Six years of progress in the oral biopharmaceutics area – a summary from the IMI OrBiTo project.

B. Abrahamsson[1], M. McAllister[2], P. Augustijns[3], P Zane[4], J. Butler[5], R. Holm[6], P. Langguth[7], A. Lindahl[8], A. Müllertz[9], X. Pepin[10], A. Rostami-Hodjegan[11,12], E Sjögren[13], M Berntsson[1], H.Lennernäs[13].

[1]AstraZeneca R&D, Sweden, [2]Pfizer , United Kingdom, [3] KU Leuven, Belgium , [4]Sanofi-Aventis, France, [5]GSK, United Kingdom, [6]Johnson & Johnson, Belgium, [7]Johannes Gutenberg University of Mainz, Germany, [8]Medical Products Agency, Sweden, [9]University of Copenhagen, Denmark, [10]AstraZeneca R&D, United Kingdom, [11]Certara, United Kingdom, [12]University of Manchester, United Kingdom, [13]Uppsala University, Sweden.

Corresponding author

Bertil Abrahamsson

AstraZeneca R&D

S-43183, Mölndal, Sweden

Email: Bertil.abrahamsson@astrazeneca.com

Key words: biopharmaceutics, PBPK, IVIVC, dissolution, drug absorption, permeability.

Abstract

OrBiTo was a precompetitive collaboration focused on the development of the next generation of Oral Biopharmaceutics Tools. The consortium included world leading scientists from nine universities, one regulatory agency, one non-profit research organisation, three small/medium sized specialist technology companies together with thirteen pharmaceutical companies. The goal of the OrBiTo project was to deliver a framework for rational application of predictive biopharmaceutics tools for oral drug delivery.

This goal was achieved through novel prospective investigations to define new methodologies or refinement of existing tools. Extensive validation has been performed of novel and existing biopharmaceutics tools using historical datasets supplied by industry partners as well as laboratory ring studies. A combination of high quality *in vitro* and *in vivo* characterizations of active drugs and formulations have been integrated into physiologically based *in silico* biopharmaceutics models capturing the full complexity of gastrointestinal drug absorption and some of the best practices has been highlighted. 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 the vision of model-informed drug development.

Introduction

The Innovative Medicines Initiative (IMI) is Europe's largest public-private initiative in the life science sector between the European Union (EU) and the European pharmaceutical industry association (EFPIA) (http://www.imi.europa.eu). The purpose of IMI is to accelerate the development of better and safer medicines for patients and to build networks of industrial and academic experts in order to boost pharmaceutical innovation in Europe. Importantly, IMI provides the legal infrastructure and platforms to create and manage large public-private partnerships for pre-competitive research in the healthcare sector. OrBiTo (http://www.orbitoproject.eu) was selected for funding as part of the 4th IMI call for projects and was designed to streamline and optimize the development of orally administered drug products with focus on the underpinning science of oral biopharmaceutics (1). OrBiTo was funded from 2012 to 2017 with an additional one year no cost extension and the consortium brought together in total 27 partner organisations which included nine universities, one EU regulatory agency, one non-profit research institution, three small/medium sized specialist technology companies together with thirteen pharmaceutical companies (see figure 1).

OrBiTo had an integrated research approach covering all the critical aspects required to establish a framework for the prediction of oral absorption (see figure 2) (1). The different work packages (WPs) included active drug and formulation *in vitro* characterisations (WP1 and 2, respectively,) which not only focused on defining new and improved tools and methods, but also incorporated standardization and validation activities to better understand and utilise existing methods more effectively. WP 3 covered *in vivo* systems understanding and *in vivo* tools mainly through novel mechanistic studies in man. WP4 focused on *in silico* based prediction models, primarily physiologically based pharmacokinetic models (PBPK) and capturing best practices in conducting modelling strategies. This holistic approach allowed fundamental learnings on the gastrointestinal (GI) tract environment, generated from *in vivo* studies in man, to be embedded within the design of new *in vitro* and *in silico* tools. Outputs from new tools and data from clinical studies in man were used to improve the quality of the *in vitro* inputs used for *in silico* predictions. An improved understanding of drug absorption from the GI tract was not only obtained by prospective *in vivo* studies, but also through extensive validation which was obtained by establishing a database based on historical *in vivo* datasets from industry partners.

