Report on the Activities of the ECFS Cystic Fibrosis Molecular & Cell Biology and Physiology Basic Science Working Group (ECFS Basic Science WG)

Mo30 Report

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1. Goals

The ECFS Basic Science WG (BSWG) was created in October 2014 with the following goals:

1) Widening the number of European scientists doing fundamental research on those areas of CF as ECFS members, in particular to attract, train and maintain younger investigators in the CF field;
2) Disseminating recommendations for best reagents (e.g. cell lines, compounds, antibodies, etc) on ECFS website and promoting best practice procedures;
3) Developing a network (jointly with ECFS-CTN and Registry) for the creation of biobanks of CF patients' materials across Europe for the generation (e.g., primary cultures of epithelial cells, intestinal organoids, etc) and distribution of resources for CF research;
4) Producing consensus guidelines for standardization of research-derived laboratory techniques that can be applied to the clinic (e.g., novel biomarkers to be used in CF diagnosis or as "surrogate endpoints" for clinical trials, etc.)
5) Prioritizing topics related to emergent needs in the field so as to create "task forces" (e.g., on CFTR structure, animal models, high-throughput screens, etc.);
6) Promoting excellence in CF research by fostering European-scale research to avoid effort duplication at national level and fragmentation and to achieve competitiveness for EU consortia
7) Liaising with basic scientists in other societies and patients association to maximize and optimize efforts)

2. Activities

2.1. Third Meeting of the BSWG

The BSWG organized its 3rd meeting which took place on 30 March as a Satellite meeting during the ECFS Basic Science Conference in Albufeira (Algarve), Portugal, 29 March – 1 April 2017. The purpose of this 3rd meeting was to discuss the contribution of the BSWG to the ECFS "*Task Force on Personalised Medicine for CF*". The following topics were proposed to be discussed by the participants:

How can Basic science help Personalised Medicine?

1. Validation/ optimization of novel biomarkers
2. Assays for improved endpoints to evaluate novel drugs: cilia beating, ASL height, patch-clamp for nasal cells
    a. Find the hub labs which can do this in different countries as a service
3. Drug discovery in academia: alternative channels

The 3rd meeting of the ECFS BSWG, counted with **140 participants** (see list in Annex 1). These split into 3 discussion subgroups (for topics 1-3) which nominated a 'rapporteur' per group, then discussed for 90 min and finally presented the conclusions (10 min each) focussed on each topic as follows.

1. **Validation and optimization of novel biomarkers Assays for improved endpoints for preclinical evaluation of novel drugs** (Discussion leader Rapporteur – Kris de Boeck, BE)

At the 2016 meeting, it was concluded that organoids and nasal cells are the most promising biomarker for *ex vivo* assessment of CFTR function for use towards personalized medicine. When asked whether in 2017 other biomarkers should be added to this list, the group decided negative, although only one member felt strongly that intestinal current measurements (ICM) should be added on to the organoid measurement. However, since reproducibility of ICM, technique to use and standard operating procedure (SOP) have not yet been fully harmonized, it was decided not to add it. Hence, we focused the discussion on validation and optimization of organoids and nasal cells as biomarkers of CFTR function.

To discuss biomarker validation, we use the methodology of previous work in the ECFS-CTN standardization group [1,2].

- **Reliability**: accuracy of the measurement: intra-test variability and between lab reproducibility; fluctuation over time (inherent variability without an intervention)
- **Validity**
    - **Concurrent Validity**- correlation with the gold standard (if that exists)
    - **Convergent Validity**- correlation with other tests that measure the same parameter
    - **Discriminative Validity**- does the biomarker differentiate between groups who differ in function
    - **Predictive Validity**- can this biomarker predict outcome or prognosis
- **Responsiveness**: how does the biomarker change when CFTR function improves

INTESTINAL ORGANOIDS

Reliability/reproducibility to do's/ to list and publish

In the context of personalized medicine, intra-patient repeatability is much more important than interpatient differences in measurements

- Intra-test variability is known and small (standard error of current readouts is small)

- Resample biopsies from the same patient and repeat measurements (Beekman has (limited?) data on this;
- Frozen aliquots of same sample show consistency over new thaws- good responders stay good responders, etc. (Beekman data);
- Fully standardize technique among labs: reliability across labs to be proven;
- Set quality criteria for organoids at start of test;
- Include internal controls (reference organoids) in each test (positive and negative);
- Use the same drug concentrations across patients (drawback to consider: potency of potentiators differs for different *CFTR* mutations e.g. S1251N more responsive than G551D);
- Use identical forskolin concentration (at present opt to use several concentrations across the entire range, until known which is optimal);
- Agree on best assay read-out: % swelling over baseline at 60 min at one forskolin (Fsk) concentration, AUC of swelling at different Fsk concentrations; initial rate of swelling
    - How to find a solution to relate back to % WT function? there is a maximum swelling potential for a given organoid and this is influenced by the organoid volume at baseline
- A strategy can be: first identify residual function for a given patient; then choose Fsk concentration to be used.
- Can use of indomethacin at baseline offer a benefit to 'normalize' baseline with no endogenous cAMP and thus have no difference in pre-swelling condition?

Validity

- No data for concurrent validity since there is no gold standard for CFTR function
- Convergent validity: correlation of read-out at baseline with sweat chloride concentration, NPD read-out, ICM read-out; is this important in the context of personalized medicine?
- Discriminate validity; how does baseline read-out differ between patients with known differences in baseline CFTR function: PS versus PI, patients with residual CFTR function; is this important in the context of personalized medicine?

