The development and application of outcome measures and biomarkers for treatment of people with a mucociliary clearance disorder

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Chapter 7

General discussion

In this thesis, we explored the use and the development of outcome measures and biomarkers in the setting of the mucociliary clearance diseases cystic fibrosis (CF) and primary ciliary dyskinesia (PCD), with the aim to monitor disease progression and treatment effects. This chapter will discuss the main findings of our research and practical considerations to be taken. Furthermore, the conclusions will be summarized and future perspectives formulated.

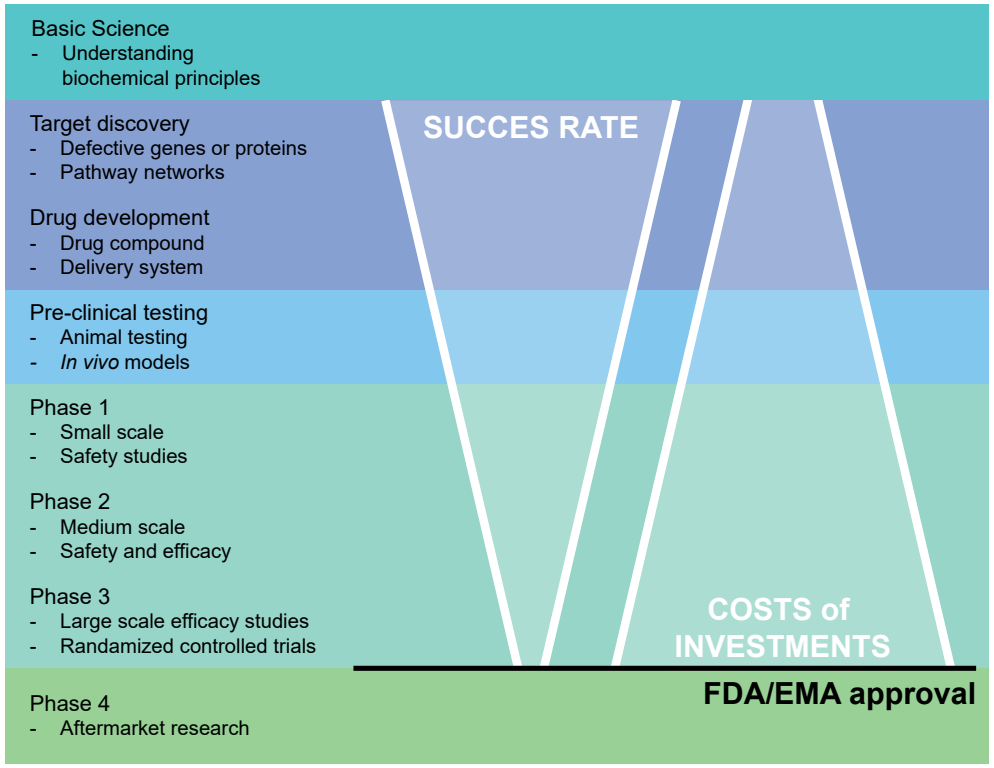

Figure 1 Drug pipeline. The further down the drug pipeline, the lower the success rate and the higher the investment costs.

1 Main findings

1.1 Clinical outcome measures and phenotypes

The classical drug pipeline has been the same for decades, and includes multiple phases of research as shown in Figure 1.[1] Basic science to understand the biochemical processes in the human body is the foundation off the drug pipeline. The first steps toward a treatment is target discovery and treatment development, followed by pre-clinical trials, before one can initiate the first in human clinical trials. Over time, it has become increasingly more expensive to move from a compound of interest towards an approved drug. Therefore, pharmaceutical companies have mainly been interested in clinical trials in which the drug, after market release, can be administered to a large population. Alternatively, in case of smaller populations such as CF, pharmaceutical companies need to drastically increase their prize to cover the cost of the trials.

