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August 9, 2018

Subject: **DDT QUALIFICATION SUBMISSION**

DDT Type: **BIOMARKER**

DDT Tracking Number: **DDTBMQ000074**

Submission Type: **LETTER OF INTENT (LOI)**

DDT Name: **Volume of the lower lobes of the lungs (V_{LLL}) as assessed by CT**

Context of Use: Volume of the lower lobes of the lungs (V_{LLL}) at maximal inspiration is a monitoring biomarker for disease staging in patients with Idiopathic Pulmonary Fibrosis (IPF) for use in IPF treatment studies.

Dear Dr. Leptak,

Please find attached a Letter of Intent (LOI) for the DDT listed above in part of the requirements of the first phase of the DDT Qualification Process.

Thank you,

Jan De Backer, MSc, PhD, MBA

CEO

FLUIDDA, Inc.

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1. ADMINISTRATIVE INFORMATION

1.1. REQUESTING ORGANIZATION

FLUIDDA, Inc.
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1.2. PRIMARY CONTACT

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1.3. ALTERNATE CONTACT

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1.4. SUBMISSION DATE

August 9, 2018

1.5. IF THERE IS A PRIOR, CURRENT, OR PLANNED SUBMISSION TO OTHER REGULATORY AGENCIES, LIST THE AGENCIES AND DATES AS APPROPRIATE.

N/A

2. PROPOSED CONTEXT OF USE

(limited to 500 characters)

Use Statement: Volume of the lower lobes of the lungs (V_{LLL}) at maximal inspiration is a monitoring biomarker for disease staging in patients with Idiopathic Pulmonary Fibrosis (IPF) for use in IPF treatment studies.

Conditions of Qualified Use: The biomarker can be used in patients diagnosed with IPF based on currently accepted guidelines. V_{LLL} is determined from a single HRCT scan taken at maximal inspiration, which must be taken in strict accordance with the imaging protocol set forth by FLUIDDA to ensure data integrity.

3. DRUG DEVELOPMENT NEED

Describe the drug development need that the biomarker is intended to address, including (if applicable) the proposed benefit over currently used biomarkers for similar COUs (limited to 1,500 characters).

Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease characterized by progressive loss of the ability of the lungs to transport oxygen into the bloodstream effectively due to the buildup of scar tissue (fibrosis). IPF is traditionally staged with terms such as “mild,” “severe,” “early,” and “advanced” which are ambiguously based on pulmonary function tests (e.g. Forced Vital Capacity (FVC)). FVC is the amount of air that can be forcibly exhaled from the lungs after taking the deepest breath possible, and currently reigns as the primary marker in IPF-treatment clinical trials.

A decline in percent-predicted-FVC (the ratio of measured FVC to the predicted value of FVC for a healthy patient of the same age, sex and height expressed as a percent, $FVC_{\%p}$) has been linked with an increase in mortality in patients with IPF, substantiating its use as a marker of disease staging. $FVC_{\%p}$, however, suffers from low sensitivity and high variability, which has resulted in large, expensive and complex clinical trials (>1200 patients enrolled in trials for each of two recently FDA approved drugs: nintedanib and pirfenidone) (FDA Reference ID: 3642131). $FVC_{\%p}$'s low sensitivity stems from the fact that test is a measure of the function of the lungs in their totality. It is unable to detect the disease early on due to the compensatory nature of the lungs, in which the upper lobes can compensate for the diseased lower lobes leading to an overall unchanged $FVC_{\%p}$. The high variability of $FVC_{\%p}$ to the disease stage can be attributed to testing

complications such as patient dependence on the test maneuver, and fact that an assessment of the overall function of the lungs oversimplifies the staging of IPF.

V_{LLL} can be expressed as a percentage of the predicted V_{LLL} of a healthy patient with the same age, sex and height to form the decision tool: percent-predicted-V_{LLL} (V_{LLL-%p}). V_{LLL-%p} has increased sensitivity over FVC_{%p}, due to its ability to detect changes in the lower lobes early on in the disease, that are invisible to FVC_{%p}. A reduction in variability is achieved through the use of “gating” at the time of CT scan acquisition to minimize dependence on patient effort.

4. BIOMARKER INFORMATION

4.1. BIOMARKER NAME AND DESCRIPTION. IF COMPOSITE, PLEASE LIST THE BIOMARKER COMPONENTS.

Volume of the lower lobes of the lungs (V_{LLL}) at maximal inspiration.

V_{LLL} is the sum of the volume of the lower left lobe and lower right lobe of the lungs at maximal inspiration.