Predictive validity and responsiveness (these topics were discussed together)

- More data are needed so as to establish correlations on organoid swelling response to CFTR modulators *ex vivo* and *in vivo* drug benefit
    - correlation with improvement in sweat chloride concentration (preference for this correlation because sweat chloride can be measured in all age groups; caveat when drug has no bioavailability in sweat gland)
    - correlation with improvement in FEV_1 *in vivo* (main target organ but cannot be assessed below age 6 years or when FEV1 is normal at baseline; it may be possible to use LCI in young children or in mild disease, but this is not available everywhere;

    - to build on database of Beekman;
    - how to solve/improve the problem of variability in FEV_1, sweat chloride (mean several measurements?); and especially NPD values - improve readout [3]; and increase sample area [4].
- Is there a linear relation between swelling response and *in vivo* benefit or is there a threshold of response that should be used?
- If a threshold is used, it is advised not to set the bar for the threshold too high (e.g. reaching mean Orkambi threshold is sufficient)
- Positive examples of predictiveness in context of personalized medicine:
    - G1249R patient organoid has strong response and patient is getting good improvement in sweat chloride, in lung function, in NPD
    - Negative examples of predictiveness in context of personalized medicine: G970R patient organoids show no response, and also no response in sweat chloride, lung function (hence superior to FRT cells)

Main advantages of organoids:

- Can be biobanked and used indefinitely
- Good correlation with in vivo benefit so far in the context of personalized medicine, plus overall in vivo benefit in clinical trials

Main drawbacks of organoids:

- Difficult to relate read out to %WT function
- Uncertainty of linearity of the assay
- It is unknown what is the best dynamic range for the assay
- 3D organoids are analysed by 2D readout only

NASAL CELLS

Reliability

Need to generate more data for this technique:

- -Standardize how nasal brushings are done (TDN protocol is available):
    - Use xylocaine to limit pain for the subject;
    - Obtain at least 250,000 cells for successful culturing;
- Standardize reprogramming of human nasal epithelial (HNE) cells:
    - Quality control for nasal cell cultures: check tight junctions, tubulin, % differentiated cells; transepithelial resistance (TEER) of at least 600 Ω;
    - Standardize how to grow HNE cells on filters;
    - Fully define the test read out: I_{sc} measurement and response to Fsk and other compounds such as VX-770; followed by change in response in presence of Inh_{172} or a mixture of inhibitors.
- Reliability of nasal cell biomarker to do's/report:
    - Sample the same patient at different time points;

- Measure same patient response at different cell passages.

Main advantages of nasal cells:

- Respiratory tissue;
- Bronchial and nasal epithelium seems to behave similarly, at least in terms of nasal PD [5], but no comparative studies between HNEs and HBEs and they may have a different range of response;
- Nasal cell I_{sc} response is more or less linear (vs organoids);
- Can be frozen and stored to some extent.

Main disadvantages of nasal cells:

- No examples in context of personalized medicine yet;
- Cannot be stored and reused indefinitely.

OPEN QUESTIONS:

- Patient acceptance of nasal brushings versus rectal biopsy;
- Concordance between nasal cell responses and organoid responses;
- Determine the dynamic range of CFTR activity in HNEs vs organoids.

2. **Alternative channels and other complementary approaches to CFTR mutation-specific modulators** (Rapporteur – Marcus Mall)

Despite major breakthroughs in CFTR modulator therapies, their efficacy remains limited and there is still a substantial portion of patients with CF genotypes that cannot be treated with CFTR modulators yet (and possibly will never be). Therefore, alternative strategies aiming to compensate or correct CFTR dysfunction remain important.

In this context, the discussion first focussed on the epithelial Na^+ channel (ENaC) and the alternative Cl^- channels TMEM16A/Anoctamin 1 (ANO1) and SLC26A9 as alternative therapeutic targets to counteract airway surface dehydration and acidification associated with CFTR malfunction in the airways. The group felt that inhibition of ENaC, especially with emerging long-acting inhibitors is a rationale and promising approach to improve airway surface hydration and mucus clearance that is currently tested in early phase clinical trials.

Further, the Ca^{2+}-activated Cl^- channel TMEM16A/ ANO1 and the constitutively active Cl^- channel SLC26A9 were discussed as promising candidates to bypass impaired anion transport in the airways and potentially other organs affected by CF such as the GI-tract and the pancreas, especially since the identification of these channels at the molecular level has opened new possibilities for the development of specific activator compounds. However, it was also felt that a more in depth understanding of the role of these alternative Cl^- channels in health and disease, and the development of reagents such as cell models, as well as

sensitive and specific antibodies will be crucial for further exploration of these alternative Cl^- channels as therapeutic targets in CF.

Second, the discussion focussed on gene editing and gene and cell replacement strategies including emerging possibilities of patient- and mutation-specific *ex vivo* editing of mutant CFTR in inducible pluripotent stem cells (iPS) using the CRISPR-Cas9 system, *ex vivo* differentiation of corrected iPS cells into airway progenitor cells and transplantation into the patient lung. However, despite tremendous progress in this area, there are still several hurdles that have to be taken including issues around safe transplantation and efficient engraftment of CFTR corrected iPS-derived cells in the lung. RNA-based strategies were identified as another promising area opening opportunities for mutation-agnostic correction of CFTR expression and function, as well as siRNA-mediated inhibition of therapeutic targets such as ENaC. Overall, these gene and cell replacement approached were considered highly promising, but also more long term compared to pharmacotherapy with respect to translation into safe and efficacious therapies for patients with CF.