In **chapter 1,** several pros and cons, of clinical trials as compared to real world studies, have been introduced. Because of the costliness of clinical trials, only certain phenotypes are included, in which it would be easiest to measure any effect of the drug. Usually, this excludes those patients that are doing too well, as there is limited room for improvement, and those patients that are too severely ill, as this is considered a safety hazard and can result in higher report of adverse events.[2] Any phenotype that is excluded from clinical trials, relies on after market research in order to assess safety and efficacy.[3]

For PCD, several genotype-phenotype relationships have been described, though given the large number of genes involved in PCD (>50) and the relatively rare nature of this disease, much is still unknown. However, we do know, for example, that mutations in *CCDC39* are associated with relatively poor lung function, and mutations in *DNAH11* are associated with relatively good lung function.[4] In CF, over 1500 mutations have been described in only one gene coding for the CFTR protein. Nonetheless, the type of mutation is clearly associated with outcome: patients with a minimal function or stop codon mutation show a worse clinical presentation and shorter life expectancy as compared to patients with residual function mutations[5]

Interestingly, in this thesis, we also found that in a genetically homogeneous PCD-population, with the exact same mutation in the *CCDC114* gene, that phenotypes could differ considerably (**chapter 2**). Overall, the phenotype linked to this mutation, is considered moderate to severe.

We found that lung function within this population varied from an FEV_1 (forced expiratory volume in 1 second) of 50 to 130 percent of predicted. Thus, several of these patients with the same genotype would have a lung function similar to that of a healthy patient, while others are limited in their daily activities.

In **chapter 4,** we discuss a very severe group of people with CF. While these patients have the same genotypes as those included in the clinical trials, they have a much more severe lung disease, with a FEV_1 of less than 40% predicted. Age, a common denominator in disease severity,[6] did not play a large role in this instance, as the median age of our population was younger than those included in the clinical trials. Thus, the phenotype does not fully depend on genotype or nature, but also depends on nurture and exposure. There are several risk factors associated with worsening lung disease, such as low weight-for-age, and type of bacterial infection (e.g. *Pseudomonas aeruginosa*).[7–9]

Overall, **chapter 4** describes a phenotype that was excluded from clinical trials that tested the CFTR modulator elexacaftor/tezacaftor/ivacaftor. Those with this CF phenotype depend on phase 4 or aftermarket study designs, to assess safety and efficacy. Our study shows that these patients have a similar effect while maintaining a similar safety profile as compared to the patients included in the clinical trials. In order to compare our results to those of the clinical trials, we chose to use the same primary outcome measure (FEV_1) in our study. In general, to compare different studies, including different phenotypes, across different phases, there needs to be a standard method of reporting.

As this is already more established for CF, study protocols are often based on predecessors, we focussed on standardizing the method of reporting in clinical trials (phase 2 and 3) for PCD in **chapter 3**. In this chapter, we build a core outcome set (COS) by consensus of a global expert panel. This panel agreed on standard reporting methods of lung function, exacerbations, microbiology and patient reported outcomes. Patient reported outcomes have been of growing interest in those studies where effect is not easily measured across patient groups. However, patient reported outcomes alone are not considered enough evidence for market approval. During an e-Delphi process with this global expert panel, several interesting outcome measures have been mentioned that have not yet been integrated in the core outcome set. This is because they are not properly validated at this time, after validation they can be reconsidered for the COS.

The validation of outcome measures, and surrogate endpoints in the form of biomarkers, is critical. As during any clinical study, focus should not be on statistically significant differences

but rather on clinically significant differences for the patients. Additionally, for personalized medicine treatments like gene or transcript therapies, only small numbers of patients may be available for recruitment; therefore, compound outcomes and novel trial designs with fewer patients may be required.

1.2 Exhaled breath analysis for detection of respiratory pathogens

Using our basic senses for diagnosis of disease, has been done for many centuries and is at the foundation of modern medicine. Though, some senses might be more obvious to use for diagnosing, as using sight to diagnose a dislocated joined, touching someone's forehead to detect fever, and listening to breathing sounds via stethoscopes, then others. Back in 1606, professor Alonso y de Los Ruyzes de Fontecha J. Diez reported on a child that tasted salty, although the diagnosis was bewitchment at the time, in hindsight it was most likely CF.[10] Next to that, Greek physicians already realized since Hippocrates' time, that the smell of human breath can reflect on the person's health. Diabetes was linked to a fruity smell, advanced liver disease causes a musty and fishy smell, kidney failure cause a urine-like smell, and lung abscesses left a putrid smell.[11] Moreover, one does not have to be a physician to smell foul breath, which is often caused by bacteria on the tongue and in the throat.[12] These notions have been at the basis of the development of exhaled breath analysis.