Oral bioavailability (F) is a major factor which contributes to the to the clinical efficacy and safety of a drug administered by the oral route. F is the result of three general processes: i) fraction dose absorbed across the apical cell membrane into the cellular space of the enterocyte, described as fa; ii) gut wall first-pass metabolism (E_G); and iii) hepatic first-pass metabolism (E_H) (equation 1) (2).

$$F = fa \cdot (1-E_G) \cdot (1- E_H) \quad (1)$$

The fraction of the dose absorbed (fa) is affected by different factors such as drug release from a formulation, dissolution of the active drug in the GI fluids, GI luminal drug degradation or complexation, and intestinal wall drug permeability (see figure 3). In general, these factors can be grouped into three categories: (i) physico-chemical factors of the drug

molecule itself, (ii) pharmaceutical factors such as design of formulations including choice of excipients and the physical/solid state form of the drug in the final product, and (iii) physiological and pathophysiological factors in the intestine. A special focus of OrBiTo has been to characterize and predict the impact of dissolution on drug absorption. Dissolution is primarily governed by drug solubility and formulation design. The composition, volumes of the GI fluids and hydrodynamic conditions generated by the GI motility controlled by endocrine and neural factors are physiological factors that influence drug release and dissolution. However, the effect of dissolution on GI absorption is also modulated in a complex manner by other factors like permeability, complexation, degradation and first-pass metabolism. For example, the overall impact of dissolution on GI drug absorption is strongly influenced by the intestinal effective permeability (3). In a similar fashion, processes, such as intestinal degradation and/or carrier-mediated transport processes through the intestinal wall, metabolism in the intestinal mucosa, hepatic first-pass metabolism, and lymphatic transport all have an inherent effect on F but they must also be considered to determine the impact of drug form and formulation on F. In addition, many of these key processes are regional specific and vary along the GI tract. As a result, their impact will differ depending on the *in vivo* drug dissolution. Due to these regional differences, transit of the API and the formulation in the GI tract is also a key factor in GI drug absorption. In a limited number of cases the pharmaceutical excipients have been proven to exert effects beyond dissolution/solubility including effects on intestinal P_{eff}, metabolism and GI transit as summarized recently by Flanagan (4). Thus, in order to predict the influence of API's physical form and formulation technologies on GI drug absorption all factors described need to be taken into consideration. For this reason, the OrBiTo project applied an integrated approach that fully considered the interplay among the above mentioned factors in order to improve the *in vivo* prediction of GI drug absorption.

The importance of the API form and formulation on the clinical performance has been magnified by modern drug discovery approaches, which yield highly potent and selective APIs that exhibit challenging biopharmaceutical properties, e.g. low aqueous solubility or higher molecular weight, sometimes in combination with limited intestinal stability (5). Such drugs often require complex formulation strategies to enable successful GI drug absorption and product performance. These dosage forms are based on high energetic solid-state forms of the API, reduction of the API particle size - sometimes to the nano-scale, lipid formulations, soluble drug complexes or permeability enhancers (6). These trends have highlighted the need for rational use of *in vivo* predictive biopharmaceutics tools at different stages of drug development, e.g. during candidate drug selection and risk assessment, product design, bridging between different formulations in clinical trials and commercial manufacturing, setting *in vivo* relevant quality criteria, and post-approval changes of the manufacturing. Most of the progress in understanding and predicting the GI absorption process during the last 15-20 years is from an industrial perspective represented by the introduction of *in vitro* permeability models (7,8), biorelevant dissolution fluids (9), the BCS (10) and *in silico* PBPK models for integration of *in vitro* data and prediction of GI drug absorption (11). However, these important advances should be viewed not as an end-point, but rather the platform for modern biopharmaceutics and signposting the strategic direction for improved *in vivo*

predictions (12). At the start of the OrBiTo project, extensive reviews were made and published within each work package in order to further define the state of art and gaps to be addressed in the project (13-16). In summary, key scientific areas that have been targeted by OrBiTo across the four WPs include;

- A refined understanding and modelling of GI fluids, compositions and volumes
- Abundance of drug metabolising enzymes and transporters in animal and human intestine
- GI hydrodynamics of importance for disintegration and dissolution and which control the release of API in the GI tract
- Intestinal drug supersaturation and precipitation, of specific relevance for BCS II/IV basic drugs, salts and other enabling formulations
- Improved permeability predictions for low solubility compounds in combination with dissolution studies
- Regional aspects of dissolution, intestinal permeability and other absorption factors not only relevant for MR formulations, but also for BCS II, III and IV drugs not achieving complete absorption in the upper GI tract
- Specific excipient/formulation effects on GI drug absorption especially for solubility enhancing formulations.

