IMI1 Final Project Report Public Summary

Project Acronym: ORBITO

Project Title: Oral biopharmaceutics tools

Grant Agreement: 115369
Project Duration: 01/10/2012 - 30/09/2018

1. Executive summary

1.1. Project rationale and overall objectives of the project

OrBiTo is a project in the area of oral biopharmaceutics tools that included world leading scientist from nine universities, one regulatory agency, one non-profit research organisation, three small/medium sized specialist technology companies together with twelve pharmaceutical companies. The OrBiTo project aimed to deliver a framework for rational application of predictive biopharmaceutics tools for oral drug delivery.

This was achieved through novel prospective investigations to define new methodologies or refinement of existing tool. Extensive validation has been performed of novel and existing biopharmaceutics tools by using historical datasets from industry partners. A combination of high quality in vitro and in silico characterizations of active drugs and formulations have been integrated into physiologically based in silico biopharmaceutics models capturing the full complexity of gastrointestinal drug absorption. This approach has given an unparalleled opportunity to deliver transformational change in European industrial research and development towards model based pharmaceutical product development in accordance with visions of Quality by Design.

Benefits include an accelerated formulation development process, particularly for challenging projects e.g. for low solubility molecules (Biopharmaceutics Classification System (BCS) II and IV) or modified-release formulations, as well as allowing optimization of clinical product performance for patient benefit. In addition, outcome from the OrBiTo project will significantly reduce the need for future animal experiments in this area and reduce the need for human bioequivalence studies for bridging between formulations.

1.2. Overall deliverables of the project

Increased understanding of the gastro-intestinal (GI) drug absorption process as a prerequisite for improved biopharmaceutical predictions.

New or refined in vitro and in silico methods contributing to improve in vivo predictions of drug absorption.

A framework for optimal use of biopharmaceutical predictive tools and preclinical models.

1.3. Summary of progress versus plan since last period

All remaining milestones and deliverables outlined in the description of work have been successfully completed.

1.4. Significant achievements since last report

OrBiTo has had a productive extension year in terms of publications with 25 manuscripts published in international high impact pharmaceutical journals and about another 32 manuscripts in the pipeline to be published (accepted papers listed on http://www.orbitoproject.eu/deliverables). OrBito published in December in the Molecular Pharmaceutics in a themed edition '*Industry-Academic Collaboration in Oral Biopharmaceutics – The European IMI OrBiTo Project*'. In addition, a special OrBiTo issue of European Journal of Pharmaceutics and Biopharmaceutics, a high impact journal in pharmaceutics science area, is in preparation including the majority of the remaining papers in the pipeline. There has also been extensive external dissemination through invited and short presentations at international scientific meetings, for example at a dedicated OrBiTo 2-hour session at the annual meeting of the American Association of Pharmaceutical Scientists (AAPS) which is the largest global event for this area. Furthermore, a two-day OrBiTo open science meeting was organised in collaboration with UK Academy of Pharmaceutical Sciences (APS) which was "sold out" (125 attendees). Finally, OrBiTo was recognized at the IMI 10th year anniversary conference with a Best Oral Presentation award (B Hens et al).

OrBiTo has continued to organise well attended webinars with European and US regulatory authorities regarding possible future use of predictive tools and approaches developed within OrBiTo, for example to reduce the number of clinical bioequivalence studies. A written progress report from OrBiTo has also been submitted to key scientists at regulatory authorities. Leading regulatory scientists from EMA and FDA were invited speakers at the OrBiTo open science meeting.

"Young OrBiTo", consisting of PhD students and post-docs in the project, successfully organised a webinar sharing "hot off the press" new science within OrBiTo as well as a poster session at the final OrBiTo face to face meeting.

OrBiTo deliverables to accelerate product development, improve product design and align with animal testing 3R principles in industrial development of new medicines are achieved through the implementation of improved in vivo predictive in silico and in vitro tools. Over the last year, efforts have been focused on translating the development and validation of new methods into useful guidance to the industry in the rational use of such methods. This has included;

- A decision tree for active pharmaceutical ingredient characterisation has been developed that will guide work needed to select compounds that have optimal properties for product development, enable more accurate dose predictions to be made for first time in human studies, provide a basis for product design strategies and improve the quality of input parameters for advanced in silico models (PBPK) of drug absorption.
- A decision tree has been developed and published for guidance on the selection of appropriate in vitro dissolution method testing strategies to provide improved predictions of in vivo performance of oral formulations. This decision tree can also be applied to select the optimal dissolution methodology to determine the impact of process changes made during scale-up or post-approval on the clinical performance of oral drug products.
- A decision framework has been developed for the use of preclinical in vivo models to predict performance in human studies. The use of this decision framework, which is based on characterisation of active drug and formulation properties, is intended to be limited to

difficult cases where the use of in vitro/in silico predictive tools alone are not providing predictions with the required accuracy and confidence for formulation performance.

- Best practice guidance for PBPK in silico modelling has been developed which will help achieve consistent outputs through the provision of extensive guidance on the appropriate selection of input parameters and modelling assumptions for different molecular classes and formulation types. This will provide a basis for a more standardised application of models and facilitate use in potential future regulatory applications.

One strength of these end-point deliverables is that they have been developed in close collaboration between academic experts and industrial scientists and are built both on industry experience and scientific progress in OrBiTo. This should strongly facilitate industrial relevance and implementation.

Another major deliverable during the current period has been the completion of a second major PBPK validation study involving around 70 modellers in both academia and industrial groups. This work incorporated several improvements and learnings since the first PBPK validation had been completed which resulted in a significant improvement of the predictions obtained. This work also provided further insights on areas for future improvement.

The maintenance of the OrBiTo database with historical in vivo data and associated biopharmaceutical characteristics and meta-data has been secured by decision to establish a new database consortium which includes a majority of the partners from OrBiTo as a starting point. This is an unparalleled knowledge source for new research beyond OrBiTo which now can be further developed and enriched. Future possibilities may also include application of artificial intelligence (AI)solutions. Some EFPIA companies and FDA have also expressed an interest to use this database format, which was a significant initial work in OrBiTo, for internal data storage and biopharmaceutics knowledge management. Work is on-going to establish an agreement for the new consortium.

EFPIA companies have continued to demonstrate a strong commitment to the project during this final year and have provided an annual in-kind contribution consistent with the in-kind levels from previous years. It should be noted that this continued contribution was made even though the budget in-kind already had been delivered and exceeded before start of this period! There has also been evidence of extensive implementation of OrBiTo tools and approaches in EFPIA companies, including about 60 examples from individual companies, which over the next years will be translated into a more effective product development process with reduced and better use of in vivo studies in addition to new products with improved clinical properties.

