade in the pemetrexed-resistant tumor cell population as well as sensitization of the entire tumor to immune attack. These events during extended Dex administration could be established and monitored in the patients through a combination of 1. FLT-PET imaging of individual lesions to monitor both initial effects of Dex on tumor cell proliferation and effects following Dex withdrawal and 2. Correlative blood tests to evaluate production of tumor senescence associated chemokines and immune activation post-Dex treatment. FLT-PET will allow us to make such measurements non–invasively and assess heterogeneity between patients and across multiple lesions in a single patient.

In summary, based on the rationale that

1. Pemetrexed-based regimens that are the standard-of-care in advanced NSCLC include Dex treatment on the day before, day of, and day after pemetrexed administration.
2. Dex, in a GRα -dependent manner, has been shown to reversibly inhibit NSCLC cell progression into the S-phase of the cell cycle, producing down-regulation of pemetrexed transporters and target enzymes, and resulting in a protection of NSCLC cells from pemetrexed cytotoxicity.
3. Primary NSCLC tumors are highly variable in their expression of GRα.
4. Extended (2 weeks) Dex treatment induces irreversible cell cycle blockade and a senescence phenotype through chronic activation of the p27Kip1 gene in GR overexpressing lung tumor cell populations
5. Induction of senescence by Dex is accompanied in high GR expressing lung adenocarcinoma cells by the release of select chemokines that attract and activate T cells, NK cells and monocytes and tumor cell cytolytic activity of NK cells
6. Pilot data that show FLT-PET is able to monitor Dex-mediated G1 arrest in patients with advanced lung cancer.

This arm of our pilot study will evaluate the effect of Dex in patients with NSCLC using FLT-PET imaging. This study will examine whether FLT-PET can accurately monitor Dex-mediated S-phase suppression in patient tumors and whether it could be routinely employed for the stratification of patients by GRα status. We will conduct correlative studies to measure the reversibility of Dex effects in NSCLC cells and will correlate measurements of tumor GRα expression with our findings from FLT-PET imaging when adequate sample is available. Additional correlative studies will utilize patient blood samples to measure Dex levels and will look for tumor senescence markers including specific cytokines and for activation of immune cells.

III. PATIENT SELECTION

Eligibility Criteria

1. Patients must have histologically or cytologically proven newly diagnosed, recurrent, progressive, refractory or non-refractory non-squamous NSCLC, which may be localized or widespread.

2. Patients must have measureable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm with conventional techniques (CT, MR or PET). Lesions in the previously irradiated area can be considered as measureable lesions as long as there has been an increase of at least 10 mm when compared to measurements obtained after completion of radiation.

3. No chemotherapy for at least 4 weeks and no radiation to the index lesion or clear progression in that lesion (greater than 20% and at least 10mm increase in longest diameter).

4. Life expectancy of greater than 4 weeks.

5. Absolute neutrophil count ≥1,000/mcL (measured within 6 weeks of registration).

6. No history of HIV or active infections.

7. Random blood glucose of ≤300mg/dl if history of diabetes mellitus. .

8. No surgery in the last 2 weeks prior to study enrollment.

9. Has not received Dex or another corticosteroid in over 4 weeks prior to enrollment.

10. Ability to understand and the willingness to sign a written informed consent document.

11. Agreed to FLT-PET imaging and signed consent and eligible FLT-PET protocol 2006-127.

IV. REGISTRATION PROCEDURES

General Guidelines

Eligible patients will be entered on the study centrally at the clinical trials office of the Karmanos Cancer Center/Wayne State University by the study coordinator.

At the time of registration:

- Patients must have signed an informed consent form.

- Confirm that all required prestudy history and physical examination, and laboratory tests have been collected.
- Document demographic information including smoking history. Also document medications.
- Patients should be scheduled for pre-treatment FLT-PET scans if patients have provided appropriate consent.
- Previous tumor biopsy should be retrieved for GRα analysis. This is not required prior to the start of therapy but should be retrieved within a month of registration.
- Inform patients about all necessary follow-up including laboratory tests and further PET imaging.

Following registration, patients should begin protocol treatment within 7 days. Issues that would cause treatment delays should be discussed with the Principal Investigator, Dr. Anthony Shields at 313-576-8735; shieldsa@karmanos.org, or contact the study coordinators. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be cancelled. The study coordinator should be notified of cancellations as soon as possible.

Registration Process

To register a patient, the following documents should be completed by the research nurse or data manager and faxed or emailed to the study coordinator:

- Copies of required laboratory tests, scans.
- Signed patient consent form.
- HIPAA authorization form.
- Eligibility checklist.
- Completed registration form.

To complete the registration process, the study coordinator will:

- Register the patient on the study.
- Contact the research nurse or data manager and verbally confirm registration.