3. **Assays for improved endpoints to evaluate novel drugs** (Rapporteur – Jeff Beekman)

This sub-group discussed various endpoints related to the use of CFTR modulators, and their efficacy in the context of individuals or groups. Most prominently, we considered the value of biomarkers of CFTR function, being both *in vitro* or *in vivo* for such purposes, and their relation to FEV1. It was also recognized that additional 'disease' biomarkers remain needed, especially those focusing on inflammation and long tissue damage.

The classical surrogate endpoint FEV1 has important strong points that still need to be established for most CFTR-biomarkers: commonly used for pulmonary diseases and many treatments in group-based clinical trials, established surrogate-endpoint with relation to pulmonary exacerbation and death. The most critical downside of this biomarker is the huge variability, which makes this biomarker unsuited for individual assessment of CFTR modulators.

Biomarkers of CFTR function on the other hand may hold the key to individually assess CFTR modulators, despite variability in these biomarkers is also considerable. It was recognized that the classical *in vivo* CFTR biomarker namely sweat chloride concentration (SCC) is effective at the group level to report on CFTR modulator efficacy, but that individual correlations between this biomarker and FEV1 remains limited or even absent. Potential causes for this lack of correlation are likely technical variability in the measurement and the impact on non-CFTR dependent variables on both SCC and FEV1. Individual readouts of cultured cells *in vitro* are likely to complement these *in vivo* readouts, by enabling a prospective analysis of CFTR modulators. In addition, these readouts can enable a more precise measurement of CFTR function due to the ability to measure strictly CFTR function-dependent readouts such as intestinal organoid swelling or electrophysiological readouts in airway cells under controlled assay conditions. Electrophysiological readouts in airway cells are the most direct readouts of CFTR function by measurement of anion transport across the

epithelium, whereas fluid secretion readouts are indirect measurement of CFTR function that rely on the coupling of ion transport to fluid secretion.

For most of the CFTR biomarkers, relations with clinical disease and CFTR modulator responses *in vivo* are lacking, and thus need further attention. Data from intestinal organoids appear to support that the impact of modulators can be predicted for individuals using *in vitro* cultured cells. Organoid swelling correlates with FEV1 improvement and in vivo SCC, and has been used in prospective settings to identify responders to CFTR modulators (*see above*). This assay may present a path forward to enable access to CFTR modulators beyond their current label, but require more follow up (see below) and input from regulators. Additional questions to focus on include:

1. What are the relations between short-term and long-term clinical improvements of CFTR modulators, and how do these link to sweat chloride concentration?
2. What are the relations between *in vitro* readouts of CFTR function and short- and long-term clinical improvements of CFTR modulators?
3. How do different *in vitro* readouts (e.g. in nasal cells or intestinal cells) compare?

2.2. BSWG Workshop

The BSWG organized a "*Hands-on Workshop on Epithelial Systems: Physiology and Pathophysiology*", which took place at the Faculty of Sciences of the University of Lisboa (FCUL), Portugal, between 18 – 22 July 2016 (see Programme in Annex 2).

This workshop aimed to elucidate researchers from the CF community on the theoretical aspects of basic CF science, as well as provide practical training in the new techniques underlying current and novel biomarkers based on CFTR activity and other molecular and cell biology parameters.

The Workshop was initially open to 12 participants, but given that it received 32 applicants, it was decided to accept 17 participants: Australia (1), Belgium (2), Brazil (1), Czech Republic (1), France (2), Germany (4), Italy (4), Poland (1), and USA (1).

The Workshop counted with the support of National Patients Organizations from Belgium, Germany, Italy and The Netherlands, in the form of travel grants for participants from the respective countries.

Based on the very positive evaluations of the 2016 BSWG Workshop (see evaluation by participants in Annex 3), a "2nd Hands-On Workshop on Epithelial Systems: Physiology and Pathophysiology" will be organized again at FCUL, Lisboa (Portugal) 24 – 28 July 2017.

References

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Annex 1 – List of Participants at the 3rd BSWG meeting (30 March 2016)

The 3rd meeting of the ECFS BSWG, counted with **140 participants**, most of which are full BSWG members (ECFS/BSWG membership to be confirmed by email)

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Anders	Westad				
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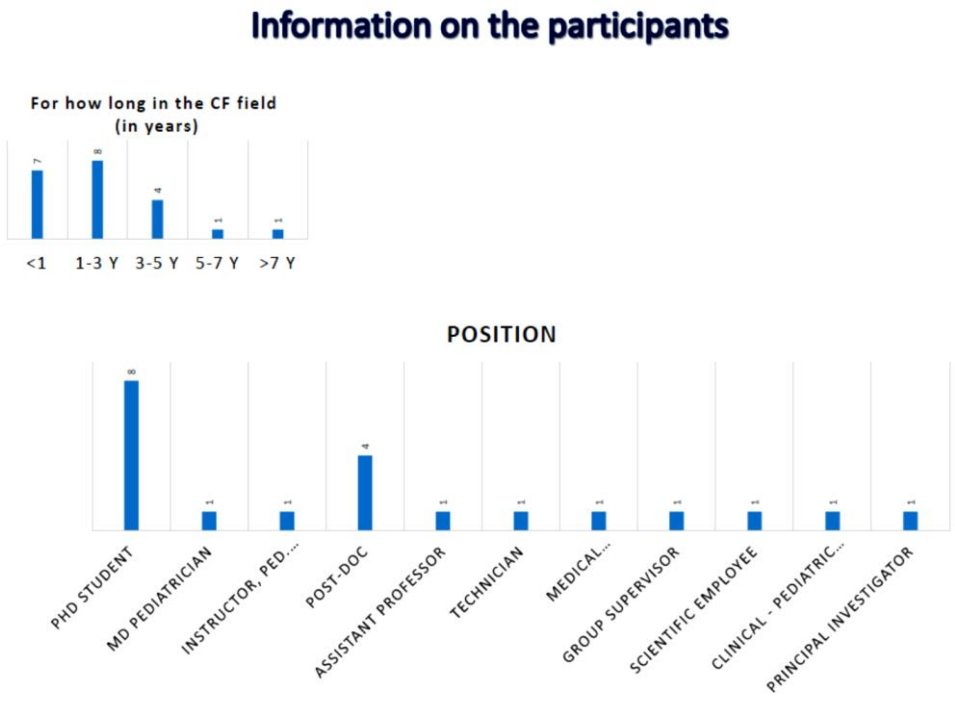
Annex 2 – Programme of the "*Hands-on Workshop on Epithelial Systems: Physiology and Pathophysiology*"

