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Executive Director

Letter of Support for Forskolin-Induced Swelling (FIS) Assay: Biomarker for Cystic Fibrosis Transmembrane Conductance Regulator Protein (CFTR)

Cystic Fibrosis (CF) is a monogenetic, rare and life shortening disease that affects many organs. Approximately 2100 variants of the Cystic Fibrosis Transmembrane Conductance Regulator Protein (CFTR)-gene (coding for a chloride and sodium channel) are known in the CFTR1 and CFTR2 database, of which 1700 are estimated to be CF-causing.

F508del on at least one allele is by far the most frequent mutation, and the first available disease-modifying drugs targeted primarily the F508del. However, many rare CF mutations have not been characterized yet. Even when characterized, the disease can show a huge variability between patients with similar genotypes.

The HIT-CF consortium developed a new in vitro method, the Forskolin-induced swelling (FIS) assay, to measure CFTR function. The FIS test is performed in patient-derived organoids (PDO) from CF patients' intestinal stem-cells to assess the level of CFTR function. In intestinal organoids, Forskolin increases intracellular cAMP, which stimulates in turn CFTR opening by binding to intracellular domains of the transmembrane CFTR protein, inducing a rapid influx of fluids into the organoid lumen as indicated by the swelling of the whole organoid which can be observed already 10 min after forskolin stimulation, and this exclusively through CFTR activation as per available genetic and pharmacological data. Consequently, the organoid lumen increases in size. It has been shown that CFTR modulators that proved to restore CFTR function in other in vitro model systems also increase FIS in intestinal organoids in a CFTR genotype-dependent fashion. Also (epi)genetic signatures contributing to individual differences in CFTR function are recapitulated in organoids and are retained during prolonged organoid culturing.

The intended use is application of the FIS assay as a biomarker for CFTR-function in three specific contexts of use (CoU):

1) The FIS assay is a CFTR-dependent, reproducible valid biomarker for CFTR function in patients with Cystic Fibrosis (pwCF);

2) the FIS assay is a biomarker of CFTR function that reflects disease severity and risk of disease progression in pwCF;

3) the FIS assay is a biomarker of CFTR function that correlates with clinical drug response in pwCF.

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Ad 1) The Applicant has presented the data to support that FIS is dependent on CFTR. The SAWP/CHMP recommends that further work is performed to determine cut-offs and the specific test conditions to determine FIS. Technical and experimental reproducibility needs additional work to determine these cut-offs, and setting of swelling thresholds, e.g., No-low swelling 0-1000 AUC; Medium swelling 1000-2000 AUC; High swelling >2000 AUC are considered an important next step in the testing of the FIS assay. With regard to the technical reproducibility, processes for performing the test, with the need to closely follow (including documentation) the STAR-protocol, should be clarified. The observed large limits of agreement (500-1000) compared to the proposed thresholds (around 1000 and 2000) FIS should be defined as an average of multiple replicates, which may require further experimentation to determine the optimal number. One of three laboratories tested had a lower agreement than other labs. This suggests that using thresholds can be possible for classification but that interval FIS ranges with an undefined classification may be needed, and/or that calibration should be done for each laboratory separately.

Therefore, while the FIS assay is demonstrated to be CFTR-dependent, its sensitivity and specificity remain to be established for various ranges of levels of CFTR activities across various mutations and various Forskolin concentrations and ideally across different laboratories.

Ad 2) The Applicant has provided literature that investigated the relation between the FIS assay and other biomarkers used for the diagnosis of CF (sweat chloride concentration (SCC), intestinal current measurements (ICM) and nasal potential difference (NPD)), and the relation with clinical disease indicators (pancreas function, lung function decline, cystic fibrosis related liver disease (CFRLD) and cystic fibrosis related diabetes (CFRD)).

The correlation of FIS with established pharmacodynamic parameters supports that FIS captures a biological process, i.e., is an ex-vivo biomarker. Residual CFTR function measured by FIS may be a fair biomarker of long-term clinical phenotype in the various organs based on the presented data.

For an assessment of the ability of FIS to capture disease progression, correlations of changes in FIS and parallel changes of clinical disease indicators over a given period of time would need to be established.