Off-Study Criteria

- Patients will be considered to have gone off of study following the completion of the third FLT-PET scan unless they elect to continue Dex therapy under the supervision of their physician.
- Patients continuing Dex (chronic Dex) will remain on study until Dex treatment has been discontinued.

V. TREATMENT PLAN

FLT-PET Imaging

- Scan 1: Baseline within 7 days prior to the start of Dex treatment.
- Scan 2: Either on day 3 or 13-14 of Dex.
- Scan 3: Continue oral Dex 4mg twice a day for a total of 14 days, then stop Dex and obtain Scan 3 6-8 days after the final dose of Dex.

Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks for Dexamethasone are described in Section VI. No investigational or commercial agents or therapies other than Dexamethasone may be administered with the intent to treat the patient's malignancy.

AEs will be collected at the time of consent, at each scan and until Dex is discontinued. Patients electing to continue on chronic Dex will be followed by their treating physician.

Agent	Dose	Route	Schedule
Dexamethasone	4 mg	Orally	Twice daily for a total of 14 days. Scans before, during Dex as directed on day 3 or 13-14 and 6-8 days after Dex.

*Patients may elect to stay on dexamethasone (4 mg bid) indefinitely following the completion Scan 3 if it is considered to provide benefit by their treating physician. Patients continuing on Dex (chronic Dex) will be followed by the treating physician.

Benefits:
Dex 4mg twice daily compared to placebo has been shown to improve cancer related fatigue, anorexia and quality of life in patients with advanced cancer (22).

Correlative Markers:
1. GRα expression in tumor tissue will be assessed from the most recent tumor tissue available, using Either one or both of 1) optimized TaqMan probes to quantify GRα mRNA; and 2) semi-quantitative immunohistochemistry to measure GRα protein. The Biobanking and Correlative Sciences (BCS) Core facility at KCI will perform immunohistochemical studies on fixed tumor tissue under the supervision of Dr. Julie Boerner, the director of the BCS Core. The BCS Core will assist with the acquisition of patient tissue as well as preparation of samples for analysis. Analyses of GRα mRNA will be conducted in the Ratnam Laboratory at in the Hudson-Webber Cancer Research Center and will be under the direct supervision of Dr. Manohar Ratnam. Testing will be prioritized depending on the amount of sample available as follows:
    i. approximately 5000 cells or more: TaqMan probes to quantify GRα mRNA
    ii. approximately 5 cells: immunohistochemistry to measure GRα protein

2. Blood (20 ml) will be drawn at the time of each scan specifically, by the personnel at the Children's Hospital of Michigan PET Center. 15 ml of blood will be placed in heparinized tubes whereas serum will be prepared from the remaining 5 ml of blood.
    i. The serum samples will be used for analysis of Dex levels and to measure levels of specific chemokines. Serum samples will be taken to the KCI Pharmacology Core facility and frozen. The concentration of Dex will be measured using liquid chromatography-mass spectroscopy. Dr. Jing Li, the director of the KCI Pharmacology Core facility, will oversee quantification of plasma Dex concentration. Dr. Ratnam's lab will measure cytokine levels in the serum.
    ii. The 15 ml of whole blood will be transported to the Ratnam lab for flow cytometric analysis of immune cells.

VI. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

This study will use the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

Reporting Adverse Events
In the unlikely event that any immediate toxicity is found during the course of treatment, it will be recorded and reported as described below. Given the dose of drug, no measurable delayed toxicity is expected.

Reaction	Reporting Obligation for A, B, and C
A. All life-threatening events (Grade 4), which may be due to drug Administration [Note 2]	Within 24 hours [Note 1]
B. All fatal events (Grade 5) while on study (or within 24 hours of treatment).	Written report to follow within 10 working days. [Note 3]
C. First occurrence of any **previously unknown** clinical event (regardless of grade).	[Notes 4 and 5]

Note 1 Telephone number available 24 hours daily: (301) 230-2330 (Recorder after hours).
Note 2 See DCTD/NCI Common Terminology Criteria for Adverse Events.
Note 3 Report to: Investigational Drug Branch, PO Box 30012, Bethesda, MD, 20824.
Note 4 A list of all known toxicities can be found in the protocol document or consent form.
Note 5 **Reactions judged *definitely* not treatment-related should not be reported.**

- Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded on the Adverse Event Case Report Form and followed as appropriate. An adverse event is any undesirable sign, symptom or medical condition occurring after starting even if the event is not considered to be related.

- Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment (any procedures specified in the protocol).

- Any serious adverse event occurring after the patient has provided informed consent and until 4 weeks after the one dose of Dex must be reported.

- Adverse events occurring before starting study treatment but after signing the informed consent form are recorded on the Medical History/Current Medical Conditions Case Report Form.

- Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or require therapy, and are recorded on the Adverse Events Case Report Form under the signs, symptoms or diagnosis associated with them.