4.2. TYPE OF BIOMARKER

Type of Biomarker: Radiologic

Scheme: n/a

I/D: n/a

Matrix (e.g. blood) or modality (e.g. MRI): CT

Primary biomarker category: Monitoring

4.3. DESCRIBE THE MECHANISTIC RATIONALE OR BIOLOGIC PLAUSIBILITY TO SUPPORT THE BIOMARKER AND ITS ASSOCIATED COU (LIMITED TO 1,500 CHARACTERS).

The fibrosing nature of IPF is already known to cause a reduction in lung compliance which manifests as an overall reduction in lung volume (Plantier, 2018). The disease is known to begin in the lower lobes (ATS, 2011), with CT honeycombing occurring in the lower lobes earlier than the upper lobes and auscultation of the lungs revealing

inspiratory crackles occurring in the lower posterior lung zones upon physical exam early on in the disease (Meltzer, 2008).

In addition, the decline in V_{LLL-%p} has been correlated with declining FVC_{%p} (section 7.1), which is a previously accepted marker of disease staging in IPF.

4.4. IF BIOMARKER IS AN INDEX/SCORING SYSTEM, PLEASE PROVIDE INFORMATION ON HOW THE INDEX IS DERIVED (E.G., ALGORITHM), THE BIOLOGIC RATIONALE FOR INCLUSION OF EACH OF THE COMPONENTS, THE RATIONALE FOR ANY DIFFERENTIAL WEIGHTING OF THE ELEMENTS, AND THE MEANING/INTERPRETATION OF THE INDEX/SCORE (LIMITED TO 1,500 CHARACTERS).

The biomarker V_{LLL} is used to form a decision tool V_{LLL-%p}. V_{LLL-%p} is calculated by dividing the measured V_{LLL} of the patient by the predicted value of V_{LLL} for a healthy patient of the same age, sex and height and expressing as a percent. The age, sex and height are the dominant parameters in determining the percent-predicted-FVC (FVC-%p) of a healthy patient, which is based on total lung volume. Age, sex, and height have also been validated by FLUIDDA as the dominant parameters for determining the predicted values of the individual lobes of the lungs.

Frequently, FVC_{%p} is used in a continuous manner, with decreasing FVC_{%p} indicating later stage IPF, however equidistant cut points at 25%, 50% and 75% have been used to divide the progression into 3 stages in some studies (Kolb, 2014).

We propose that V_{LLL-%p} be used similarly. That is, continuously when appropriate, and discretely defined by the following stages, with increasing stage having decreased V_{LLL-%p} and increased mortality:

V _{LLL-%p}	Stage
V _{LLL-%p} > 75%	1
50% < V _{LLL-%p} ≤ 75%	2
V _{LLL-%p} ≤ 50%	3

Table 1. IPF staging using percent-predicted-Volume of the lower lobes of the lungs percent predicted (V_{LLL-%p})

5. BIOMARKER MEASUREMENT INFORMATION

Provide a general description of what aspect of the biomarker is being measured and by what methodology (e.g., radiologic findings such as lesion number, specific measure of organ size, serum level of an analyte, change in the biomarker level relative to a reference such as baseline) (limited to 1,500 characters).

V_{LLL} is assessed by mathematically processing a single low-dose high resolution computed tomography (HRCT) scan of the lungs taken at maximal inspiration. In the 2D HRCT scan, the lungs are first identified by grouping together pixels in the scan having Hounsfield units between -1024 and 500. The 2D lungs are converted to 3D models (Figure 1) through a process known as segmentation.

From the 3D models of the lungs, fissure lines (shown in red in Figure 1) which divide the lungs into the 5 lobes are identified. These fissure lines are used to construct 3D models of each of the five lobes from the 3D models of the lungs. The volume of each of the lobes is calculated from its 3D model and the volume of the two lower lobes is summed to form V_{LLL} .

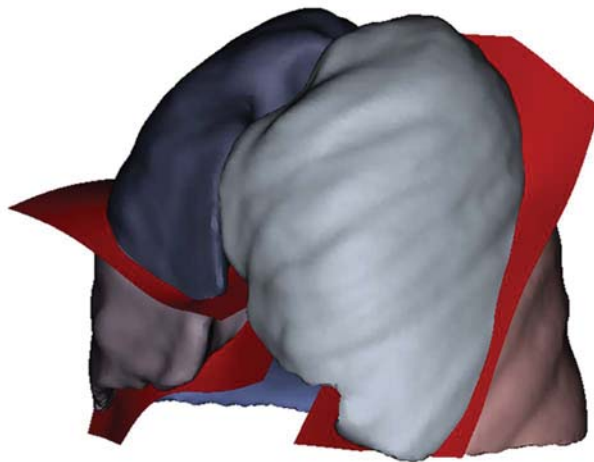


Figure 1. 3D model of lungs showing fissure lines (in red).