During the last two decades, there has been extensive research on volatile organic compounds (VOCs) in relation to respiratory pathogens. Due to the efforts of many researchers globally, extensive lists have been comprised of VOCs associated with pathogens, distinguishing between different settings, media, and tissue types.[13–15] This has resulted in hundreds of compounds associated with respiratory infections. Researchers have been able to comprise this list thanks to the two dimensional separation of mass spectrometers, that obtained increasingly higher resolution over the years, resulting in a detailed list of compounds present in a sample.[16] However, the large amount of data collected from breath analysis is by nature prone to false positives, classic statistics using allows for a 5% error rate, indicating that 5 out of every 100 associated compounds are false positives. More developed statistical methods can take this into account and allow for multiple testing.[17] Nonetheless, validation of these compounds is key in proving association with respiratory pathogens.

In another field that works with large datasets, the field of genetics, a movement towards targeted analysis has been ongoing over the past few years. This utilizes the results of large studies performing whole genome sequencing for determining genes/mutations of interest,

after which a study is designed that is focused on validating those findings.[18] In this thesis, **chapter 5 & 6**, we have taken this same approach and applied it to the field of exhaled breath analysis. Unfortunately, there is no such thing as whole metabolome sequencing yet; however, we have shown that by performing a systematic literature review, a list of VOCs of interest can be identified. In **chapter 5,** we validated one of these VOCs of interest for detecting *Pseudomonas aeruginosa* in paediatric patients with CF, and a combination of these VOCs had a strong enough association to distinguish between adult patients with and without *P. aeruginosa*. In **chapter 6** we broadened our scope to other common CF pathogens and found several combinations of VOCs for detecting these pathogens in children with CF. In the same chapter, we also discussed the importance of analysing these results in the context of other pathogens being present as many CF patients suffer from co-infections.

The results of these last two chapters, though focussed on people with CF, are also promising for patients with other mucociliary clearance diseases, such as PCD, that often suffer from respiratory infections. Though, it is unknown how the metabolites of these bacteria change when present among patients with a different disease, these studies provide a new blueprint on how to approach exhaled breath analysis.

2 Practical considerations

2.1 Clinical trials for rare diseases

In this thesis, as introduced in **chapter 1**, we discuss two obstacles that researchers face when designing and executing clinical trials for rare diseases: comparability (**chapter 2**) and variability in phenotypes (**chapter 3 & 4**). These are not the only challenges these trials face; another major limitation, that the studies in this thesis suffer from as well, is the limited availability of patients resulting in a small sample size. This becomes increasingly challenging when considering that different trials are often necessary for different genotypes and phenotypes.

Therefore, for the future design and execution of these types of trials, collaboration is key. Over the years, several large initiatives have been setup to increase collaboration on national and international level. Both for PCD and CF, large international registries have been set up for the collection of clinical data on these patients. For CF, this includes an American, European, and several national registries, including a Dutch CF registry which includes 95% of Dutch patients.[19–21] For PCD, which is less prevalent than CF, there is one large

collaborative cohort, and a worldwide registry, along with several national registries.[22–24] These datasets, in both fields, have resulted in a multitude of research papers. However, unfortunately, not all registries use the same definitions and reporting standards, which can make it difficult to compare or unite datasets.

Moreover, to increase collaboration across academic hospitals and universities, in partnership with pharmaceutical companies, several clinical trials networks (CTNs) or clinical research collaborations (CRCs) have been set up. For CF, the European CTN judges several clinical trials each year and has centres across Europe that collaborate in the execution of these trials.[25] The Dutch CF CTN also coordinates the patient recruitment to available slots on a national level.[26] Such collaborations make it easier to meet the sample size requirements. Similarly, there is also the PCD-CTN, this network has evaluated several study-protocols and has facilitated the collaboration and execution of multiple trials in PCD.[27] This network was established within the framework of ERN-LUNG. a European initiative that aids research and clinical care on rare respiratory diseases.[28] Moreover, another clinical research collaboration has been established for PCD, the BEAT-PCD CRC, **chapter 2** is a direct result from this.[29]