In addition, the lack of standardization and insufficient validation of existing methods were recognized as a gap at the start of the OrBiTo project and remedies were suggested regarding best practices. Finally, it was recognised that use of tools (i.e. dissolution or modelling alone) in a discreet, isolated way was a significant limitation for achieving accurate predictions for oral absorption and it would require the adoption of a more integrated approach to significantly improve the predictive power of biopharmaceutics tools.

In summary, the main objectives of the OrBiTo project were;

- to increase the understanding of the GI drug absorption process as a prerequisite for improved biopharmaceutical predictions
- to create new or refined *in vitro* and *in silico* methods contributing to improved *in vivo* predictions
- to develop a framework for the optimal use of predictive tools and preclinical models.

It was envisaged that successful progress in the areas detailed above would deliver the desired outcomes of reducing the number in vivo or clinical studies required to define drug product performance during a typical development programme and would provide a deeper knowledge of the critical biopharmaceutical attributes for the design of future drug products.

Outcome from OrBiTo

WP1: Physico-chemical tools – Understanding the active pharmaceutical ingredient (API)

WP1 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 provide input parameters for advanced *in silico* models (PBPK) of drug absorption. While methods such as dissolution, solubility and permeability measurements have been well established in the industry, a need to refine and standardise methods to attain consistency in data interpretation was identified as a gap which must be addressed to aid decision-making and increase the physiological relevance of the methods. Furthermore, new tools to accurately characterise supersaturation and precipitation behavior was a critical need. Finally, current biorelevant media to simulate the physico-chemical conditions in the small intestine have been developed to simulate the average/median physiological conditions. This 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 a focus on poorly soluble compounds (BCS class II and IV) was established. The second cornerstone was a set of simulated GI 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 (13).

Several new physico-chemical tools were developed in WP 1 that will be further exemplified below. Several new as well as existing tools have been extensively validated. These validations were to a large extent conducted by the EFPIA partners thereby confirming industrial relevance and facilitating more widespread implementation. Furthermore, standardised protocols have been developed for several already existing key methods selected through EFPIA surveys in order to derive a best practice for achieving consistent output across different laboratories. One example of an area selected for standardisation was API dissolution and solubility measurements (17). This work benefited greatly from extensive EFPIA input sharing internal procedures followed by interlaboratory validation to confirm and refine the proposed procedures. Finally, a decision tree is under development which proposes a framework to guide the rational use of the API characterisation tool box in an industrial setting.

A novel approach to incorporate GI physiological variability (pH, bile salt and phospholipid concentration etc.) 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. This was later reduced to a more streamlined approach which is more suitable for implementation in a high-throughput industrial setting (18-23).

For the determination of the APIs 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, was established and characterized using equipment available at most of the EFPIA partners, ie the µDISS (24,25).

Novel, standardized small volume assays and pH-shift methods have been explored and refined to determine the propensity of a given API to supersaturate, nucleate and precipitate in SGIM. These assays include the possibility to determine the solid state of the precipitate by in-line or off-line methods using Raman and/or XRPD. The tool was subjected to an interlaboratory comparison including both academic and EFPIA partners, which demonstrated the suitability and robustness of the method in both settings. This work further provided insights into both the method and the industrial needs for one of the SME partners, Sirius (now a part of Pion Inc), to refine their commercial equipment to better meet the needs of the customer. 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 risk evaluation of precipitation and the excipients that could be used to avoid this phenomenon (26).

Based on the extensive wet lab experimental data generated and collected in OrBiTo, molecular dynamics (MD) models were developed to understand the chemical mechanisms leading to solubilisation of low solubility drugs in intestinal fluids. This also included colloidal systems generated both from endogenous bile acids as well as formulation components providing solubilization, such as lipids and surfactants. Furthermore, this work has addressed the propensity for supersaturation and precipitation and providing important mechanistic insights into relationships between API structure and solubilisation required for oral absorption to allow this tool be used as an early screen in the selection of new molecules with the appropriate biopharmaceutics properties for further development (27, 28).

WP2: *In vitro* Tools – Understanding the formulation

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 reflecting oral dosage form performance 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.

A wide range of biopharmaceutics *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 predictive methods was sparse. 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 behavior of IR and MR formulations, 2) dissolution in the lower gastrointestinal tract, 3) the performance 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 both existing and novel *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 are further exemplified below. Typically, tool development also included validation at both academic and industry laboratories. 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 defined best practice guidance which would achieve 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 (29).