1.5. Scientific and technical results/foregrounds of the project

OrBiTo has developed a toolbox for prediction of oral drug absorption with an emphasis on the impact of drug form and formulation. This includes not only development of some new methods (e.g. doi/10.1021/acs.molpharmaceut.7b00422), but also significant validations and standardisations of both new and already existing methods (eg https://doi.org/10.1016/j.ejpb.2018.12.010). The future use of this toolbox has been facilitated by the development of decision tools and best practice guidance which rationalize the application of this array of methods (eg doi.org/10.1016/j.ejpb.2018.07.003). These tools are explained in more detail in the following work

package specific sections regarding early in vitro characterization of active drugs (WP1), in vitro product characterisations of formulations (WP2), preclinical in vivo models (WP 3) and in vivo predictive in silico tools (WP 4). In addition, a number of clinical studies in WP 3 has revealed important new insights in understanding the absorption process. For example, the dynamics of changing contents in the GI tract for different conditions of relevance for bioavailability studies, characterization of shear and pressure forces in the GI tract, studying details of drug the drug absorption in the GI tract through local administration and sampling. Among many other things, a gastric magenstrasse was revealed in fed state that allows rapid bypassing of food contents and thereby avoidance of the delay of onset of drug action with food (doi: 10.1021/mp500022u). Finally, a new database (doi: 10.1016/j.cmpb.2016.11.006, doi: 10.1016/j.ejps.2016.09.027.) with in vivo data from the industry partners have been established as source for improved understanding and validation of predictive tools for oral drug absorption. This database was used in OrBiTo for extensive and unique gap analysis and validation of physiologically based pharmacokinetic in silico tools used for prediction of oral drug absorption (eg doi: 10.1016/j.ejps.2016.09.037).

WP 1 - Physico-chemical tools – Understanding the active pharmaceutical ingredient (API)

WP 1 focused on the development and standardization of small-scale physico-chemical tools to characterise the critical properties of the active pharmaceutical ingredient (API) to understand and predict its absorption. Such characterisation is mainly performed during candidate selection and early product development to select compounds that have the best pre-requisites for product development, enable accurate dose predictions to first time in man studies, establish a basis for product design strategies and inform input parameters for advanced in silico models (PBPK) of drug absorption. While such methods like dissolution, solubility and permeability measurements have been well established in the industry, there was a need to refine and standardise methods to attain consistency in data interpretation to aid decision-making and increase the physiological relevance of the methods. Furthermore, with the increasing number of low solubility drugs requiring enabling formulation approaches, new tools to accurately characterise supersaturation and precipitation behaviour was a critical need. Finally, biorelevant physico-chemical tools developed to date have been developed to simulate the average/median physiological conditions, which means that the extremes of physiological variability, even within a group of healthy subjects, are usually been neglected. Therefore, new approaches focused on capturing the impact of true physiological variation were developed by WP1.

WP1 was built on two cornerstones. Primarily, a structurally diverse set of APIs with focus on poorly soluble compounds (BCS class II and IV) was established. This was initially selected from open access compounds well described in the literature and during progress of the project enriched with additional compounds from EFPIA partners and the OrBiTo Database. The second cornerstone was a set of simulated gastro-intestinal media (SGIM), reflecting compositions of the human gastro-intestinal (GI) fluids in the fasted and the fed state to form the basis for the standardized, validated physico-chemical tools developed in WP1. SGIM simulating the composition of GI fluids were defined by an extensive literature search and further enriched by unpublished data from EFPIA partners. This was published in an initial review paper.

WP1 has developed a number of new physico-chemical tools that will be further exemplified below. These tools have been extensively validated to a large extent by work at EFPIA laboratories thereby confirming industrial relevance and facilitating and more widespread implementation. Furthermore, standardised protocols have been developed for several already existing key methods selected through EFPIA surveys in order to ascertain best practice with consistent output from different labs. One example of an area selected for standardisation is API dissolution and solubility measurements. This work has benefited greatly from extensive EFPIA input sharing internal procedures followed by interlaboratory validation to confirm and refine the proposed procedures. Finally, a decision tree has been outlined proposing a rational industrial usage of the API characterisation tool box taking a science and risk-based approach focusing efforts on the critical cases. Again, a key strength of this work was the close collaboration between academic experts and industry scientists assuring a meaningful output from an industry point of view.

High-level summaries of the small-scale methodologies developed within WP 1 are provided below;

- A novel approach to incorporate GI physiological variability in the determination of API GI solubility has been established based on a Design of Experiment (DoE) approach. This work first elucidated all relevant sources of variation in extensive multifactorial design experiments and later reduced to a more limited approach which is more suitable for implementation in industrial product development. This work has been described in 5 published research manuscripts, while 2 more manuscripts are in press/preparation.
- For the determination of API intrinsic dissolution rate and solubility, standardized small volume tools have been developed using API powder or discs. A strategy to select the most appropriate tool, based on API solubility, has been established. Overall 3 manuscripts have been published in this area.
- Novel, standardized small volume assays and pH-shift methods have been developed to determine the propensity of a given API to supersaturate, nucleate and precipitate in SGIM. This assay includes the possibility to determine the solid state of the precipitate by in-line or off-line methods through the use of Raman and/or XRPD. The tool was subjected to an interlaboratory study with EFPIA partners, showing that it was indeed suitable for the industry. This has also provided bases for one of the SME partners, Sirius, to refine their commercial tools. For more mechanistic studies of nucleation and precipitation, a video microscopy approach with image analysis has been developed. This approach, based on a high-throughput multiple well plate format, also provides a tool particularly suitable for early screening of the risk of precipitation and the excipients which could be used to avoid this phenomenon. Overall 3 research manuscripts have been published in this area, and 4 more are in press/preparation.
- Based on the extensive wet lab experimental data generated and collated in OrBiTo, molecular dynamics (MD) models have been developed for solubilisation of low solubility drugs in intestinal fluids including colloidal systems generated both from the endogenous bile acids as well as formulation components providing solubilisation such as lipids and surfactants. Furthermore, this work has also addressed the measurement of the propensity for supersaturation and precipitation. This work has provided important mechanistic insights in relationships between API structure and solubilisation required for oral absorption and

should also be possible to use as a first screen in selection of new molecules. Two manuscripts have been published in this area, and one more is in preparation.

- Whilst the majority of work in WP 1 has been focused on accurately measuring the solubilisation process for low solubility drugs in the GI fluids as a prerequisite for oral absorption, some significant work has also been done in the area of API permeation over the gut wall. First of all, a new method of combing dissolution and permeability by a cell culture model has been developed. This model has proven especially useful for early evaluation of supersaturating drugs and use of enabling formulation approaches like nanoparticles, amorphous drug forms and precipitation inhibiting excipients. This work has also introduced mucus producing cells to improve the in vivo relevance of this model since this could be an important barrier e.g. for nanoparticulate formulations. Another WP 1 task developed a new approach to predict intestinal permeability based on surface active profiling. This has been successfully developed to provide mechanistic insight to structure relationships with permeability and for possible use in early assessment in drug discovery. In this area, 1 manuscript has been published and further 3 are in press/preparation.