Nevertheless, despite these collaborative efforts for standardizing research and getting easier access to patients, some genotypes/phenotypes are still too rare, to get a large enough sample sizes for clinical trials. For example, class III, IV and V mutations are only present in 3% of the CF population each.[30] Therefore, in most trials these types are grouped based on certain traits. For CF these are often grouped among remaining CFTR function, such as minimal or residual function mutations. However, this still poses several issues amongst trial design and drug registration. For CF specifically, several minimal function mutations have been registered for use of elexacaftor/tezacaftor/ivacaftor. Nonetheless, not all of the minimal function mutations have been registered within the label, as they were not included in the trials. These mutations need after-market entry authorization.[31]

In the field of respiratory medicine, we can learn from other research areas, such as oncology. A drug rediscovery protocol has been set up by researchers to prescribe drugs off-label, which are registered for other types of tumours, to patients for whom they can assess *ex vivo* if markers are present that would predict a response to the therapy. Similarly, for CF, individual patient derived intestinal organoids are used to predict response to therapy by the forskolin-induced swelling assay.[32] The HIT CF Europe project aims on collecting organoids from rare mutations across Europe to assess therapy effectiveness.[33] If patients show a response they can be invited into clinical trials, after which labels can be extended to include their

genotype. The organoid model is also under investigation by the EMA if it can be used by itself to extend the drug label. However, this is still ongoing. Next to intestinal organoids, research is performed in human airway organoids, from both CF and PCD patients.[34,35] These organoids display ciliary beating and therefore show potential for the development of a drug screening assay. However, it is important to mention that organoids cannot mimic systematic symptoms and, therefore, only show effect of interventions that alter the disease causing defects or genes. Finally, validation of a correlation between *ex vivo* outcomes and clinical impact is key.

2.2 Exhaled breath analysis for monitoring in clinical practise

Respiratory infections have a major impact on patients with a mucociliary clearance disease, potentially leading to hospitalization, a decreased quality of life, and increased mortality. As discussed in **Chapter 1**, it is important to start eradication therapy as early as possible, but current pathogen detection methods are limited. In **Chapter 4 & 5** we discuss exhaled breath analysis as a potential source of biomarkers for the detection of several pathogens commonly seen in CF patients.

Several challenges are met when the aim is to implement exhaled breath as monitoring tool. The first of which is the transition from research devise to a point-of-care application tool.[36] In this thesis, a gas chromatography mass spectrometer is used. This equipment provides a lot of information and data, but is due to its size, price, and complexity, difficult to use in clinical practise. In general, the more information a machine produces (such as most mass spectrometer based equipment), the more difficult it is to interpret the results and therefore to implement it at point of care. n overview of detection methods is depicted in Figure 1.[37] Exhaled breath analysis based on a single sensor is the easiest to implement, an example of this is the breathalyser used for the detection of alcohol in breath by the police. A breathalyser consist of a single sensor that measures the concentration of a single volatile organic compound (VOC) in exhaled breath, in this case ethanol. A device using a sensor array, such as an eNose, shows more potential for the use in clinical practice, but often suffers from a black box in which there is no knowledge on exactly which VOCs are creating the distinctive pattern.[38] Nonetheless, sensors can be selected based on VOCs discovered by spectrometry-based techniques, combining the best of both worlds.

Another challenge that exhaled breath analysis faces is the contribution of internal and external factors to the exhaled breath composition. As discussed in **Chapter 1**, VOCs can

originate from several sources, such as systemic processes and the ambient air. However, we do not fully understand to which extent all these factors affect our breath measurements. While factors like food intake and medication use have been studied for several years, other more unexpected factors also show an effect on exhaled breath. Recent research has shown that people have a different exhaled breath pattern when they experience stress, the hypothesis behind this is that a faster breathing pattern, an increased pulse rate, and elevated blood pressure are accountable for these changes.[39] The clinical implication of this is large, when considering that most patients are met in a hospital, which is a stressful environment in general. In addition, when examining children, you must consider the stress you inflict when collecting the exhaled breath and try to minimize this as much as possible. Another factor impacting the clinical implications is the effect of the circadian rhythm on the exhaled breath profile.[40] As the circadian rhythm affects metabolomic processes in the body, it also effects the metabolites present in the exhaled breath. Thus, when designing studies, visit times should be either randomized or standardized to reduce bias. Not only within the same day, but also across several days, individuals show fluctuations in their exhaled breath patterns. However, several studies have shown that if you expose these patients to a viral challenge, the change in exhaled breath pattern is larger than seen in the day-to-day variations, and the shift in exhaled breath pattern occurs before the actual symptoms arise.[41,42] Thus, using patients as their own standards can allow for recognizing if they are deviating from their baseline health based on exhaled breath analysis.