5.1. IS THE BIOMARKER TEST/ASSAY CURRENTLY AVAILABLE FOR PUBLIC USE?

Yes.

5.2. INDICATE WHETHER THE BIOMARKER TEST/ASSAY IS ONE OR MORE OF THE FOLLOWING:

Laboratory Developed Test

5.3. IF THE BIOMARKER IS QUALIFIED, WILL THE TEST/ASSAY BE PERFORMED IN A CLINICAL LABORATORY IMPROVEMENT AMENDMENTS (CLIA)-CERTIFIED LABORATORY?

No

5.4. IS THE BIOMARKER TEST CURRENTLY UNDER REVIEW BY THE CENTER FOR DEVICES AND RADIOLOGICAL HEALTH OR THE CENTER FOR BIOLOGICS EVALUATION AND RESEARCH?

No

5.5. IS THERE A STANDARD OPERATING PROCEDURE (SOP) FOR SAMPLE COLLECTION AND STORAGE?

Yes

5.6. IS THERE A LABORATORY SOP FOR THE TEST/ASSAY METHODOLOGY?

Yes

5.7. DESCRIBE THE EXTENT OF ANALYTICAL VALIDATION THAT HAS BEEN PERFORMED (E.G., SENSITIVITY, SPECIFICITY, ACCURACY, AND/OR PRECISION OF THE ASSAY OR METHOD) (LIMITED TO 1,500 CHARACTERS).

Functional Respiratory Imaging has been validated across a variety of studies. The technical variability associated with FRI was assessed by scanning an imaging phantom using two different scanners in different positions. Results indicated that the variation in the tube volume was $0.45 \text{ mm}^3 \pm 0.68\%$ and variability across different scanners was $0.99 \text{ mm}^3 \pm 0.68\%$. The minimum resolution of the segmentation approach is 1/6th

of the pixel size due to the partial volume effect; where a typical pixel size of 0.6 mm corresponds to a segmentation resolution of 0.1 mm.

FRI-based lung volume measurements were validated against conventional lung function testing in a cohort of IPF patients. The FRI-based lung volumes were compared to lung volume measurements obtained using helium dilution. A strong correlation ($R = 0.96$) was found between the two methods.

Additional validation data can be found in the supporting documents.

6. ADDITIONAL CONSIDERATIONS FOR RADIOGRAPHIC BIOMARKERS

6.1. HOW HAS THE METHOD FOR IMAGE ACQUISITION, ANALYSIS, AND INTEGRATION OF THE DATA BEEN OPTIMIZED? (LIMITED TO 1,000 CHARACTERS.)

FLUIDDA maintains a variety of quality assurance protocols to ensure data reliability, validity and integrity. Technical variability across scanners is normalized through the use of an imaging phantom, subsequent analysis, and maintenance of a library of individual scanner parameters. Clinical variability (patient-related variability) is minimized through the use of “gated” breathing during the scan. All CT technicians are trained and qualified by FLUIDDA prior to data acquisition in full 13485 ISO compliance. Data integrity is managed through maintenance of secure upload protocols. After file integrity has been validated, the images are subject to a battery of tests to ensure image quality before FRI analysis commences. These tests include, but are not limited to: verification of fully captured anatomical region, lack of motion artifacts, and comparison of lung volumes at maximal inspiration and maximal expiration to ensure dynamics are as expected (NOTE: the scan taken at maximal expiration is not used in the calculation of V_{LL}).

6.2. DOES DATA CURRENTLY EXIST TO SUPPORT THE PROPOSED CUT POINT(S), IF IMAGING RESULTS ARE NOT REPORTED AS A CONTINUOUS VARIABLE?

Yes

6.3. PROVIDE THE NAME AND VERSION OF THE SOFTWARE PACKAGE TO BE USED FOR IMAGE ACQUISITION AND ANALYSIS (LIMITED TO 500 CHARACTERS).

CT scans are segmented using Mimics, version 20, a segmentation program that has been cleared by the FDA's Center for Devices and Radiological Health (CDRH) under the 510(k) process (Food and Drug Administration, K073468) and has been CE marked in Europe (Conformité Européenne certificate, BE 05/1191.CE.01). Subsequent analysis is performed using FLUIDDA's proprietary software.