In summary, WP1 has successfully obtained all deliverables, with several of the new tools having already been adopted among industrial partners. The impact of this workstream will continue to grow over the next few years as tools become more widely implemented. The main industrial impact of this WP is to improve earlier decision-making during compound selection and to enable the early formulation development strategy to be developed using less drug compound and wet lab experimentation. The provision of more precise, high quality, inputs into integrated in silico absorption modelling (see WP4) is also important in this context. In summary, WP1has generated 34 research and review papers, including those currently in press/review, clearly advancing the science of this field.

<u>WP2 – In vitro Tools – Understanding the formulation</u>

The general objectives of WP2 were (i) to establish a functional array of in vivo predictive in vitro tools for formulation evaluation, with a primary focus on mimicking oral formulation behaviour in the GI tract, including drug release, supersaturation, precipitation and permeation, and (ii) to develop a decision tree for selecting the most appropriate in vitro model(s) in industrial product development for a given drug/formulation/prandial state combination.

The industrial use of such methods is fundamental in the development of a formulation with desired clinical performance, to ascertain unchanged clinical performance during scale-up of manufacturing and post-approval changes, as a surrogate for in vivo bioequivalence studies between different versions of formulations (biowaivers), and as part of the quality control in commercial manufacturing. In these roles, biorelevant in vitro testing has the potential to replace preclinical in vivo studies used to characterise in vivo performance of oral formulations during industrial development. In addition, this area also has a significant regulatory interest in terms of replacing mandatory vivo studies with predictive tools and developing quality control methods and acceptance criteria that are clinically relevant assuring desired drug performance in patients while not imposing requirements leading to waste and increased costs.

A wide range of such in vitro methods was available prior to OrBiTo. However, besides the standardised methods described in various pharmacopoeia, which generally do not aim for in vivo prediction, the systematic validation and standardisation of such methods was lacking. Furthermore, a need for novel methods existed, considering the increasing number of challenges in drug product development with very low solubility drugs and an increasing interest in novel complex or modified release formulations. High priority gaps that were addressed included (1) the impact of GI motility and hydrodynamics on the behaviour of IR and MR formulations, (2) dissolution in the lower gastrointestinal tract, (3) the behaviour of supersaturating drug delivery systems, (4) drug permeation from complex luminal samples, and (5) the role of lipid digestion in drug absorption.

The in vitro tools developed in WP2 focused on maximizing the biorelevance and thus the power to predict in vivo results whilst retaining simplicity and ease of use. Two synergistic approaches were adopted to achieve the general objectives: (1) systematic testing and validation of existing, optimised and new in vitro models, and (2) translating an improved understanding of gastrointestinal processes underlying absorption (WP 3) into optimised and/or new biorelevant in vitro models.

Within WP2, several new methods have been developed, which will be further exemplified below. Typically, the development also included validation at both academic and industry labs. Furthermore, standardised protocols have been established and extensively validated for several already existing key biorelevant dissolution tests (biorelevant fluids, two-stage dissolution, TIM®, models for evaluating extended release). This determined best practice with consistent output from different labs for several biorelevant dissolution methods of varying complexity. Finally, a decision tree has been developed proposing a rational industrial usage of the tool box to evaluate oral formulations taking a science and risk-based approach focusing efforts on the critical cases. This decision tree is freely available on the internet and is also published as a research manuscript.

Like WP1, one great strength of the WP2 work has been the close collaboration between academic experts and industry scientists including sourcing of test material, selection of test methods, experimental validation and agreement on best practice decision trees. This assured a meaningful output from an industry perspective and facilitated the implementation of the WP 2 outputs in product development. Another success factor has been the close interaction with WP3 where new in vivo data was generated that served both as input to the design of in vitro tests (with respect to GI motility and GI fluid volume/composition/transfer) and as high quality in vivo data for validation of tools to assess intestinal drug dissolution/precipitation. Finally, cross-WP groups ensured the coordination of efforts across WPs in a few focused areas: (a) in vitro dissolution data integration in PBPK modelling, (b) supersaturation and precipitation models, and (c) integrated permeation/dissolution models.

Here, a high-level summary is given of some of the outstanding achievements of WP2:

- Improved implementation of the hydrodynamics in the GI tract in in-vitro dissolution tools to better capture the impact of hydrodynamics on drug release.
- A dynamic flow through in vitro system has been developed that simulates intragastric flow profiles and gastric pressures in the region of the antrum and pylorus. Through comparisons with in vivo data, this method has been shown to successfully predict the intragastric disintegration and drug release behaviour of dosage forms. Within OrBiTo, seven papers

have been published for this method, which is now commercially available as GastroDuo® from Physiolution, GmbH, Greifswald, Germany.

- A new method has been developed to capture the impact of increased GI fluid viscosity in the fed state. This has proven particularly useful in predicting negative food effects for BCS class III drugs.
- Development of new biorelevant fluids and dissolution methods simulating the ileo-caecal environment. This was based on unique work by means of characterisation of human intestinal fluids in OrBiTo WP3 and was described in four publications.
- Development and validation of biorelevant yet simple multi-compartmental models to determine luminal drug release profiles from extended release (ER) dosage forms in both fasted and fed state. These methods capture the change of fluid composition that an ER tablet experiences during transport through the GI tract. Development was based on previously established biorelevant fluids, extensive literature compilation of GI fluids and new in vivo characterizations performed within the framework of OrBiTo WP3.
- Development, optimization and validation of models to predict gastrointestinal supersaturation/precipitation from enabling formulations of low solubility drugs. For instance, a two-stage method named the biorelevant gastrointestinal transfer system (BioGIT), has successfully been established; ten papers have been published from this work.
- Integration of permeation within dissolution/precipitation models for the evaluation of enabling formulations. This enables improved in vivo predictions by mimicking the dynamics of dissolution and absorption over the gut wall. An Artificial Membrane Insert (AMI) system has been developed within OrBiTo as a time- and cost-effective alternative to cell-based models for easy implementation into dissolution testing. It has been successfully applied to capture the potential of poorly soluble drugs to permeate after solubilization.
- Improved implementation of lipolysis in dissolution testing to improve prediction of the impact of lipids, either from food or as vehicles for drug delivery, on drug absorption. In particular, a two-stage method including both a gastric and an intestinal lipolysis step was developed. Another more mechanistic method was established to monitor the colloidal structures formed between lipids and bile components during dissolution testing with use of dynamic light scattering (DLS) or small-angle neutron scattering (SANS).
- Improved understanding of the complex multicompartmental TIM-1 model, including its predictive ability for human performance of different drugs and dosage forms, and an evaluation/comparison with a newer simplified version (Tiny TIM)
- An improved disintegration tester for solid dosage forms as an alternative to the pharmacopoeial fixed velocity disintegration testing device allowing hydrodynamic control of tablet movement and thus forces acting on the solid dosage form. In addition, the new device integrates direct forces acting on the dosage form thus simulating the effect of stomach contractions and pressure waves.