Adverse Event (AE) Characteristics

Serious adverse events
A serious adverse event is an undesirable sign, symptom or medical condition which:
1. Is fatal or life-threatening.
2. Required or prolonged hospitalization
3. Results in persistent or significant disability/incapacity
4. constitutes a congenital anomaly or a birth defect
5. are medically significant, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

Not considered to be serious adverse events are hospitalizations for the:
1. Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition.
2. Treatment, which was elective or pre-planned, for a pre-existing condition that did not worsen

3. treatment on an emergency, outpatient basis for an event **not** fulfilling any of the definitions of serious given above and **not** resulting in hospital admission

Commonly Reported Side Effects of Dexamethasone

- Stomach upset, irritation or stomach ulcers
- Increased blood sugar
- Increased blood pressure and swelling from fluid retention
- Decreased production of cortisol
- Increased susceptibility to infection
- Insomnia
- Mood changes, depression, anxiety
- Restlessness
- Vomiting
- Diarrhea
- Fever
- Decreased platelet count
- Increased risk of cataracts and glaucoma
- Osteoporosis
- Muscle loss
- Problems with healing
- Acne
- Weight gain
- Easy bruising
- Irregular or absent menstrual periods.
- Headache
- Dizziness
- Increased hair growth

Serious Side Effects Requiring Medical Attention

- Skin rash, itching, or hives
- Swollen face, lower legs, or ankles
- Vision problems
- Black or tarry stool
- Loss of consciousness
- Difficulty breathing
- Difficulty swallowing

'Attribution' of the AE will be defined by the investigative team

- Definite – The AE *is clearly related* to the study treatment
- Probable – The AE *is likely related* to the study treatment
- Possible – The AE *may be related* to the study treatment
- Unlikely – The AE *is doubtfully related* to the study treatment
- Unrelated – The AE *is clearly NOT related* to the study treatment

VII. MEASUREMENT OF EFFECT: FLT-PET SCANS

FLT-PET scans will be performed and data will be collected and analyzed as described in protocol 2006-127.

[^{18}F]-FLT [3'-deoxy-3'-^{18}F-fluorothymidine] is a thymidine analog used in PET imaging. After transport into the cells, it is phosphorylated by TK1 and trapped intracelluarly. Due to the lack of a 3' hydroxyl

group on its sugar, it cannot incorporate into the DNA structure, and therefore its retention reflects intracellular TK1 (31-33). FLT accumulates in tissues with high expression of TK1, such as bone marrow and tumors, and is a proliferation-imaging tracer (31, 33, 34). Kinetics data has shown that FLT is useful as a predictor of response to therapy in lung cancer (35).

FLT was originally developed for the treatment of HIV infection (36). In the initial human trial a starting dose of 0.125 mg/kg q12 hours resulted in grade 3 hematologic toxicity in 6/10 subjects. Other cytopenias have also been noted. The dose to be given during FLT-PET scans is about 0.02 mg or <1% of the dose used in the initial trials. Toxicity took weeks to develop even at the high therapeutic doses used in the phase I study. Based on this work no toxicity is anticipated as part of these imaging studies. In the unlikely event that any toxicity is found it will be recorded and reported.

PET imaging will be conducted over 60 minutes after the injection of approximately 10 mCi or tracer, once before therapy, and twice after the Dex administration. The first scan will be within 7 days prior to the start of Dex treatment 4 mg twice daily. The second scan will be during Dex, on day 3 or 13-14. The third and final scan will be done 6-8 days after the final Dex treatment.

Data Collection

Tumor uptake will be measured as Standard Uptake Value (SUV). SUV is calculated by the following formula:

SUV = Radioactivity concentration in ROI (μCi/mL) / Injected Dose (mCi) / Body Weight (kg)

The Standard Uptake Value maximum (SUVmax) will measure the most active pixel in the tumor and the two adjacent planes. Regions of Interest (ROIs) for the SUVmean will be drawn using isocontours set at 50% of the SUVmax. Results will be indicated as percent change in the uptake between the two scans. A pilot study of FLT-PET in NSCLC has shown that the measurement of SUVmean is reproducible in untreated patients, with errors of <10% when imaged twice within one week (24). SUVpeak considers the variation within a tumor. ROIs for the SUVpeak may be drawn with variations such as changing size, location and shape of the ROI. Results will be indicated as an average over a circle of approximately 1 cm around the hottest pixel and averaged over three adjacent or consecutive planes. One study of FLT-PET in solid tumors has shown that intratumor variations caused minimal variations in average tumor response but depending on how the ROI was drawn may be indicative of tumor response (37).