7. SUPPORTING INFORMATION

7.1. PLEASE SUMMARIZE EXISTING PRECLINICAL OR CLINICAL DATA TO SUPPORT THE BIOMARKER IN ITS COU (E.G., SUMMARIES OF LITERATURE FINDINGS, PREVIOUSLY CONDUCTED STUDIES) (LIMITED TO 2,000 CHARACTERS).

Fibrogen FG-3019:

A phase-II clinical trial (NCT01262001) to evaluate the safety, tolerability and efficacy of FG-3019 (Fibrogen, San Francisco, CA) in subjects with IPF concluded in June, 2017. In this open-label, single-arm multicenter study, 66 patients (predominantly male and white, with a mean age of 67.9 years) with an IPF diagnosis, completed 48 weeks of treatment with pulmonary function testing (PFT) and HRCT occurring at regular intervals. It was hypothesized that FG-3019 treatment could reverse parenchymal fibrosis, and as such, the inclusion criteria stipulated an extent of whole-lung parenchymal fibrosis ($\geq 10\%$ and $< 50\%$), assuming that some fraction of that fibrosis was reversible, with no more than 25% honeycombing (HC), which was assumed to be irreversible. FVC was measured and HRCT paired inspiratory/expiratory scans were taken at baseline, 24 weeks and 48 weeks.

Figure 2 shows $V_{LLL-\%p}$ vs $FVC_{\%p}$ for all patients at all three visits. The blue dotted line is a linear fit with ($R^2 = 0.36$, $p < 0.001$). The following key points are observed:

1. Based on the fit, for $FVC_{\%p} = 100\%$, $V_{LLL-\%p} = 60\%$ (green lines), indicating that up to 40% of the volume of the lower lobes can be lost to IPF while $FVC_{\%p}$ remains normal. (It has been shown that early on in the disease, the upper lobes can compensate for the diseased lower lobes, resulting in an overall unaffected $FVC_{\%p}$.)

- All but 7 of the data points fall below the unit line (orange), indicating that V_{LLL-%p} is more sensitive than FVC_{%p}.
- Thirteen points have FVC_{%p} > 100%, even though all of the patients were diagnosed with IPF.

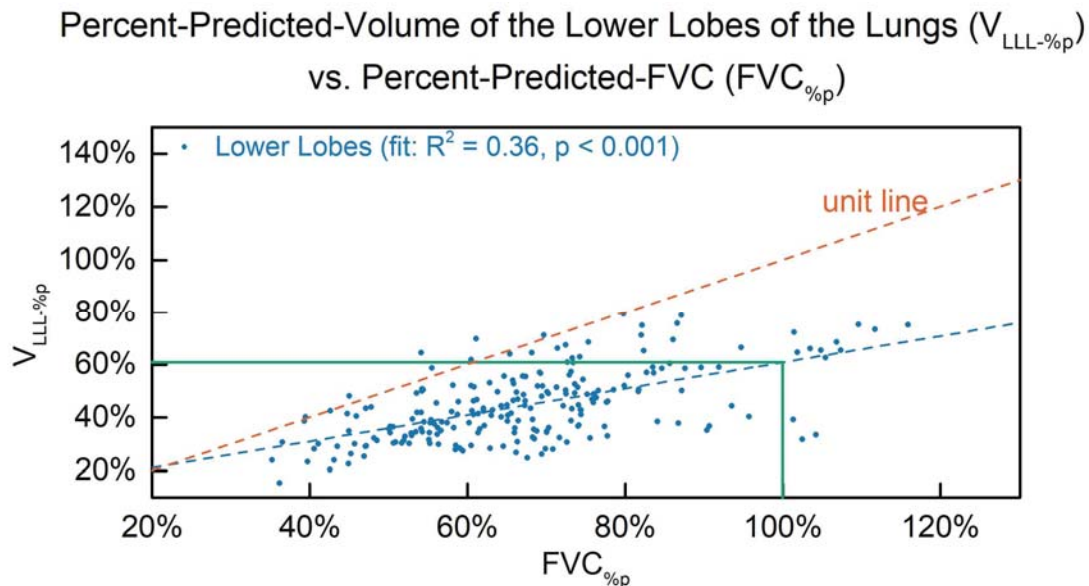


Figure 2. Percent-Predicted-Volume of the Lower Lobes of the Lungs (V_{LLL-%p}) vs. Percent-Predicted-Forced Vital Capacity (FVC_{%p}) from Fibrogen study. (---) Linear fit (R² = 0.36, p < 0.001). Up to 40% of the lower lobes can be lost to IPF with unaffected FVC_{%p}. All but 7 points fall below the unit line indicating V_{LLL-%p} increased sensitivity over FVC_{%p} to assess disease staging in IPF.