In summary, WP2 has successfully obtained all planned deliverables, and several of the new tools and procedures are already being adopted among industrial partners. The outcome of WP2 has an important impact on the pharmaceutical industry, including a reduced need for in vivo studies in designing formulations, more rational and effective formulation development (“getting it right first time”) even for challenging drugs and drug products, and high-quality input into integrated in silico

absorption modelling (see WP4). Furthermore, the standardisation and validation of biorelevant dissolution methods provide a basis for future extended regulatory usage of such methods replacing mandatory clinical bioavailability/bioequivalence studies. Finally, WP2 has generated more than 60 research and review papers clearly advancing the science of this field.

WP3 – In vivo tools – Systems characterization & understanding

The main objectives in WP3 can be summarized as follows:

- The collection of in vivo data derived from animals and humans regarding physiological characterization of the gastrointestinal tract and the behaviour of compounds and formulations, regional absorption and human GI fluid data
- Mechanistic experiments to improve understanding of the GI system
- Measurement of in vivo dissolution and comparison with in vitro experiments to obtain a better understanding of pharmaceutical effects on drug absorption
- Advances in predictability of human oral absorption by use of animal models
- An improved understanding of excipient effects on drug absorption beyond their already more or less well understood effects on drug dissolution. This includes effects on drug permeability, and water retention and transport within the GI tract.

A primary gap in our current knowledge regarding the predictivity of in vitro and in silico assessments for in vivo drug behaviour is the level of biorelevance needed for such predictive models. While in vitro and in vivo tools at start of OrBiTo included some biorelevant aspects, failure in predictions especially for challenging cases like low solubility/permeability drugs or modified release formulations clearly indicated that improved understanding of the in vivo system is needed. In OrBiTo that has been addressed by more than 20 mechanistic studies in man using intubations, imaging and telemetric “smart” capsules with sensors to provide an enriched dataset describing key properties of the GI environment. For example, the following studies have been performed;

- Fluids has been sampled and characterised from poorly studied regions in the GI tract
- Studies of some basic physiological characteristics for standardized conditions of drug intake such as motility patterns, pressure, temperature and volumes
- Drug permeability in different regions of the GI tract has been characterized for drugs with different hydrophilic/lipophilic balance
- Drug dissolution and precipitation in the GI tract by sampling of GI fluids and simultaneous monitoring of pharmacokinetics in plasma

Traditionally in vivo assessment of drug absorption is performed based on drug plasma concentration- time data. However, such profiles are the result of a multifactorial chain of biological processes which can make it very difficult to distinguish the influence of the many different factors involved in the drug absorption process from systemic pharmacokinetic effects. Therefore, the latter type of studies with direct measurement of drug concentrations and physical forms in the GI tract have been especially important to reveal the true chain of events in the GI tract as a starting point

and data sets for validation in the development of predictive models (WP1,2,4). Finally, some parts of this work also aimed to improve standardized protocols for biopharmaceutic in vivo studies.

Whilst it was a key theme of OrBiTo to replace in vivo studies with in vitro/in silico predictive tools it was realized that the need for some pre-clinical in vivo studies will remain. For example, in vivo studies will still be needed when initial validations of predictive tools or acceptable predictions have not been reached. A subsidiary aim of WP3 was therefore to better understand the suitability of animal models for different types of APIs and formulations. Such an understanding would lead to the rational selection of the best animal models for a given API or dosage form and would ensure translation to man.

The main part of the in vivo studies man was performed at the academic labs while EFPIA contributed in some cases with study material. A significant part of the preclinical in vivo work, both validating translation to man of animal models as well as allowing additional mechanistic investigations was performed by EFPIA partners. In the development of a best practice and guidance in use of animal models for absorption studies there was a significant input from several EFPIA partners sharing internal practices as a starting point for further improvements.

The work in WP3 mainly fed into the other WPs to generate improved design or validation of predictive in vitro/in silico methods as well as having a significant impact also on such work outside OrBiTo. Therefore, there are a very limited number of methods generated with direct implementation in the industry. One exception is the animal model decision guide that has been presented at several meetings and a research manuscript is in preparation. Another more generally applicable outcome is the compilation of a simple physiological database of relevant information for the GI tract both consisting of OrBiTo data and historical literature data including both preclinical models and man.

A non-comprehensive list of some key findings in the in vivo work are listed below;

- The unique sampling of fluids from the ileo-ceacal region revealed that the reduction in available bile acids in this region results in a significant decrease in the solubilization of low solubility drugs present in the ileum.
- The gastric conditions after a standardized high fat meal used in food interaction studies was characterized for the first time. Interestingly the fed state remained in the stomach in all subjects for more than six hours for example having implication for future dosing regiments for drug requiring fasting conditions.
- Another very interesting finding was obtained in the high fat meal study mentioned above. It is well known that the gastric emptying of gastric content is much slower in the fed state compared to the fasting situation often reflect by slower onset of drug action with concomitant food intake. However, in the current study using in vivo imaging, administration of water immediately after meal intake, showed that the water was rapidly emptied similar to fasting conditions in contrast to slow gastric emptying of the meal slurry! Thus, this implies novel formulation opportunities to allow rapid onset of action also when the drug is taken with a meal!
- Studies with ibuprofen, a frequently used anti-inflammatory and pain-killing drug, using sampling in the stomach and small intestine, showed that when the drug was given as a

solution, it was precipitating in stomach to solid drug particles thereby taking away potential benefits of such administrations. However, when reaching the small intestine, the precipitated drug particles were relatively rapidly re-dissolved and delivered absorption of the dose. A new finding was the fact that dissolving ibuprofen particles in the intestine did lower the intestinal pH, indicating the very limited buffer capacity of intestinal fluid when compared to buffers used in in vitro dissolution testing.