A high-level summary of some of the achievements of WP2 is given below:

Improved hydrodynamics to capture the impact on drug release were implemented by developing a dynamic flow through *in vitro* method 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 behavior of dosage forms. The model developed is now available as GastroDuo® in a spinout company from the University of Greifswald (30-36).

A novel 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 (37-40).

Biorelevant fluids have so far been confined to represent stomach, upper small intestine and colon. New biorelevant fluids and dissolution methods simulating the ileo-caecal environment were therefore developed. This was based on unique work in OrBiTo to characterize human intestinal fluids (41,42).

Development and validation of biorelevant multi-compartmental models was performed to determine luminal drug release profiles from extended release (ER) dosage forms in both fasted and fed state. These methods captured 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 characterization of GI fluids performed in humans during the OrBiTo project **(**43-47).

Development, optimization and validation of models to predict GI supersaturation/precipitation and intraluminal concentrations after administration of weak bases and/or enabling formulations of low solubility drugs including the development of a new model, the biorelevant GI transfer system (BioGIT) **(**48-56).

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 **(**57-60).

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 **(**61).

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), (62,63).

Improved understanding of the multicompartmental TIM-1 model, including its predictive ability for human performance of different drugs and dosage forms, under fasted and fed state conditions, and an evaluation/comparison with a newer simplified version (Tiny TIM), (64,65).

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 (66-68).

Biorelevant methods using a compendial paddle apparatus were consolidated from methods already used by OrBiTo partners and evaluated in a set of interlaboratory studies on two poorly soluble weak acids to establish method robustness and identify sources of variability. A dual media method, which utilizes a media addition step for gastric to intestinal transfer, is likely to be particularly useful in the future as a standardized, robust biorelevant method for industry to use. Subsequent work evaluated a poorly soluble weak base in the dual media method **(**70,71).

A more comprehensive review of WP2 work can be found elsewhere (72)

WP3: *In vivo* tools and Systems characterization & understanding

The main objectives in WP3 can be summarized as follows;

- The collection of *in vivo* data derived from animal and human trials focused on physiological characterization of the gastrointestinal tract including regional absorption and availability of GI fluids. The data collection had a particular emphasis on compounds and formulation performance.
- Mechanistic experiments to improve understanding of the GI system with relevance to formulation processing and drug dissolution and absorption.
- Measurement of *in vivo* dissolution and comparison with *in vitro* methods to obtain an improved understanding of biopharmaceutical 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, 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 behavior is the level of biorelevance needed for such predictive models. While *in vitro* and *in vivo* tools at the start of OrBiTo included some biorelevant aspects, failure in predictions especially for drugs/drug products with challenging properties like low solubility/permeability drugs or modified release formulations clearly indicated that an improved understanding of the *in vivo* system was needed. Within OrBiTo that aspect has been addressed by more than 20 mechanistic studies in man using intubations for drug administrations and sampling, 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 have been sampled and characterized from distal regions in the GI tract.
- Investigations to determine the basic physiological characteristics for improved understanding of GI conditions at time of drug dosing such as motility patterns, pressure, temperatures and volumes.

- Regional permeation of drugs with different physico-chemical and BCS properties has been characterized.
- Drug dissolution and precipitation in the GI tract by sampling of GI fluids and simultaneous monitoring of pharmacokinetics in plasma.
- Targeted and global proteomics of drug metabolising enzymes and transport proteins in human gut wall (jejunum and ileum).

Traditionally *in vivo* assessment of drug absorption is performed based on drug plasma concentration- time profiles. However, such data are the result of a multifactorial chain of pharmacokinetic processes which can make it very difficult to distinguish the influence of the many different factors involved in the drug absorption process. Therefore, the latter type of studies with direct measurement of drug concentrations and the physical drug forms in the GI tract have provided the community with novel *in vivo* data allowing more precise and mechanistically insightful assessment of drug absoprtion (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 animal *in vivo* studies with *in vitro* and *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 a more rational selection of the most valuable animal models for a given API or dosage form and would ensure successful translation to human. Biopharmaceutical decision trees available in four EFPIA partners were discussed by seven companies of which 3 had no decision tree currently defined. The strengths, weaknesses and opportunities for improvement were summarised and offer a perspective on the use of *in vivo* models in biopharmaceutic decision trees for development of new oral drug products (73).

A non-comprehensive review of selected key findings from the *in vivo* work are given below.