Analysis and Interpretation of Results

SUVmean will show the average standard uptake in the region of interest of the tumor and is representative of the total tumor uptake. Comparison between SUVmean in scans before and after therapy allows for measurements of the effect of treatment on the whole tumor tissue. Conversely, SUVmax will show the highest uptake in the selected region of interest of the tumor and will allow for comparisons of the effect of therapy within the most active sites of the tumor. The difference in SUVpeak between scans will be determined. SUVpeak may be an indication of tumor response with intratumoral measurements in scans before and after therapy.

VIII. CORRELATIVE STUDIES

1. Tumor GRα expression in tumor tissue obtained from the most recent tumor sample available, when sample size is adequate.
   As discussed above, GRα status is the principal determinant of the sensitivity of NSCLC to suppression of the S-phase of the cell cycle by Dex (13). As such, FLT-PET imaging studies will be correlated to patient specimens when available to verify that the imaging method is providing results consistent with cellular signaling within tumors. GRα will be measured using either 1) optimized TaqMan probes to quantify GRα mRNA or 2) semi-quantitative immunohistochemistry to measure

GR protein. GRα expression by stain requires approximately 5 cells while TaqMan needs approximately 5000 or more. Consideration for each sample size will determine which correlative studies will be done. If GRα is negative on the first sample then testing subsequent samples is not indicated.

As detailed above, the Ratnam laboratory will be responsible for measurements of GRα mRNA levels and the BCS core will conduct immunohistochemistry on fixed patient biopsy samples.

2. Measurements of blood Dex levels.
Of a total of 20 ml of blood drawn at the time of each FLT-PET scan, 5 ml will be used to generate serum. A part of the serum will be used for analysis of Dex levels using LC-MS. As mentioned, these experiments will be carried out at the KCI Pharmacology Core facility.

3. Measurements of tumor senescence markers and immune cell status
In addition to its anticipated effects on FLT-PET imaging, extended dexamethasone treatment has been shown to induce cellular senescence in cells expressing very high-level GR resulting in secretion of chemokines (senescence markers) in the plasma that in turn activate immune cells. The correlative studies will focus on the senescence markers and T cell and NK cell frequency (proliferation) and activation profile during long-term Dex treatment compared with the pre-treatment samples.

Peripheral blood (20 ml) will be drawn at the following time points: (1) Pre-treatment; (2) At scan 3 on Day 3 or 13-14 of Dex treatment and 1 week (6-8 days) after stopping Dex treatment. 5 ml of the blood will be used to prepare serum, a part of which will be used to measure Dex levels as noted above. The remaining serum will be used to measure levels of specific cytokines previously found to be sectreted by the senescent tumor cells, including CCL2 (MCP-1), CCL4(MIP-1b), CXCL1 (GROa) and CXCL2 (GROb).

Blood from blood drawn at the time of each FLT-PET scan will be collected in heparin tubes and plasma and PBMC will be isolated using Ficoll gradient centrifugation following a standard protocol. With the PBMC, multi-parametric flow cytometry will be performed for phenotypic and functional characterization, including activation markers, intracellular cytokine profile, of immune cell subsets, as follows:

Surface stain:

CD3	FITC
CD56	PE
NKG2D	BV510
CD16	APC-Cy7
PD-1	BV421

Intracellular staining:

IFN-γ	BV711
Perforin	AF647
Ki67	PerCP-eFluor710

IX. STATISTICAL CONSIDERATIONS

Study Design

This a small pilot study of 6 patients with NSCLC, with each patient serving as his/her own control. The primary goal will be to use FLT-PET to determine the change, if any, in tumor SUVmax between the baseline scan prior to treatment, and the second scan on day 3 or 13-14 of Dex, 4 mg twice daily. Comparison between SUVmax in scans before and after Dex therapy makes it possible to measure the

effect of Dex on the whole tumor tissue. In addition, patients will be imaged about 7 days after discontinuation of Dex and tumor senescence markers will be assessed using biopsy samples and blood drawn at the time of imaging.

CT anatomic landmarks will be used to assess the same lesion post-treatment if the lesion is difficult to visualize on PET imaging. SUVmean measurements will also be recorded. The reproducibility of tumor uptake based on SUV analysis is estimated to be on the order of 10%, and a true effect in an individual case can be defined as a change in tracer uptake greater than 3 times the standard deviation (SD) of the tracer ([^{18}F]-FLT) uptake without treatment.

Statistical Analysis

The primary analysis is one sample t-test for the change of SUVmax. Secondary analyses will be carried out with descriptive statistics such as mean, SD, range. Correlation analysis will be descriptive. Spearman correlation coefficient will be calculated and scatter plot will be plotted. Subgroup analyses for Dex withdrawal will be performed within the GRα high group. All subgroup analyses will be descriptive due to the expected small sample size.

Based on epidemiological studies, described previously, we expect that about 50% of patients will have high GRα and thus will be susceptible to Dex-mediated S-phase suppression and therefore reduced FLT uptake at the time of the second scan. High GRα expression, for the purposes of this protocol, will be measured by immunohistochemical staining and will be defined as 75% of the sections scoring 3+ or 4+ based on a recent lung adenocarcinoma tissue microarray.