- The relationship between drug permeability in the human jejunum and different pre-clinical in vivo models, e.g. rat perfusion, or in vitro cell layer or artificial membrane models have been well established. However, in the OrBiTo project, such relationships were established also for more distal parts of the GI tract in a structured way for the first time. These data provided further validation of preclinical models and will be very useful in refining in silico modelling especially for extended release formulations where absorption from the entire GI tract is needed.
- Population models of individual variability have been built on large historical data sets for some of the key variables in drug absorption such as the rate of gastric emptying in some disease states. Another factor is the bile acid concentration in the gut which determines solubilization of low solubility drugs. The individual variation had been elegantly determined from markers in plasma reflecting the gut bile concentration. These population models are a great asset for inclusion in integrated in silico modelling of absorption for example allowing virtual clinical trials to be performed with a statistical analysis of the effects of bile acid variability.
- The rate of absorption for many drugs is dependent on the rate of gastric emptying. Gastric emptying in the fasted state is dependent on a recurring motility cycle with longer periods of very low activity followed by shorter periods of increasingly intense motility leading to more rapid gastric emptying and absorption. A new study showed that sparkling water immediately induces a more intense gastric motility pattern, prompting consistent rapid gastric emptying and drug absorption (exemplified with paracetamol). This provides new opportunities for future design of rapid onset drugs and also provides suggestions for improved standardisation of bioavailability studies. The work performed by Leuven University also generated great local media interest with articles in several journals and national radio interview.
- The effect of various critical excipients on intestinal permeability and absorption was assessed in ex vivo and in vivo animal models. The investigations showed clear concentration and transit time dependence. It is clear that effect of excipient needs to be assessed by in vivo models as the net effect will be strongly affected by a complex interplay with multiple physiological and biopharmaceutical factors.

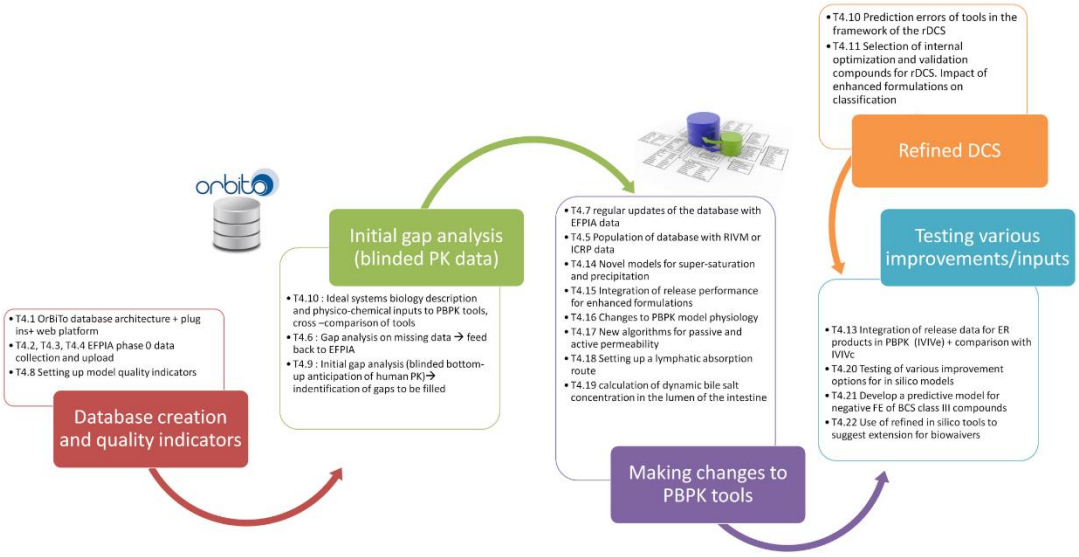
All deliverables have been successfully achieved and the results of each task/milestone/ deliverable have been reported in the progress reports with the accompanying publications / manuscripts. As of the 1st of August 2018, there were in total 47 publications (original articles and reviews), 27 poster contributions and 22 podium presentations as output from WP3 related tasks. Additional publications are in preparation.

WP4 - In silico tools – Integrating data towards in vivo predictivity

The OrBiTo workpackage 4 (in silico tools) was organized around 5 key streams of activity (Figure 1).

1. Database creation and population with novel EFPIA data
2. Initial gap analysis of the pre-existing in-silico tools and processes in the bottom-up anticipation of human pharmacokinetics
3. Changes to the in-silico models and procedures
4. Test of the various improvements
5. Creation of a refined Developability Classification System

Figure 1 - WP4 organization

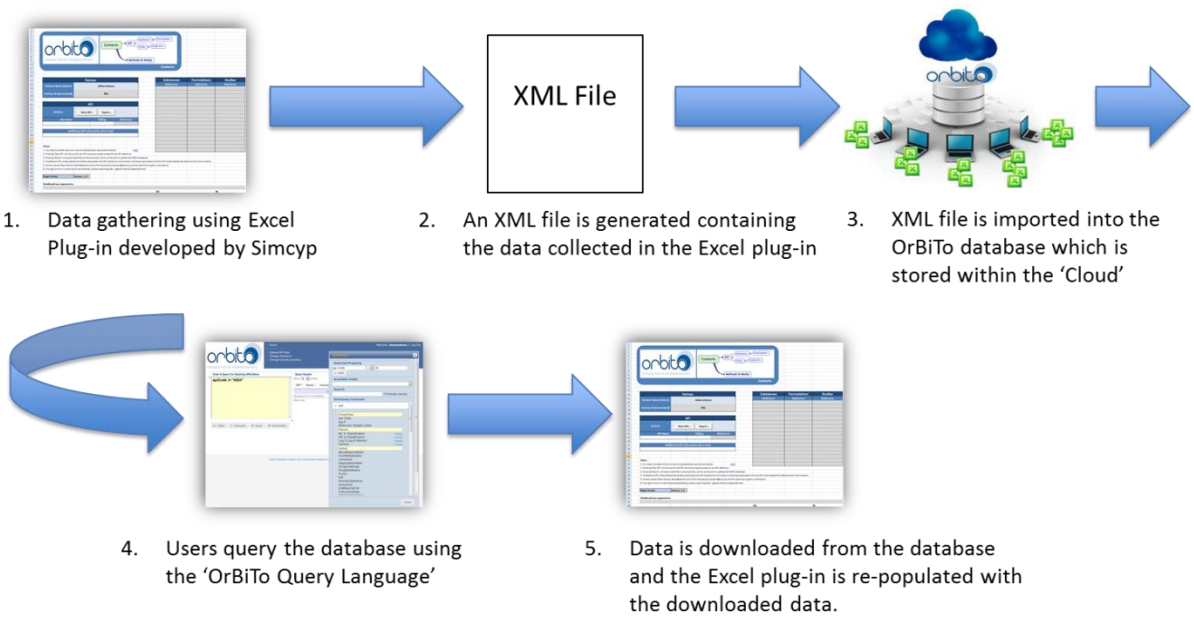


Database creation

The first set of activities related to the creation of a novel database holding partnering EFPIA foreground data on physico-chemical characteristics of drug substance and drug products, disposition and distribution data (in vitro), preclinical and clinical pharmacokinetics. The objectives of this database were to allow the in-silico model evaluation work to be performed using the same dataset (before and after improvement) and to foster collaboration and continuous improvement of the data and metadata needed for running appropriate modelling of human absorption of drug products.