The sampling of fluids from the ileo-caecal region in man 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 **(**74). This reduction in solubilization capacity occurs already in the terminal ileum, a region of the small intestine where particulate material can stay for a significant part of the small intestinal transit. Thus, the use of simulated intestinal fluids with jejunal bile acid levels are typically only relevant for a time period of 1 – 2 hours.

The gastric conditions after a standardized high fat meal (75) used in food interaction studies was characterized for the first time (76). Interestingly the fed state remained in the stomach in all subjects for more than six hours which has clear implications for future dosing regimens for drugs requiring fasting conditions

Another very interesting finding was obtained in the high fat meal study mentioned above (76). 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 diclofenac, 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 **(**77). However, when reaching the small intestine, the precipitated drug particles were relatively rapidly re-dissolved and complete absorption of the dose was obtained. An additional important 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 (78). A number of similar studies was performed with other drugs also including several solubility enhancing formulation principles (65, 79-86) providing an excellent basis for validation of predictive *in vitro* and *in silico* tools.

In vitro dissolution testing in up to 40% ethanol has been introduced as a regulatory requirement for extended-release formulations in the later years. A study was performed in healthy volunteers to determine the actual levels of ethanol in the stomach and small intestine in fasted and fed state after intake of beer, wine or whiskey. As expected, the highest level was achieved in the fasting stomach after a double whiskey (80 ml). Still the average ethanol concentrations barely exceeded 10 % at max concentrations and it was almost back to zero within one hour (87, 88). Thus, requirements of 2 hours *in vitro* testing in 40 % ethanol seems to be a stress test beyond reasonable relevance at least for patients not being on co-medications strongly influencing gastric emptying.

The relationship between drug permeability in the human jejunum and different pre-clinical *in vivo* models, e.g. rat intestinal single-pass perfusion, *in vitro* cell mono-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 **(**89-94).

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 diabetes (95). Another factor is the bile acid concentration in the gut which determines solubilization of low solubility drugs (96).
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 creates gastric pressure events, prompting consistent rapid gastric emptying and drug absorption (exemplified with paracetamol) **(**97). 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 the University of Leuven 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 like surfactants was assessed in *ex vivo* and *in vivo* animal models (98, 99). The investigations showed clear concentration and transit time dependence. It is clear that effect of excipients 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.

The levels of proteins for a a of drug metabolizing enzymes and transporter proteins in the human intestine were quantified using QconCAT proteomics technology. Relative mRNA expression for drug transporters which also had been measured did not correlate with the abundance of their cognate protein except for P-gp and OST-α, highlighting the limitations of RNA as a surrogate for protein expression in dynamic tissues with high turnover. Significant inter-correlations were found within CYP (2C9–2C19, 2C9–2J2, 2D6–2J2) and UGT (1A1–2B7) family of enzymes. There were also correlations between P-gp and several other proteins (OST-α, UGT1A6, and CYP3A4). Incorporating such correlations into building virtual populations within PBPK models is crucial for obtaining plausible characteristics of simulated individuals (manuscript in preparation).

The soft Bioperm™ intubation method, a well-established tool for investigation of permeability, absorption, metabolism, and drug interactions at predefined locations in the gastrointestinal tract, was modified to include pump-controlled infusion of pharmaceutical suspensions as well as simultaneous pH monitoring in the upper small intestine (78). This technique was used in a proof of concept study in healthy human volunteers. A comparison of three different ibuprofen drug products, one solution and two suspensions with different particle size distributions, as well as two different infusion rates simulating different gastric emptying rates was performed. A thorough kinetic analysis of plasma-concentration time data revealed that the dissolution of both suspensions was similar and ibuprofen input from suspensions was slightly slower compared with the solution dosage form. This knowledge about the similarity/differences of *in vivo* dissolution of suspensions with different particle sizes is important, since it permits higher flexibility in setting particle size specifications when formulating products with a particular API. Finally, based on the *in vivo* study, an *in vitro* dissolution method for ibuprofen suspensions was developed allowing estimation of the *in vivo* dissolution (to be published).