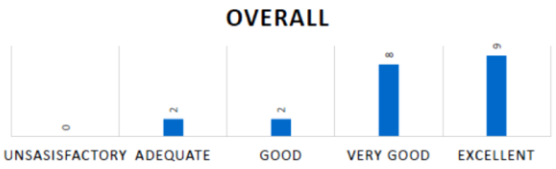
Time	Monday (18 Jul)	Tuesday (19 Jul)	Wednesday (20 Jul)	Thursday (21 Jul)	Friday (22 Jul)	Time
8.30-9.00	Course Introduction					
9.00-9.30	Lecture LC	Lecture 04 RT	Lecture 06 KK	Lecture 08 MH	Lecture 10 KK MG	9.00-9.30
9.30-10.00						9.30-10.00
10.00-10.30	Coffee Break	Coffee Break	Coffee Break	Coffee Break	Coffee Break	10.00-10.30
10.30-11.00	Lecture 02 PM	Lecture 05 JB	Lecture 07 KK	Lecture 09 MG	Lecture KK MG	10.30-11.00
11.00-11.30						11.00-11.30
11.30-12.00	Coffee Break	Coffee Break	Coffee Break	Coffee Break	Coffee Break	11.30-12.00
12.00-12.30	Lecture 03 MDA	Research Seminar 01 RT	Research Seminar 02 JB	Research Seminar 03 KK	Research Seminar 04 MG	12.00-12.30
12.30-13.00						12.30-13.00
13.00-13.30	Lunch Break	Lunch Break	Lunch Break	Lunch Break	Lunch Break	13.00-13.30
13.30-14.00						13.30-14.00
14.00-14.30	Lab 01.1 - Nasal Cells (C8) LC VF	Lab 03 - Immuno (C8) HB	Lab 05 - Swelling Assay (C8) JB NA or Lab 06 - Ussing Chamber (C8) KK MR	Lab 05 - Swelling Assay (C8) JB NA or Lab 06 - Ussing Chamber (C8) MH MR	Tutorial 01 JB HB NA	14.00-14.30
14.30-15.00						14.30-15.00
15.00-15.30	Lab 01.2 -ASL (C2) RT					15.00-15.30
15.30-16.00		Lab 03 - Diff/ Reg (C8) IP				15.30-1600
16.00-16.30	Coffee Break				Coffee Break	16.00-16.30
16.30-17.00	Lab 02 - Organoids (C8) NA JB				Tutorial 02 MH KK MR	16.30-17.00
17.00-17.30		Coffee Break	Coffee Break	Coffee Break		17.00-17.30
17.30-18.00	Meet the Expert (C8) - MDA, PM	Meet the Expert (C8) - RT, JB	Meet the Expert (C8) - KK	Meet the Expert (C8) - MG		17.30-18.00
18.00-18.30						18.00-18.30
18.30-19.00						18.30-19.00
19.00-19.30						19.00-19.30
19.30-20.00					Course Dinner	19.30-20.00