The initial requirements for the OrBiTo database were that it should be novel, be secure and accessible to all partners, be compatible with offline capture of data using industry standard tools, be searchable, allow the selective blinding of some fields, allow interaction between data users and data owners whilst maintaining full or partial anonymity, be flexible to allow new fields and associated metadata to be captured, be fast since the database architecture, functionalities needed to be created prior to data capture from the EFPIA partners. Because this activity was on the critical path for WP4, the ideation work started prior to the official project start with involvement of Sanofi and

Simcyp. A novel architecture was developed and placed in a cloud environment for web-access. Searching functionalities were added using SQL searches through a user-friendly interface yielding a list of relevant APIs, and summary data sheets where also proposed, which extract live pre-defined type of information over the entire database. The actual data could be uploaded and downloaded via the web interface using an xml script generated from an Excel "plug-in". These Excel sheets were used to analyse the data or capture it in view of the data upload (Figure 2).**Figure 2 - OrBiTo database: how does it work**



The clinical data contained in the OrBiTo database is in compliance with the GDPR since all individual data are anonymized and randomized. The architecture of the OrBiTo database and data-blinding strategy was reviewed during the course of the OrBiTo project by the IMI Ethic's midterm review committee.

As of August 2018, 97 APIs were present in the database, 512 preclinical or clinical studies covering 1638 different administration conditions. This data was partly published in literature. The API physico-chemical and biological data in conjunction with formulation information and human and animal PK data were used to establish how current PBPK absorption modelling tools perform in a blinded exercise (T4.9) using minimal guidance for modellers and, following model improvements and standardization of model inputs in task (T4.20). The selection of compounds and associated formulations allowed to probe certain model gaps and evaluate model performance on a variety of drug substances and formulations.

The use of the current data was focussed on delivery of the tasks described in the Description of Work (DoW) for OrBiTo WP4. There are a number of other ways this data could be used in the future:

- Provide a valuable tool for validation of future biopharmaceutics PBPK predictions in conjunction with other future participants which may include regulators.
- Prevent re-work in the future to replicate cross-company database
- Provide a forum for continued research and enrichment of dataset across companies

- Could be used by companies to compare compound performance / build understanding of a wider biopharmaceutics design space

Concrete examples cover the use of individual PK to understand variability and their sources by type of formulations or API, the link between human and animal exposure…etc.

A decision has been taken in OrBiTo to maintain the database beyond the end of the project with a majority of the partners continuing as members of the database group. A project agreement, based on the principles defined in the IMI OrBiTo PA, is in preparation and is intended to enable future collaborative research based on the use of data within the OrBiTo database. The main principles of this agreement will be that data will be accessed for research and publication purposes to improve our understanding of the biopharmaceutics models. Data will continue to evolve, and new fields and metadata will be added in the future. The contribution to partners will allow the data access to be maintained and the main administration of the database to be done to ensure continuity after the OrBiTo project stops.

Another interesting indirect outcome of the work with database is that this has highlighted in some companies the poor internal integration of relevant data across different functions, prompting internal initiatives to improve modelling practices based on OrBiTo experience. Another related outcome is that some companies are considering implementing the OrBiTo database also for internal data storage and biopharmaceutics knowledge management. Finally, at a recent presentation of this work, FDA expressed interest in gaining access to the database format for internal use in addition to becoming a member in the new database group formed post OrBiTo!

Bottom-up anticipation of human PK

As a first step in improving the prediction performance of *in silico* tools, a large-scale simulation exercise was performed (T4.9) with an aim to identify the weaknesses and strengths of these modelling platforms and approaches and suggest improvements. For the task, 43 APIs were found to satisfy the defined selection criteria. The 43 APIs chosen represents over 165 human studies, and over 600 human study arms. Over 4000 simulation files had been generated by the participating institutions, representing over 2550 study arm-institution-software combinations. The study was performed according to a blinded bottom-up approach. The participating institutions uploaded these simulation output files to SharePoint. Simulated plasma concentration-time profiles were extracted from simulation output files using Matlab and transferred into MS Excel templates which contained automated calculation of pharmacokinetic (PK) parameters. In order to facilitate the analysis of the results with respect to inputs selection, compound properties and study design, a summary spreadsheet containing this information was generated. A list of topics for focused analysis was generated and results were published in 3 papers. Results identified focus areas for further improvements such as integration of *in vitro* release profiles, training of modellers, and input data quality. Finally, standard operating procedures (SOPs) of software use were updated based on the findings.

The task was repeated with updated data and SOPs (Task 4.20). In this case, 58 APIs were chosen for the simulation task, representing over 200 Human Studies, 700 Human Study Arms. Guidance documents for both software use, selection and calculation of input parameters were provided to

the modellers along with guidance on reporting the performed simulations containing all information regarding inputs used and output. Before performing the simulations, modellers were asked to define strategies for the calculation and selection of input parameters for allocated APIs and software. They were asked to discuss these strategies with fellow modellers simulating the same API in other software in order to harmonize the inputs across the software. These "strategy documents" were discussed and updated during the course of monthly strategy meetings between modellers and a team of experts (core team members). Simulations were performed by the modellers based on these strategy documents once signed off by the core team members. This important information and discussions were used as one source in further updating and improving a standard operating procedure (SOPs) of software use, created in a previous simulation exercise as a function of data availability and software options. Each participating institution was asked to generate bottom-up predictions for every human study arm associated with their allocated APIs using a population representative of healthy volunteers built into the programs. Extraction of input parameters and simulated plasma-concentration profiles from the software files was carried out in an automated way and PK parameters were calculated for each study arm. Data cleansing was performed in order to prepare data sets for final analysis. The performance of the models to predict the PK parameters was evaluated using already defined metrics.

Around 2000 simulation files had been generated by the participating institutions representing 700 unique study arms and 58 API simulated in the three software packages. Findings will be published in two papers.

This has been a unique study gathering extensive interest from the scientific community not least among people at the regulatory authorities. PBPK has shifted during the "OrBiTo era" from being an internal company decision/de-risking tool to an additional emerging use in regulatory context to replace in vivo studies. OrBiTo has clearly played a role in this development and for example been invited to a FDA organised meeting on the topic with OrBiTo work referenced in recent FDA material. Pilots of successful regulatory examples have also been shared by EFPIA members in OrBiTo.