Galapagos GLPG1690:

A clinical trial (NCT02738801) to assess safety, tolerability, pharmacokinetic and pharmacodynamic properties of GLPG1690 (Galapagos NV, Belgium), an autotaxin inhibitor, in patients with IPF concluded in May, 2017 (Galapagos_results). In this multicenter randomized, double-blind, parallel group, placebo-controlled, exploratory phase II-a study, 17 patients were treated with GLPG1690 and 6 patients were treated with placebo over 12 weeks.

FVC was measured and HRCT paired inspiratory/expiratory scans were taken at baseline, at 12 weeks of both the treatment and placebo arms. V_{LLL-%p} vs. FVC_{%p} is shown in Figure 3, where ~50% of the lower lobes have been lost with an unaffected FVC_{%p}. V_{LLL-%p} is shown to be more sensitive than FVC_{%p} with all but 2 data points falling below the unit line.

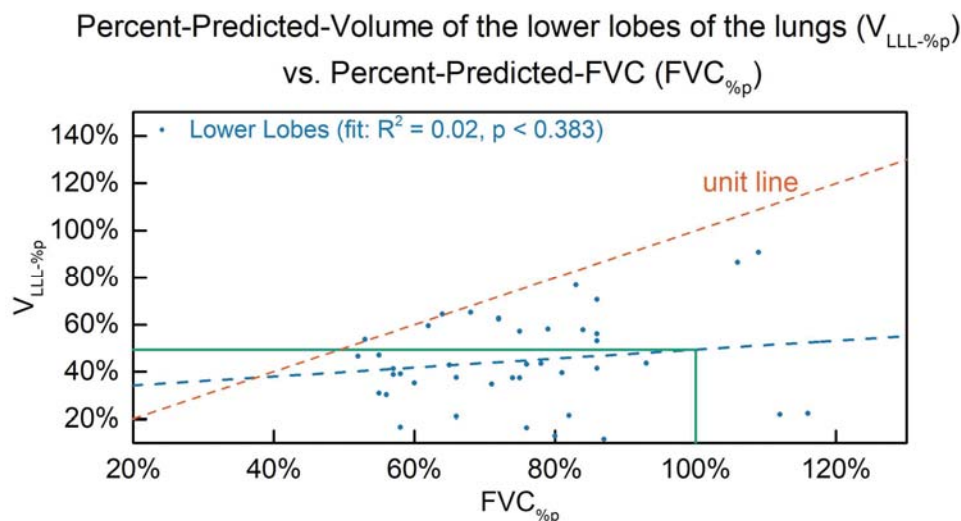


Figure 3. Percent-Predicted-Volume of the Lower Lobes of the Lungs ($V_{LLL-\%p}$) vs. Percent-Predicted-Forced Vital Capacity ($FVC_{\%p}$) from Galapagos study. (---) Linear fit ($R^2 = 0.02$, $p < 0.383$). Up to 50% of the lower lobes can be lost to IPF with unaffected $FVC_{\%p}$. All but 2 points fall below the unit line indicating $V_{LLL-\%p}$ increased sensitivity over $FVC_{\%p}$ to assess disease staging in IPF.

7.2. PLEASE SUMMARIZE ANY PLANNED STUDIES TO SUPPORT THE BIOMARKER AND COU. HOW WILL THESE STUDIES ADDRESS ANY CURRENT KNOWLEDGE GAPS? (LIMITED TO 2,000 CHARACTERS.)

Fibrogen is currently conducting another phase-II trial (NCT01890265) to evaluate the safety and efficacy of FG-3019 in a randomized, double-blind, placebo-controlled study. The results of this study will be used to further validate $V_{LLL-\%p}$ as a continuous staging biomarker of IPF as well as validate the proposed discrete cut points (50%, 75%).

FLUIDDA is also planning to collaborate with the Three Lakes Partners who have a published mission to “End Idiopathic Pulmonary Fibrosis.” The collaboration will provide FLUIDDA access to thousands of HRCT images of patients at various stages of IPF.

8. PREVIOUS REGULATORY INTERACTIONS

Letter of Support Issued for this Biomarker on date: June 6, 2016.