Sample Size and Accrual Rate

A sample size of 10 achieves 80% of power to detect a large effect size of 0.89 at a significance level of 0.05 using a two-sided one-sample t test (38). Accrual rate and study duration: We estimated the accrual rate at 4 patients per year but accrual may be as low as two patients per year. The total study duration is expected to be 42 months including 36 months for accrual and 6 months minimum follow-up for primary and secondary endpoints.

X. DATA AND SAFETY MONITORING/STUDY AUDITS/STUDY ETHICS

Data and Safety Monitoring

1. Scheduled meetings will be held monthly or more frequently depending on the activity of the protocol. These meetings will include the protocol investigators and research staff involved with the conduct of the protocol.
2. During these meetings the investigators will discuss:
    - Safety of protocol participants (adverse events and reporting)
    - Validity and integrity of the data (data completeness on case report forms and complete source documentation)
    - Enrollment rate relative to expectation of target accrual, (eligible and ineligible participants)
    - Retention of participants, adherence to the protocol and protocol deviations
    - Protocol amendments
3. Data and Safety Monitoring Reports (DSMR) of the research meetings will be completed by the Study Coordinator and submitted to the Data and Safety Monitoring Committee quarterly for review.
4. The Barbara Ann Karmanos Cancer Institute, Data and Safety Monitoring Committee (DSMC) provide the primary oversight of data and safety monitoring for KCI Investigator-initiated trials.

Data Management

Study data including patient images, biopsy slides, and blood samples will be maintained by the Shields' laboratory. In addition, a KCI CTO monitor specialist will remotely essential clinical trial data. Frequency of monitoring will be based on accrual but will occur at least once every 2 months if a patient

has been enrolled. Monthly screening and enrollment logs will be sent to the KCI lead Study Coordinator.

Study Audits

Authorized representatives of FDA, a regulatory authority or Wayne State University Institutional Review Board (IRB) may visit the center to perform audits or inspections, including source data verification. The investigator should contact Clinical Trials Office immediately if contacted by a regulatory agency about an inspection at his center regarding this study.

Ethics

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, applicable regulatory requirements.

The final study protocol, including the final version of the Written Informed Consent Form, must be approved in writing by Wayne State University IRB.

The principal investigator is responsible for informing the Wayne State University IRB of any amendments to the protocol. The protocol must be re-approved by the IRB annually. Progress reports and notifications of serious, unexpected adverse drug reactions will be provided to the IRB. The Principal Investigator is also responsible for providing the IRB with reports of any serious adverse drug reactions from any other study conducted with the investigational product.

The principal investigator will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided. In accordance with the Health Information Portability and Accountability Act (HIPAA), the Written Informed Consent Form must include a subject authorization to release medical information to Karmanos Cancer Institute, Wayne State University, the Detroit Medical Center, University Physician Group, or McLaren Health Care or Institutional Review Board access to subject's medical information that includes all hospital records relevant to the study, including subjects' medical history.

The principal investigator must store the original, signed written informed consent form. A copy of the signed written informed consent form must be given to the subject.