In silico model modifications and improvements

Several modifications were done to in silico models to support findings of WP1, WP2 and WP3. The way in which drug solubility is calculated in complex biorelevant media was modified to incorporate the contribution of the ionized and unionized drug.

Ways of calculating drug permeability based on mechanistic models were defined, and the way we integrate precipitation of drug substances and products in PBPK was extensively studied. In the absence of a common current consensus on the best predictive method for precipitation, several correlations were established between the transfer models and Stella or between the BioGIT and Simcyp.

The way in which dissolution is integrated in PBPK and the mechanistic interpretation of dissolution has progressed during OrBiTo. The consideration of fluid hydrodynamics for immediate release or prolonged release formulation and the type of apparatus or media which could provide better in vitro in vivo correlation through the use of PBPK were studied.

Finally, changes to how we model gastro intestinal transit, gastric emptying, pH variability in the stomach and the amount of bile in the small intestine were also progressed during OrBiTo which will allow more patient centric in silico approaches and a better evaluation of human variability.

rDCS – the revised developability classification system

Although the BCS is a useful regulatory tool for determining whether bioequivalence studies can be waived, it has several drawbacks when applied to drug development. For example, the dose of the drug must be known to determine the BCS class. Additionally, the classifications are both stringent and categorical, whereas the spectrum of drug characteristics is a continuum. Last but not least, the BCS classification does not provide any guidance to formulation. To overcome these drawbacks, an alternative approach was taken in the OrBiTo Project: The Refined Development Classification Scheme (rDCS).

While offering a pragmatic method which can be easily applied at the bench level, the rDCS enables most key questions during development of oral dosage forms to be addressed, such as: What if the final dose differs from the projected dose? If the drug is poorly soluble, should I aim for a dosage form with faster dissolution or an enabling formulation? Should I worry about precipitation occurring during passage of the drug through the GI tract? Further, the refined rDCS provides standard data sets for both solubility and permeability, against which data generated in-house can be correlated. This approach offers the formulator maximum flexibility in his/her choice of methodology. For many drugs a set of standard experiments will be sufficient, while for others customized experiments may be added to build a more comprehensive picture of what will be required to generate a successful oral dosage form.

Overall conclusion for WP4

Overall, the work performed in WP4 fostered excellent collaboration between industry academia and regulators. The way in which we use PBPK models, their current limitations and opportunities for improvement were highlighted in conjunction with data coming from WP1, WP2 and WP3. The way in which we integrate in vitro and in vivo data changed together with some standardization and best practises in the actual measurements needed to feed the models. The modelling exercises during the course of WP4 and the preparation that went into them served for many partners as a learning tool for their modellers.

The fact that OrBiTo Industry partners considered it worthwhile to share proprietary and confidential information on almost a hundred APIs in a pre-competitive space, is an achievement and a great legacy for future research if the work around the biopharmaceutical data can continue and develop further after OrBiTo. This is particularly important as the industry and regulators prepare/evaluate themselves to conduct more absorption modelling work to waive human clinical trials for future submissions, in the fields of formulation bridging, specification justifications, post approval changes and impact of food or acid-reducing agents on exposure.

Some clear deliverables and improvements of the in -silico models were achieved during OrBiTo such as:

- A database format for gathering and storing relevant data in a practically useful way

- Modelling of gastric emptying and reaction of gallbladder following different type of food ingestion
- Strategy to integrate dissolution in PBPK models in a more mechanistic way than was achieved before
- In vitro and in silico tools to model and integrate drug precipitation
- Modelling drug solubility in complex media
- Modelling specific patient populations such as patients undergoing bariatric surgery
- Definition of a refined Developability Classification System
- SOPs and guidance related to inputting data into models and modelling procedures.

Overall, WP4 successfully reached all deliverables and enriched the scientific area through a number of key manuscripts. The WP4 efforts as part of the overall OrBiTo project, allowed a greater interaction between industry, academia, regulators and specialist technology companies engaged in in silico model development. WP4 has provided a greater awareness of the limitations of the different PBPK models and has delivered some improvements but also identified key areas for further advancement. This experience and awareness will enable future collaborations to happen and will drive future research in this field for the next years to come.

1.6. Potential impact and main dissemination activities and exploitation of results

OrBiTo has developed a toolbox for prediction of oral drug absorption with an emphasis on the impact of drug form and formulation. The future use of this toolbox has been facilitated by the development of decision tools and best practice guidance which rationalise the application of this array of methods. The EFPIA partners have been strongly involved in the research work throughout the project, which has facilitated implementation of OrBiTo output into industrial practice. At the ending of the project, we have gathered around 60 examples of implementation of OrBiTo tools, methods and best practice guides at industrial partners. This implementation process is expected to continue for still some time after ending the project reflecting the onward impact of the significant research delivered during the final year of OrBiTo. Furthermore, the strong involvement of EFPIA partners in the research work as well as extensive sharing of science and industry examples through various meetings have also contributed to a significant uplift of expert knowledge within the industry.

The future impact of this improved toolbox on pharmaceutical product development can be summarised as:

a) reduced need for in vivo studies during product development through increased confidence and rationale use of predictive in vitro/in silico tools
b) products with better clinical performance in patients underpinned by the enhanced understanding and improved tools of drug absorption that has been obtained in OrBiTo.

Regarding the reduced need for in vivo studies; this will clearly lead to less animal experimentation in line with agreed 3R principles in addition to reducing the need for human pharmacokinetic studies

typically performed in healthy volunteers. In the latter case, aside from the ethical benefits of minimising drug exposure to healthy subjects, this also enables accelerated development and would be expected to contribute to a significant reduction in the time through the various stages of development, and ultimately, to market. It should also be noted that although in vivo studies in man still will be needed for verification of results from predictive methods, the risk of failing such in vivo studies will be significantly reduced, thereby avoiding unnecessary reformulation and repeating of human studies and extremely costly delays to market. To put this into context, if the application of OrBiTo tools lead to the avoidance of a one-year delay to just one significant NCE to market, we estimate that the OrBiTo project will have made a return on investment of about 10-fold!

The improved insight in the drug delivery and absorption in the GI tract will clearly improve opportunity for patient-centric product design that optimises the clinical performance in individual patients. For example, the following potentially desired product characteristics may be obtained more readily:

- Rapid onset of action
- Improving extent of absorption such that efficacy is reached in consistent manner
- Controlling exposure levels over time to extend the effect and avoid adverse effects related to temporary excessive drug levels in the body.
- Minimise influence of food, other drugs, disease state on drug actions and thereby reduce variability in patient response

OrBiTo is the most significant research effort in the oral biopharmaceutics area in the world. As such, it has gained much attention outside the EU. For example, in Japan a new consortium in this area, Cobito, has been started. In US, the FDA regulatory agency has been closely following and has interacted with this project as well as initiated complementary research efforts in this field.