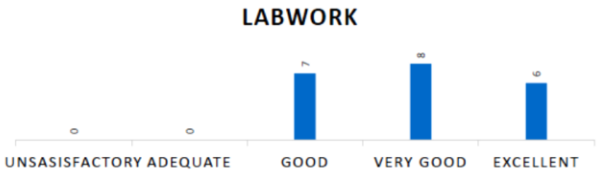
Professor	Class	Title	Type	Day	Time	time per session
Luka Clarke	Lecture 01	Cell Culture of Epithelial Cell Lines, Primary Cultures & Organoids	Lecture & Tutorial	18th	09:00	1h
	Lab 01.1	Culture of Primary Human Nasal Cells	Lab	18th	14:00 to 18:30	1h (1h x 4 groups)
Paulo Matos	Lecture 02	Polarized Cells: Specific Characteristics and Pathways	Lecture	18th	10:30	1h
	Meet the Expert			18th	14:00 to 18:30	1h (1h x 4 groups)
Margarida Amaral	Lecture 03	Cystic Fibrosis: a Disease of Epithelial Tissues	Lecture	18th	12:00	1h
	Meet the Expert			18th	14:00 to 18:30	1h (1h x 4 groups)
Rob Tarran	Lab 01.2	ASL Microscopy Measurements	Microscope Lab	18th	14:00 to 18:30	1h (1h x 4 groups)
	Lecture 04	Physiology of Airway Surface Liquid	Lecture	19th	09:00	1h
	Research Seminar 01	Effect of Tobacco Smoke on the Airways	Seminar	19th	12:00	1h
	Meet the Expert			19th	14:00 to 18:30	1h:15m (1h:15m x 3 groups)
Jeff Beekman	Lab 02	Culture of Murine Intestinal Organoids & Cryocuts		18th	14:00 to 18:30	1h (1h x 4 groups)
	Lecture 05	Organoids as Model Systems to Epithelia	Lecture	19th	10:30	
	Meet the Expert			19th	14:00 to 18:30	1h:15m (1h:15m x 3 groups)
	Research Seminar 02	TBA	Seminar	20th	12:00	
	Lab 05	Organoids Swelling Assay	Lab	20th	14:00 to 17:00	3h
	Lab 05	Organoids Swelling Assay	Lab	21st	14:00 to 17:00	3h
	Tutorial 01	Analysis of Organoids Swelling Assay Data	Tutorial	22nd	14:00 to 18:30	2h (2h x 2 groups)
Rainer Schreiber	Lecture 06	Physiology of the Airway Epithelial Cells	Lecture	20th	09:00	1h
	Lecture 07	Physiology of the Intestinal Epithelial Cells	Lecture	20th	10:30	1h
	Lab 06	Ussing Chamber Analysis of Murine Native Tissues and Polarized Epithelial Cells	Lab	20th	14:00 to 17:00	3h
	Meet the Expert			20th	17:30 to 18:30	1h
	Research Seminar 03	TBA	Seminar	21st	12:00	1h
	Lecture 10	Electrophysiology techniques: from tissues to cells and single-channel	Lecture	22nd	9:00 to 11:30	2h (1h x 2)
	Tutorial 02	Analysis of Ussing Chamber Data	Tutorial	22nd	14:00 to 18:30	2h (2h x 2 groups)
Martin Hug	Lecture 08	Functional Analysis of Cultured Epithelial Cells by Ussing Chamber	Lecture	21st	09:00	1h
	Lab 06	Ussing Chamber Analysis of Murine Native Tissues and Polarized Epithelial Cells	Lab	21st	14:00 to 17:00	3h
	Tutorial 02	Analysis of Ussing Chamber Data	Tutorial	22nd	14:00 to 18:30	2h (2h x 2 groups)
Michael Gray	Lecture 09	Physiology of the Pancreatic and Sweat Gland Epithelial Cells	Lecture	21st	10:30	1h
	Meet the Expert			21st	17:30 to 18:30	1h
	Lecture 10	Electrophysiology techniques: from tissues to cells and single-channel	Lecture	22nd	9:00 to 11:30	2h (1h x 2)
	Research Seminar 04	TBA	Seminar	22nd	12:30	1h

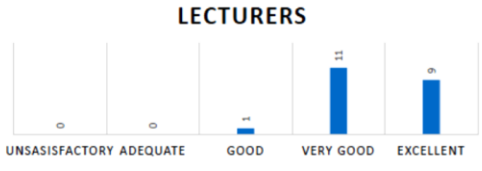
Annex 3 – Evaluation by participants of the "Hands-on Workshop on Epithelial Systems: Physiology and Pathophysiology"