3 Concluding remarks and future perspectives

This thesis shows that it is possible to standardize the reporting of outcomes in clinical trials for specific populations. Nonetheless, this thesis also shows that there are large varieties in genotype and phenotype, which can affect study results, making it very difficult to conduct a one trial fits all method. We discuss how biomarkers can play a potential role in deciding which patients can enter in a clinical trial. Moreover, this thesis discusses the development of exhaled breath analysis as source of potential biomarkers, by using a hypothesis driven approach.

Ideally, we would do one large phase III trial, in which all genotypes and phenotypes were included, to create an inclusive label for drug registration. However, if we include all patients, disregarding any potential factors that would predict either effect or risk, the trial might not be able to statistically prove any effect as the population is too heterogeneous. A poorly

designed trial potentially results in the drug not reaching the patients who would benefit. Therefore, it is important that genotypes and phenotypes are selected of which a response to therapy can be hypothesised, based on method of action or other *ex vivo* studies, that these would respond to treatment. This, however, can lead to a multitude of smaller trials, which makes it extra important that the trials themselves are designed as such that they are comparable and reproducible. Core outcome sets can be an important tool for designing such trials, but are still limitedly available; many trials are currently based on predeceasing trials rather than a defined set of outcomes by experts. These should take into account the aim of the treatment, which can be either disease altering, or focussing on symptom reduction in for example the lungs or intestines. For the construction of COSs and for the execution of these trials, it is incredibly important that different institutes collaborate, especially in rare diseases such as CF and PCD.

While initial trials are important to measure treatment effect, it is equally important to monitor the disease over time. In mucociliary clearance diseases, it is especially important to monitor whether any respiratory pathogens are acquired. Based on the results of this thesis, exhaled breath analysis can play a role in this, especially in children. However, although the technique is already very advanced, there are two major hurdles to overcome. First, is that the field should move from discovery research to a validation research, both independent clinical validation, but also validation by *in vitro* methods to establish the biochemical association between the pathogen infection and the exhaled metabolites. By using the targeted approach in this thesis, we made a step towards this goal. However, this approach should not be limited to the mucociliary clearance disease field only, or even the respiratory field only. Secondly, we should think about the use of exhaled breath in a real-life setting, which does not only include a tool that is easy to use in the clinic, but also includes taking into account all the factors from real-life that potentially affect our breath pattern without our knowledge. In order to reach this goal, it is important to shift the focus from case control studies to longitudinal studies, which use individuals as their own control. Events that largely impact our health, such as a respiratory infection, will most likely cause a metabolomic shift larger than the daily fluctuations within an individual. Therefore, it might be necessary to measure more frequently than only when a patient is seen in the hospital. Home monitoring and eHealth can play a large part in this, but should be initiated on a personalized level, to those that are comfortable with this. eHealth in general, with the measurement of lung function at home and video calls with physicians, can allow for less hospital visits.[43] This is especially important for patients with a mucociliary clearance disease, who are at a higher risk to contract a pathogen in the hospital. For eHealth and home monitoring, a tool that is easy to access and

simple to use, should be developed to measure patients frequently and recognize large shifts in patterns that could indicate a decrease in health.

In conclusion, to monitor effectiveness of therapy, within patients with a mucociliary clearance disease, a hypothesis driven approach is key. This is important in the selection of patients for inclusion in trials based on genotype and phenotype, and the selection of outcome measures that would reflect clinically significant changes. Moreover, a hypothesis driven approach is also key in clinical care and monitoring disease progression, especially for the development of biomarkers that can act as surrogate measures. To ensure the development of treatment and clinical care in general, collaboration is of great importance, especially in rare diseases such as CF and PCD.

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