With regard to FIS as a potential prognostic factor for clinical disease severity, demonstration of an association with the current disease status (e.g., FEV1, BMI) is important. While some data were presented that could differentiate patients with predominantly pulmonary or pancreatic involvement, and while within specific mutation groups FIS values correlated with some key clinical features (correlation with FEV1, PRAGMA-CT), this was not the case for other important outcomes (e.g., no correlation was found with chest radiography or exacerbations). Overall, for FIS to be considered a valid biomarker of disease severity, correlation with a validated staging system remains to be established. It should be assessed whether this correlation would be valid for all patients or would be specific of a genotype or of a disease severity/stage.

Overall, conclusions on individual level predictions cannot be made based on the presented data and replication of the findings for severity and risk of progression is considered warranted before the findings can be regarded as conclusive and can be used in clinical practice as a qualification measure for disease severity and disease progression.

Moreover, the Applicant claims that FIS measurements on patient-derived organoids is a precise tool to quantify a given patient's CFTR function, and that as such it may have a prognostic value that is more capable to capture all the epigenetic factors that determine severity than traditional classification. To accept such a statement, further investigation(s) will be needed. For instance, it would need to be

shown that patients with similar FIS would have similar severity even if defined by different traditional classifications. However, it is acknowledged that FIS can be relevant for individuals with (ultra)rare uncharacterized CFTR genotypes or with genotypes associated with varying clinical consequences. The new FIS assay can be – once properly validated - a valuable addition to other parameters for the determination of disease severity and, in the end, of the risk of disease progression at a group level. On the individual level, the FIS-based prediction of the risk for progression and the classification of disease severity is anticipated to be limited considering the size of the prediction error as per the current results.

Ad 3) Currently, there is no clear consensus on thresholds that define the individual responders or non-responders. The Applicant proposes two ways to identify thresholds for responders based on organoid FIS. The first method is the classical route by which sensitivity, specificity and positive and negative predictive values of the test are derived from individual data measurements. The second proposed application of the organoid FIS assay, and associated definitions of the thresholds, is to use the test to predict the response of patients with rare mutations (too rare to be assessed through a conventional clinical trial) to CFTR modulators that are already approved for more common mutation(s) based on conventional clinical trials (as a predictive biomarker for drug response). Both approaches need further validation work, which is referred to in the qualification advice (EMA/SA/0000069719). The Applicant presented data of FIS for various genotypes in untreated patients, and ivacaftor-, lumacaftor- or combination (ivacaftor- and lumacaftor-) treated patients. Data collected with other CFTR modulators is becoming available, and the (ongoing/planned) CHOICES trial will provide further data to determine individual level predictions that will constitute the necessary replication of data that may support validation of FIS as a predictive biomarker.

Additionally, the FIS assay may have a third potential application by being used as a surrogate efficacy endpoint in young pre- or pauci-symptomatic patients, in whom respiratory function can hardly be assessed and is not yet sufficiently impaired to show an effect during a reasonable time frame in a clinical trial.

In conclusion, the FIS assay seems a reproducible in vitro biomarker with a strong biological rationale. Based on the currently available results, the FIS results correlate, however, only moderately, with disease severity and the clinical course. Considering the wide prediction errors and as regards the correlation with clinical response, the limited number of patients and modulators investigated, a direct application for prognosis and prediction is considered premature. Further investigations using more diverse patients and modulators are advised, for instance alongside current practice of treating patients and drug development.

Yet, provided that it is fully validated for those purposes (see above), it is considered that the FIS assay could be valuably used in the proposed Context of Use as unmet needs would be answered:

- to select responders in vitro, because the FIS assay predicts drug response, and
    - propose treatments to patients with rare mutations not assessed in clinical trials with CFTR modulators, as proposed by the Applicant (high throughput screening of personalised treatment)
    - identify low/non-responders among those patients with more frequent mutations who are part of the indications of marketed disease-modifying drugs
    - non-clinical drug screening
    - baseline stratification of in vitro responders

Overall, the CHMP supports the development of the FIS assay towards a full validation.

The letter of support is issued on the basis of this qualification advice.

Yours sincerely,

Emer Cooke

Executive Director