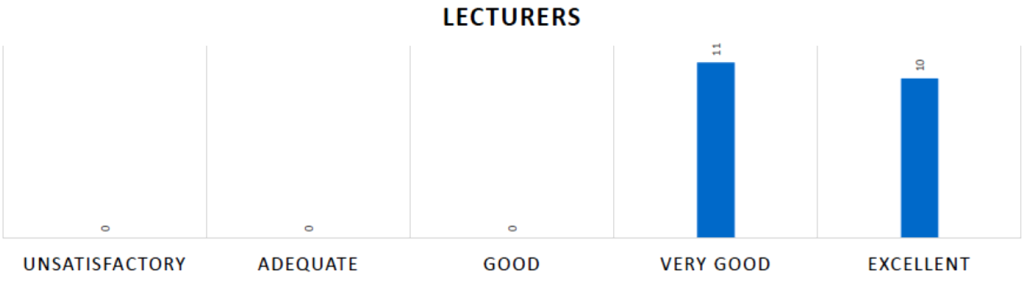
Organization of the programme



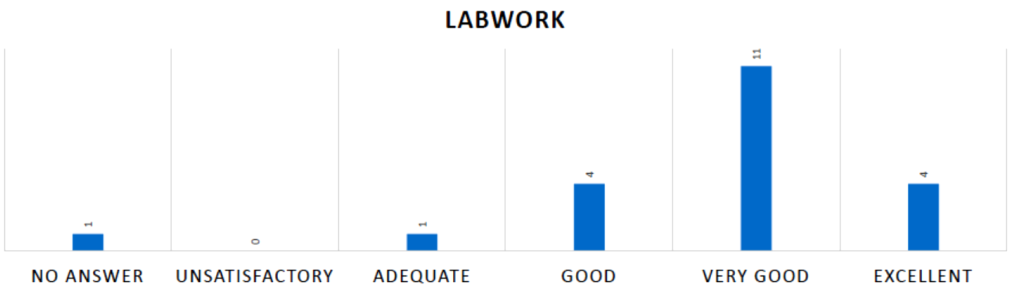


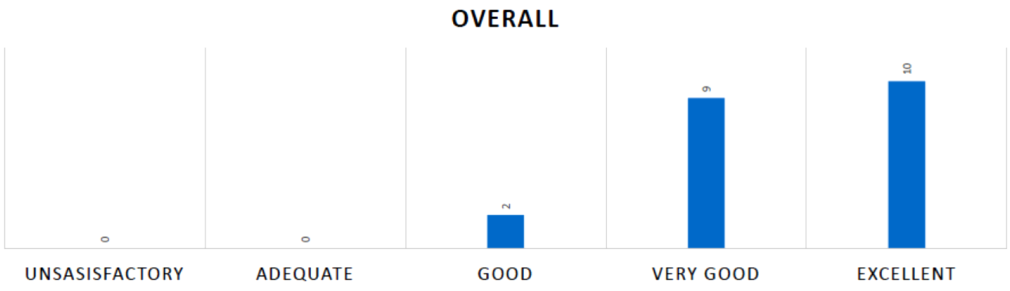


Quality of the programme

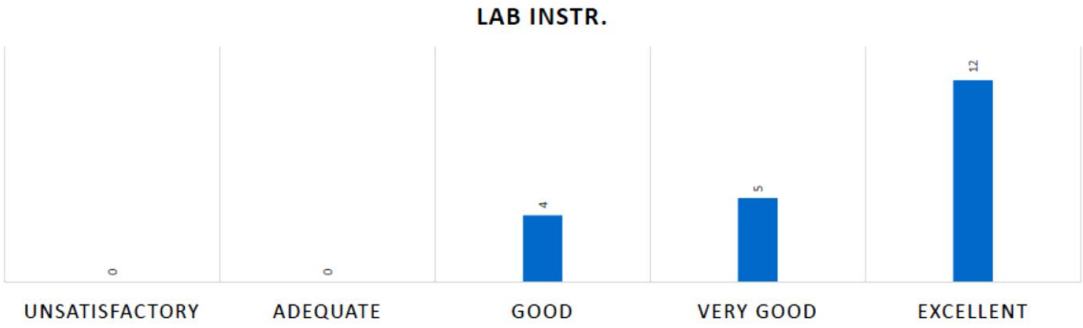


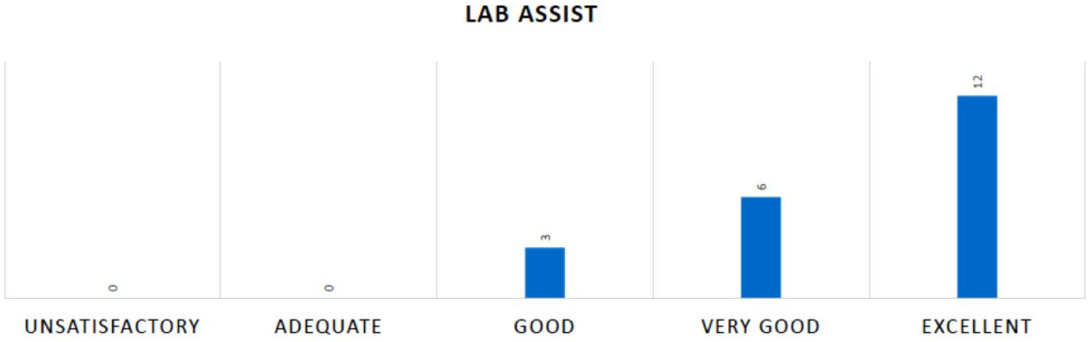
Quality of the programme



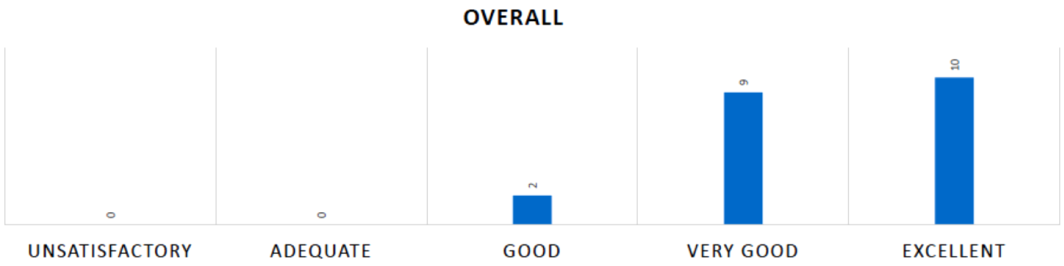