Clearly, OrBiTo has strengthened the position of Europe as the leading area for oral biopharmaceutics research in the world. Besides the significant amount of new knowledge as reflected by a total output of about 170 papers in scientific journals, this has also been obtained by the strengthened network between various partners which will facilitate future collaborations. The “Young OrBiTo”, the PhD students in the project, is also a great outcome providing an immediate source for recruitment to both industry and academia of very well-trained experts with a great international network but also a basis for future leaders in this field. OrBiTo has also provided an important platform for several talented, junior academic researchers to progress theirs career in the academy towards increased recognition, new other funding and better positions.

We can also foresee that OrBiTo output will have an impact on future regulatory guidelines in the biopharmaceutics area, especially in context of replacing mandatory in vivo clinical studies with predictive tools. There has been close interaction both with representatives from EMA (with the Swedish Medical Product Agency being a partner in OrBiTo) and US FDA, throughout the duration of the project. This has included a regulatory stakeholder board, regulatory speakers at OrBiTo face-to-face meetings, frequent webinars for regulators and publication on future use of predictive tools from OrBiTo which included regulatory authority scientists from FDA and EMA. One particular emerging area that has gained regulatory traction during the “OrBiTo era” is the use of integrated in silico absorption models (PBPK) in combination with high quality biorelevant in vitro data to justify

product specifications or formulation/process changes. This approach has been the key strategic scientific focus in OrBiTo. The work done in the project has influenced the increasing interest and understanding of this area from regulators as well as underpinning early case examples of regulatory submissions from the industry. OrBiTo representatives were invited to an FDA workshop on this topic and we can expect to see new guidance in this field during coming years.

1.7. Lessons learned and further opportunities for research

Lessons learned and reflections on collaboration in public private partnership (PPP)

The IMI program provide an unique opportunity to bring together academia, industry and regulatory authorities to work side by side on challenging tasks that could improve the speed at which we get new, better medicines to patients. The benefits of bringing these groups together are many-fold. For example, it ensures that the research areas explored, and approaches taken are relevant from an industrial point of view, it utilises a wealth of industrial experience and data which would not typically be available to outside partners, it brings scientists together to formulate and work towards common goals, and it also hugely facilitates adoption and implementation within industry compared to output from traditional academic work. Furthermore, it facilitates a scale of research private-public research projects not easily achievable by other means and provides access to multiple methods, datasets and diverse skill-sets across a large consortium group. For example, a historical database could be generated from the combined contributions of EFPIA partners which was not possible to achieve for any individual partner. The in-silico tools gap analysis and validation work were performed by almost 100 scientists resulting in a rapid, widespread dissemination of knowledge and awareness of best practice across academic and industrial partners which would have been difficult to achieve through more constrained presentation/publication approaches.

One of the key success factors in OrBiTo has been the strong involvement of industry partners in research activities as reflected by the in-kind delivery from the EFPIA partners which exceeded budget. It has been a sustained effort by the OrBiTo EXCO team to facilitate EFPIA involvement through extensive communication at all levels.

Another benefit of a public-private partnership like OrBiTo is it provides a neutral platform to interact with regulatory agencies on science of relevance for future regulatory guidance and practice. In OrBiTo, the Swedish Medical Product Agency was a full partner and throughout the entire project there were extensive interactions with other agencies including US FDA which were made possible by having a regulatory agency embedded in the project as a partner.

OrBiTo had an integrated research approach covering all important aspects to establish a framework for the prediction of oral absorption based on active drug and formulation in vitro characterisation (WP1,2) in vivo systems understanding (WP3) and integration of these data into in silico absorption models (WP4) represented by different workpackages (WPs). This holistic approach has allowed the project team to connect the different strands of the research areas and to focus on embedding the fundamental learnings on the gastrointestinal tract environment generated from in vivo studies in man into the new in vitro and in silico tools developed as part of the project. On an organisational level, efforts have also been made within the project to ensure connectivity between the different workpackages through work in EXCO and initiation of cross-WP groups focused on a few key topics.

Overall, OrBiTo has leveraged the whole oral biopharmaceutics area in the industry. For future research projects in the biopharmaceutics area it is recommended to take same holistic research approach, but perhaps try to limit the scope to more focused questions. One area of focus for the future could be work towards complete elimination of the need for in vivo studies in certain scenarios. Examples of these scenarios might include human studies to understand drug interactions with food, establishing particle size limits, bioequivalence studies for low solubility drugs (BCS II), paediatric bioavailability studies, etc.

Future research opportunities

OrBiTo has resulted in a step change in informed drug product development by significantly improving the use of, and confidence in, in vivo predictive in vitro/in silico tools. A new paradigm has been established where predictive in vitro tools and modelling in combination is at the core of the development process and in vivo studies can be limited to verify outcome or validate models at critical stages. The work performed within OrBiTo in this context is not the end-point as further research will be needed to reach the full potential of this approach. We envisage that in the future, drug product development will use approaches common in other industries such as aerospace and automotive, where development is mainly performed by predictions and only verified by physical tests at the end of the development. Future regulatory implementation of the predictive tools will also require more formal scientific qualifications for example to fully implement virtual bioequivalence trials. Another important aspect is that OrBiTo has been mainly focused on predicting average outcome in healthy volunteers. Variation in responses between individuals are also driven by factors such as age, disease, concomitant medications and life style. Although some activities in OrBiTo addressed such aspects, this remains an extensive research field which will be important for implementing patient centric product designs and precision medicine.

The current drug discovery pipeline includes a large variety of new modalities being of higher molecular weight and not suitable for oral delivery. Historically this has mainly been limited to peptides and proteins, but the modern portfolio now also includes modalities such as various sized nucleotides, conjugated hybrid molecules, and nanoparticles with functional properties for delivering the drug. There is a need for an in vivo predictive tool box firstly for parenteral administrations which is the key delivery route for many such modalities. The knowledge and toolbox in this area is only very rudimental compared to the oral area. For these new modalities, fundamental biopharmaceutics knowledge is needed for transforming the innovative delivery systems required for such molecules into useful products to patients. Secondly, for these new modalities which today are at best limited to parenteral delivery, there is from a patient-centric point of view a great demand for more convenient administration forms ultimately as conventional dosage forms for oral administration. Developing delivery systems which can augment the oral absorption of such molecules remains a significant challenge. While this has been a research area for decades, progress as measured by clinically useful products has been minimal. The approaches currently in clinical development, typically provide bioavailabilities around 1% and the large variability between individuals limit their effective use for patient populations. The expertise in Europe on understanding oral drug absorption is an excellent foundation for future innovative work in this space.