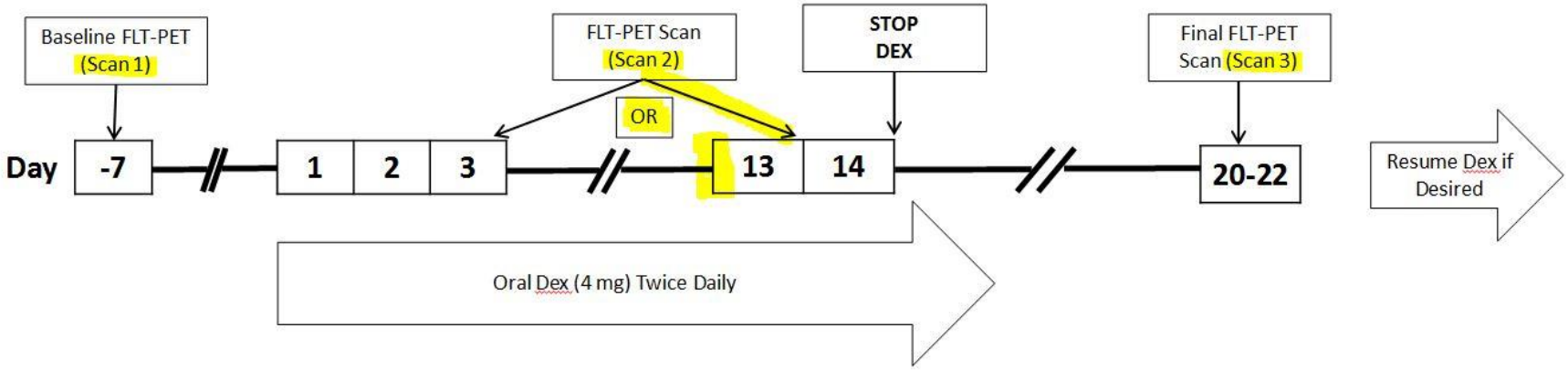
XI. REFERENCES

1. Adler LP, Blair HF, Makley JT, Williams RP, Joyce MJ, Leisure G, Alkaisi N, Milraldi F. Noninvasive grading of musculoskeletal tumors using PET. J Nucl Med. 1991;32:1508-12.
2. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csoszi T, Fulop A, Gottfried M, Peled N, Tafreshi A, Cuffe S, O'Brien M, Rao S, Hotta K, Leiby MA, Lubiniecki GM, Shentu Y, Rangwala R, Brahmer JR, Investigators K-. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med. 2016;375(19):1823-33. Epub 2016/10/11. doi: 10.1056/NEJMoa1606774. PubMed PMID: 27718847.
3. Gandhi L, Rodriguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, Domine M, Clingan P, Hochmair MJ, Powell SF, Cheng SY, Bischoff HG, Peled N, Grossi F, Jennens RR, Reck M, Hui R, Garon EB, Boyer M, Rubio-Viqueira B, Novello S, Kurata T, Gray JE, Vida J, Wei Z, Yang J, Raftopoulos H, Pietanza MC, Garassino MC, Investigators K-. Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. N Engl J Med. 2018;378(22):2078-92. Epub 2018/04/17. doi: 10.1056/NEJMoa1801005. PubMed PMID: 29658856.
4. Lilly E. Alimta (pemetrexed) package insert2005.
5. Basch E, Hesketh PJ, Kris MG, Prestrud AA, Temin S, Lyman GH. Antiemetics: american society of clinical oncology clinical practice guideline update. Journal of oncology practice. 2011;7(6):395-8. doi: 10.1200/JOP.2011.000397. PubMed PMID: 22379425; PMCID: 3219469.
6. Miranda TB, Morris SA, Hager GL. Complex genomic interactions in the dynamic regulation of transcription by the glucocorticoid receptor. Molecular and cellular endocrinology. 2013;380(1-2):16-24. doi: 10.1016/j.mce.2013.03.002. PubMed PMID: 23499945; PMCID: 3724757.
7. Kadmiel M, Cidlowski JA. Glucocorticoid receptor signaling in health and disease. Trends in pharmacological sciences. 2013;34(9):518-30. doi: 10.1016/j.tips.2013.07.003. PubMed PMID: 23953592; PMCID: 3951203.
8. Schaaf MJ, Cidlowski JA. Molecular mechanisms of glucocorticoid action and resistance. J Steroid Biochem Mol Biol. 2002;83(1-5):37-48. PubMed PMID: 12650700.
9. Vilasco M, Communal L, Mourra N, Courtin A, Forgez P, Gompel A. Glucocorticoid receptor and breast cancer. Breast Cancer Res Treat. 2011;130(1):1-10. Epub 2011/08/06. doi: 10.1007/s10549-011-1689-6. PubMed PMID: 21818591.
10. Lu NZ, Cidlowski JA. Glucocorticoid receptor isoforms generate transcription specificity. Trends in cell biology. 2006;16(6):301-7. Epub 2006/05/16. doi: 10.1016/j.tcb.2006.04.005. PubMed PMID: 16697199.
11. Ge H, Ni S, Wang X, Xu N, Liu Y, Wang X, Wang L, Song D, Song Y, Bai C. Dexamethasone reduces sensitivity to cisplatin by blunting p53-dependent cellular senescence in non-small cell lung cancer. PLoS One. 2012;7(12):e51821. doi: 10.1371/journal.pone.0051821. PubMed PMID: 23272171; PMCID: 3525662.
12. Greenstein S, Ghias K, Krett NL, Rosen ST. Mechanisms of glucocorticoid-mediated apoptosis in hematological malignancies. Clin Cancer Res. 2002;8(6):1681-94. PubMed PMID: 12060604.
13. Patki M, Gadgeel S, Huang Y, McFall T, Shields AF, Matherly LH, Bepler G, Ratnam M. Glucocorticoid receptor status is a principal determinant of variability in the sensitivity of non-small-cell lung cancer cells to pemetrexed. J Thorac Oncol. 2014;9(4):519-26. Epub 2014/04/17. doi: 10.1097/JTO.0000000000000111. PubMed PMID: 24736075.
14. Pang D, Kocherginsky M, Krausz T, Kim SY, Conzen SD. Dexamethasone decreases xenograft response to Paclitaxel through inhibition of tumor cell apoptosis. Cancer biology & therapy. 2006;5(8):933-40. PubMed PMID: 16775428.
15. Wu W, Chaudhuri S, Brickley DR, Pang D, Karrison T, Conzen SD. Microarray analysis reveals glucocorticoid-regulated survival genes that are associated with inhibition of apoptosis in breast epithelial cells. Cancer Res. 2004;64(5):1757-64. PubMed PMID: 14996737.
16. Wu W, Pew T, Zou M, Pang D, Conzen SD. Glucocorticoid receptor-induced MAPK phosphatase-1 (MPK-1) expression inhibits paclitaxel-associated MAPK activation and contributes to breast cancer cell survival. J Biol Chem. 2005;280(6):4117-24. doi: 10.1074/jbc.M411200200. PubMed PMID: 15590693.