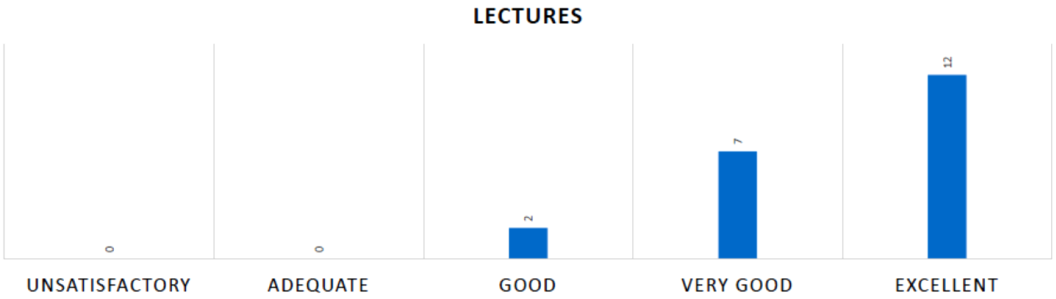
Quality of the Faculty



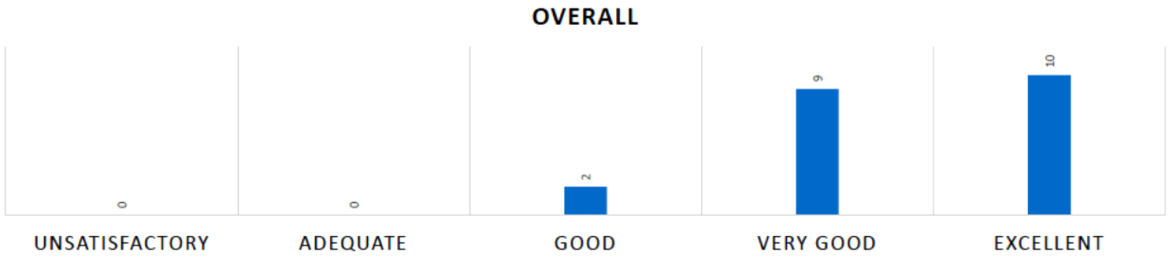


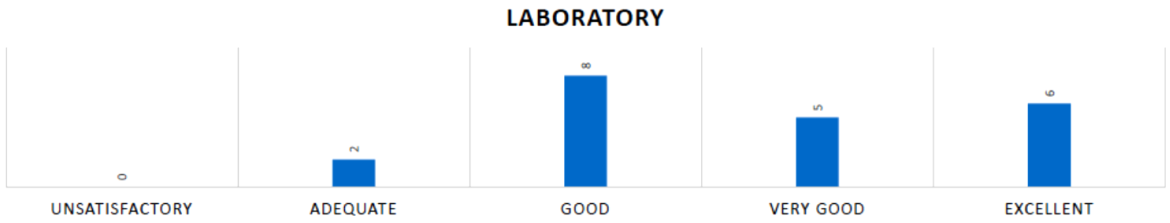
Quality of the Faculty



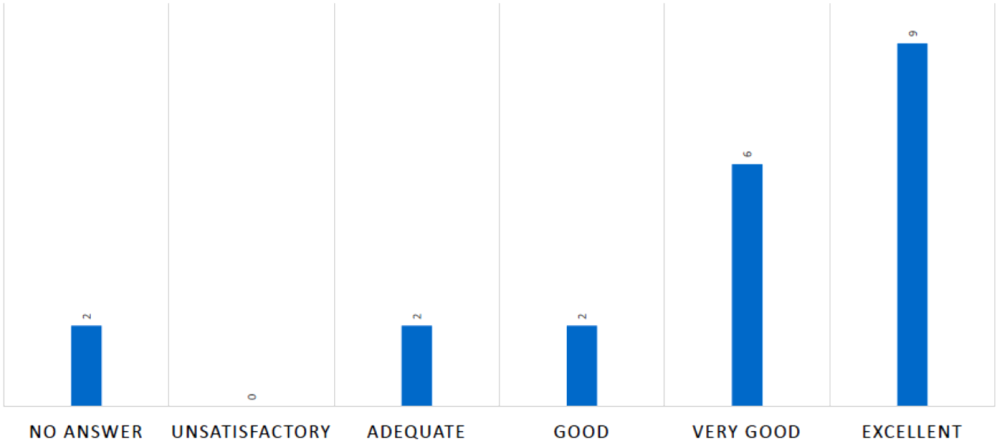


Quality of the facilities

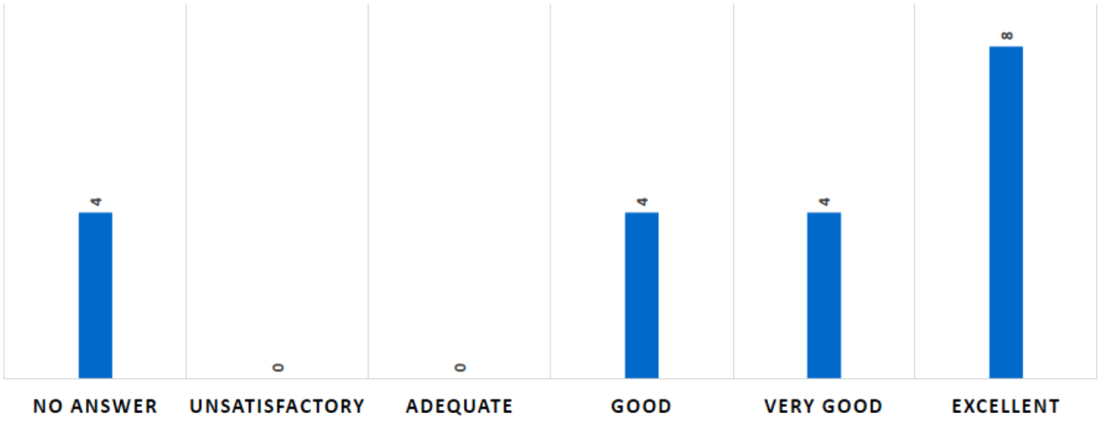




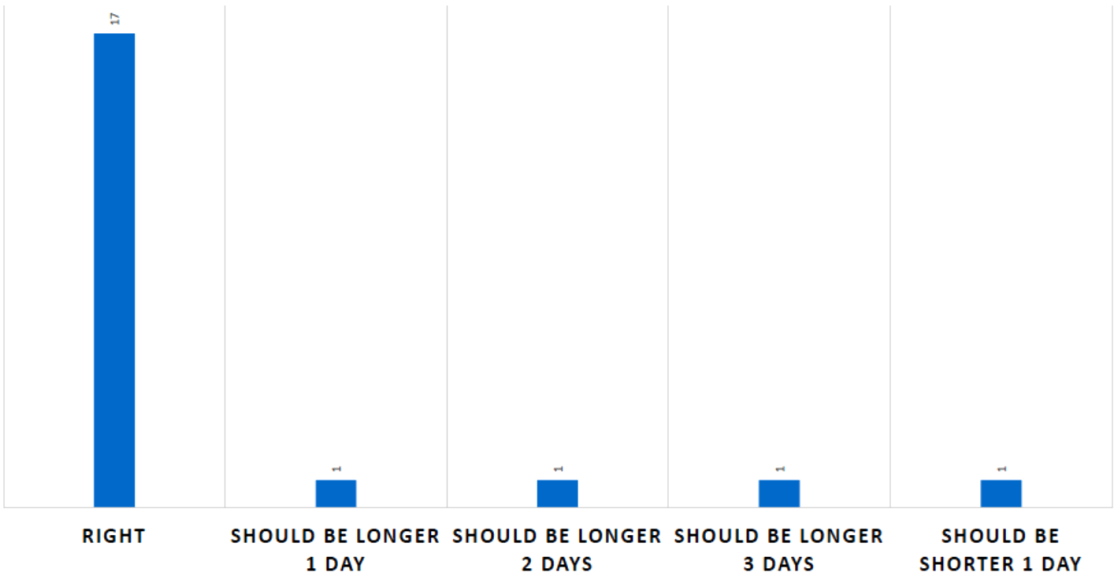