17. Lu YS, Lien HC, Yeh PY, Kuo SH, Chang WC, Kuo ML, Cheng AL. Glucocorticoid receptor expression in advanced non-small cell lung cancer: clinicopathological correlation and in vitro effect of glucocorticoid on cell growth and chemosensitivity. Lung Cancer. 2006;53(3):303-10. Epub 2006/06/30. doi: 10.1016/j.lungcan.2006.05.005. PubMed PMID: 16806572.
18. Patki M, McFall T, Rosati R, Huang Y, Malysa A, Polin P, Fielder A, Wilson MR, Lonardo F, Back J, Li J, Matherly LH, Bepler G, Ratnam M. Chronic p27Kip1 Induction by Dexamethasone Causes Senescence Phenotype and Permanent Cell Cycle Blockade in Lung Adenocarcinoma Cells Overexpressing Glucocorticoid Receptor. Scientific reports. 2018. Epub October 30, 2018.
19. Huang WW, Zhu WZ, Mu DL, Ji XQ, Nie XL, Li XY, Wang DX, Ma D. Perioperative Management May Improve Long-term Survival in Patients After Lung Cancer Surgery: A Retrospective Cohort Study. Anesth Analg. 2018;126(5):1666-74. Epub 2018/03/09. doi: 10.1213/ANE.0000000000002886. PubMed PMID: 29517574; PMCID: PMC5908254.
20. Loew D, Schuster O, Graul EH. Dose-dependent pharmacokinetics of dexamethasone. European journal of clinical pharmacology. 1986;30(2):225-30. PubMed PMID: 3709651.
21. Yennurajalingam S, Frisbee-Hume S, Palmer JL, Delgado-Guay MO, Bull J, Phan AT, Tannir NM, Litton JK, Reddy A, Hui D, Dalal S, Massie L, Reddy SK, Bruera E. Reduction of cancer-related fatigue with dexamethasone: a double-blind, randomized, placebo-controlled trial in patients with advanced cancer. J Clin Oncol. 2013;31(25):3076-82. doi: 10.1200/JCO.2012.44.4661. PubMed PMID: 23897970.
22. Cuaron J, Dunphy M, Rimner A. Role of FDG-PET scans in staging, response assessment, and follow-up care for non-small cell lung cancer. Frontiers in oncology. 2012;2:208. Epub 2013/01/15. doi: 10.3389/fonc.2012.00208. PubMed PMID: 23316478; PMCID: 3539654.
23. Weber WA, Gatsonis CA, Mozley PD, Hanna LG, Shields AF, Aberle DR, Govindan R, Torigian DA, Karp JS, Michael Yu JQ, Subramaniam RM, Halvorsen RA, Siegel BA, Acrin, teams MKR. Repeatability of 18F-FDG PET/CT in Advanced Non-Small Cell Lung Cancer: Prospective Assessment in 2 Multicenter Trials. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2015;56(8):1137-43. doi: 10.2967/jnumed.114.147728. PubMed PMID: 25908829.
24. Shields AF, Lawhorn-Crews JM, Briston DA, Zalzala S, Gadgeel S, Douglas KA, Mangner TJ, Heilbrun LK, Muzik O. Analysis and reproducibility of 3'-Deoxy-3'-[18F]fluorothymidine positron emission tomography imaging in patients with non-small cell lung cancer. Clin Cancer Res. 2008;14(14):4463-8. Epub 2008/07/17. doi: 14/14/4463 [pii]
10.1158/1078-0432.CCR-07-5243. PubMed PMID: 18628460.
25. Tehrani OS, Shields AF. PET imaging of proliferation with pyrimidines. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2013;54(6):903-12. doi: 10.2967/jnumed.112.112201. PubMed PMID: 23674576.
26. Zanetti KA, Haznadar M, Welsh JA, Robles AI, Ryan BM, McClary AC, Bowman ED, Goodman JE, Bernig T, Chanock SJ, Harris CC. 3'-UTR and functional secretor haplotypes in mannose-binding lectin 2 are associated with increased colon cancer risk in African Americans. Cancer Res. 2012;72(6):1467-77. doi: 10.1158/0008-5472.CAN-11-3073. PubMed PMID: 22282660; PMCID: 3306468.
27. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, Barlesi F, Kohlhaufl M, Arrieta O, Burgio MA, Fayette J, Lena H, Poddubskaya E, Gerber DE, Gettinger SN, Rudin CM, Rizvi N, Crino L, Blumenschein GR, Jr., Antonia SJ, Dorange C, Harbison CT, Graf Finckenstein F, Brahmer JR. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. N Engl J Med. 2015;373(17):1627-39. doi: 10.1056/NEJMoa1507643. PubMed PMID: 26412456.
28. Rinaldi DA. Overview of phase I trials of multitargeted antifolate (MTA, LY231514). Semin Oncol. 1999;26(2 Suppl 6):82-8. Epub 1999/12/22. PubMed PMID: 10598560.
29. Schneider E, Ryan TJ. Gamma-glutamyl hydrolase and drug resistance. Clinica chimica acta; international journal of clinical chemistry. 2006;374(1-2):25-32. doi: 10.1016/j.cca.2006.05.044. PubMed PMID: 16859665.

30. Weijtens O, Schoemaker RC, Cohen AF, Romijn FP, Lentjes EG, van Rooij J, van Meurs JC. Dexamethasone concentration in vitreous and serum after oral administration. American journal of ophthalmology. 1998;125(5):673-9. PubMed PMID: 9625551.
31. Buck AK, Schirrmeister H, Hetzel M, Von Der Heide M, Halter G, Glatting G, Mattfeldt T, Liewald F, Reske SN, Neumaier B. 3-deoxy-3-[(18)F]fluorothymidine-positron emission tomography for noninvasive assessment of proliferation in pulmonary nodules. Cancer Res. 2002;62(12):3331-4. PubMed PMID: 12067968.
32. Grierson JR, Schwartz JL, Muzi M, Jordan R, Krohn KA. Metabolism of 3'-deoxy-3'-[F-18]fluorothymidine in proliferating A549 cells: validations for positron emission tomography. Nucl Med Biol. 2004;31(7):829-37. PubMed PMID: 15464384.
33. Shields AF, Grierson JR, Dohmen BM, Machulla HJ, Stayanoff JC, Lawhorn-Crews JM, Obradovich JE, Muzik O, Mangner TJ. Imaging proliferation in vivo with [F-18]FLT and positron emission tomography. Nature medicine. 1998;4(11):1334-6. doi: 10.1038/3337. PubMed PMID: 9809561.
34. Barthel H, Perumal M, Latigo J, He Q, Brady F, Luthra SK, Price PM, Aboagye EO. The uptake of 3'-deoxy-3'-[18F]fluorothymidine into L5178Y tumours in vivo is dependent on thymidine kinase 1 protein levels. European journal of nuclear medicine and molecular imaging. 2005;32(3):257-63. doi: 10.1007/s00259-004-1611-0. PubMed PMID: 15791434.
35. Vesselle H, Grierson J, Muzi M, Pugsley JM, Schmidt RA, Rabinowitz P, Peterson LM, Vallieres E, Wood DE. In Vivo Validation of 3'deoxy-3'-[(18)F]fluorothymidine ([(18)F]FLT) as a Proliferation Imaging Tracer in Humans: Correlation of [(18)F]FLT Uptake by Positron Emission Tomography with Ki-67 Immunohistochemistry and Flow Cytometry in Human Lung Tumors. Clin Cancer Res. 2002;8(11):3315-23. PubMed PMID: 12429617.
36. Flexner C, van der Horst C, Jacobson MA, Powderly W, Duncanson F, Ganes D, Barditch-Crovo PA, Petty BG, Baron PA, Armstrong D, et al. Relationship between plasma concentrations of 3'-deoxy-3'-fluorothymidine (alovudine) and antiretroviral activity in two concentration-controlled trials. The Journal of infectious diseases. 1994;170(6):1394-403. PubMed PMID: 7995977.
37. Vanderhoek M, Perlman SB, Jeraj R. Impact of the definition of peak standardized uptake value on quantification of treatment response. J Nucl Med. 2012;53(1):4-11. Epub 2012/01/04. doi: 10.2967/jnumed.111.093443. PubMed PMID: 22213818; PMCID: PMC3308343.
38. Harrell F. Regression Modeling Strategies: With Applications to Linear Models, Logistic Regression, and Survival Analysis. . New York: Springer Science and Business Media, Inc.; 2001.

APPENDIX A: STUDY SCHEMA



- Scan 1: Baseline FLT-PET imaging within 7 days before Dex treatment
- Scan 2: After the 5th dose of Dex on Day 3 or 13-14 at Dex completion
- Stop Dex
- Scan 3: Final scan 6-8 days after the last dose of Dex
- Dex (4 mg bid) may be resumed if agreed to by the patient and MD