Quality of the secretariat



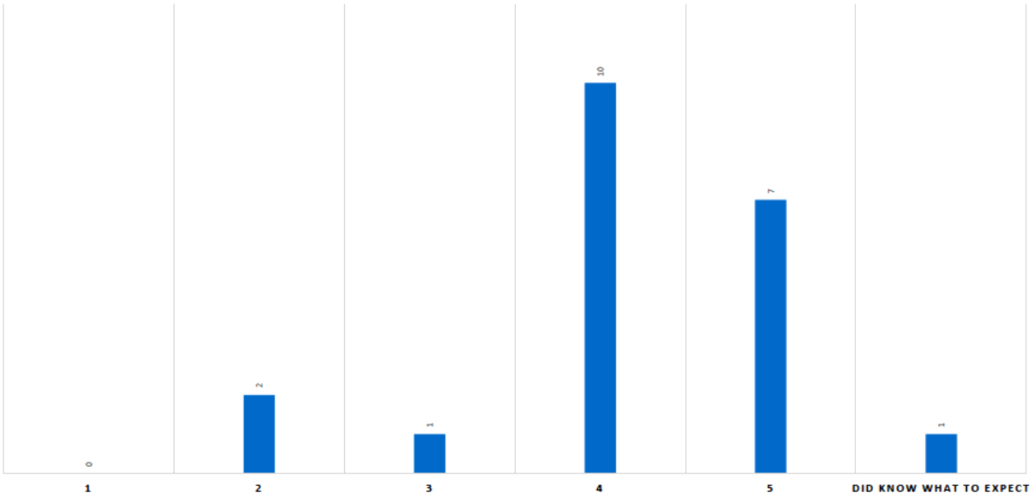
Quality and adequacy of the catering (lunches)



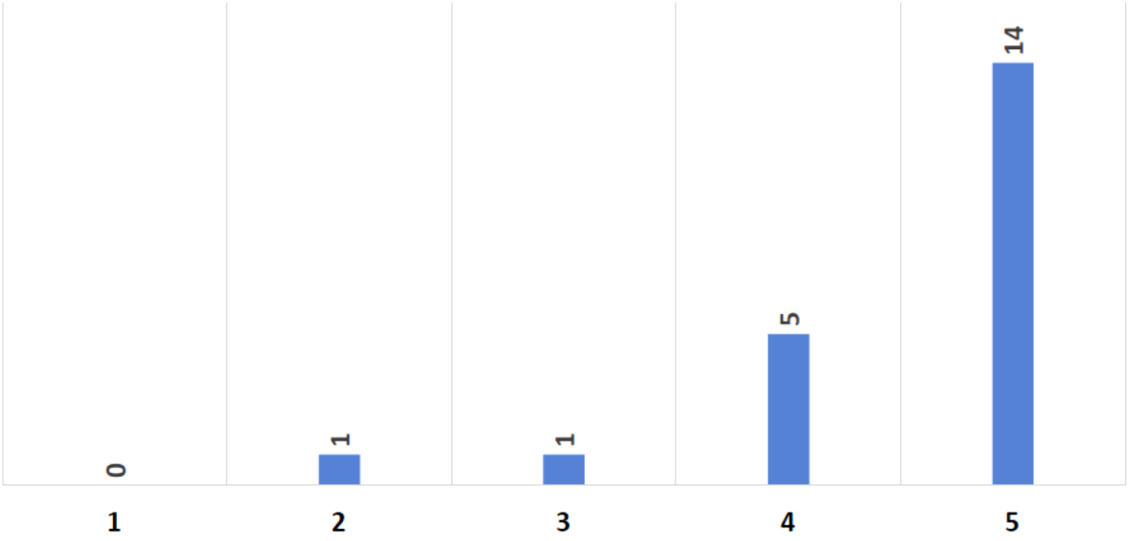
Duration of the Workshop



How did the Workshop fulfil your expectations? (5-Maximum; 1 - Minimum)



Would you recommend this Workshop to a colleague? (5-Maximum; 1 - Minimum)



Was there...