The subject of *in vitro / in vivo* correlations (IVIVC/IVIVR) was also addressed in a survey among 13 EFPIA companies and 3 Regulatory Agencies in order to capture the perspectives and experiences of industry scientists and agency members on this particular drug product development subject (100). The majority of the companies acknowledged the importance of

IVIVC/IVIVR throughout the drug development stages and a well-balanced rate of return on investment. Successful models mainly served to support formulation development and to provide a better mechanistic understanding of the *in vivo* fate of dosage forms. As a result from the survey, the IVIVC/IVIVR approach seemed to be still underutilized in regulatory submissions but, the responses from both industry and agencies indicated that there might be a chance for improved regulatory framework to guide the application of traditional and novel approaches towards IVIVC and biowaivers, such as inclusion of safe-space IVIVRs as well as the use of physiologically based modelling in the field of IVIVC. The relevance of IVIVC/IVIVR for oral IR drug products was recognized by most of the companies.

Within WP3 a working group consisting of seven EFPIA partners elaborated on the role of *in vivo* preclinical animal models and biopharmaceutic decision trees and their value for the development of new oral drug products. Key physicochemical and biopharmaceutic substance properties, the need for their *in vivo* assessment and the necessity for developing enabling drug formulations for the marketed drug product were intensely discussed with examples from in-house databases from EFPIA companies as reference. It was concluded that the *in vivo* performance of a particular drug substance and its formulation in First in human (FIH) studies cannot always be well predicted from *in vitro* and/or *in silico* tools alone at the time of selection of a new chemical entity (NCE). Nevertheless, a generalized decision approach starts with analyzing the physicochemical features of the drug substance like solid state and solubility parameters. In a next step, a relation between solubility and dose (DN) as well as a first exploratory bioavailability study via the targeted application route in animals come into play but may be omitted in case of biopharmaceutic non-challenging development compounds, such as those with high solubility / high permeability. It was concluded that a combination of physicochemical, computational, *in vitro* and *in vivo* tools may sometimes be necessary to decide whether a conventional or an enabling formulation should be developed (73).

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 4).

- Database creation and population with novel EFPIA data
- Initial gap analysis of the pre-existing in-silico tools and processes in the bottom-up anticipation of human pharmacokinetics
- Changes to the in-silico models and procedures
- Test of the various improvements
- Creation of a refined Developability Classification System
- Suggestions for best practices related to integration of modelling and simulation to the project team activities.

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 design of the database and the content of the database has been published in two separate papers (101,102).

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. 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 5).

The clinical data contained in the OrBiTo database is in compliance with the GDPR (https://eugdpr.org/) since all individual data are anonymized. Interestingly the design of the database also in most aspects are in accordance with the recently published FAIR principles outlining best practice of data management (103).

As of August 2018, 97 APIs were present in the database, 512 preclinical or clinical studies covering 1638 different administration conditions.

The API physico-chemical and biological data in conjunction with formulation information and human and animal PK data in this database were mainly used to establish how current PBPK absorption modelling tools perform in a blinded exercise using minimal guidance for modellers and, following model improvements and standardization of model inputs in a subsequent study. The selection of compounds and associated formulations allowed the OrBiTo team to probe certain model gaps and evaluate model performance with a variety of drug substances and formulations.

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 with the aim to identify the weaknesses and strengths of these modelling platforms/approaches and then 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. 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. In this case, 58 APIs, including the original 43 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, modelers were asked to define strategies for the calculation and selection of input parameters for allocated APIs and software. These strategies were discussed with an expert panel in order to harmonize the inputs between modellers and across the software. The outputs from the strategy discussions were used as one source for 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. 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 are summarized in two manuscripts (in preparation) but the chief findings are summarized below:

Around half of the simulations were within the 2-fold error for AUC0-t_{last} and around 90% of the simulations were within 10-fold error for AUC0-t_{last}. Oral bioavailability (F) predictions, which were limited to 19 APIs having intravenous (i.v.) data, showed average fold error of values 1.37y. Across different APIs, and when compared across different formulations and routes of administration, AUC0-t_{last} for oral controlled release and i.v. administration were better predicted than that for oral immediate release formulations due to higher variability and complexity in the first-pass processes for the latter. Average predictive performance did not clearly differ between software packages but some APIs showed a high level of variability in predictive performance across different software packages. This variability could be related to many factors such as compound specific properties, the quality and availability of information, and errors in scaling from *in vitro* and preclinical *in vivo* data to human *in vivo* behavior which will be explored further. The results are explained in more detail in two companion papers (104,105).

In silico model modifications and improvements

Several modifications were made to *in silico* models primarily based on findings of WP1, WP2 and WP3 and include the following;

- Ways of calculating drug permeability based on mechanistic models were defined (106, 107), and the way we integrate precipitation of drug substances and products in PBPK was extensively studied (48, 108). 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 (54,56).
- The way in which dissolution is integrated in PBPK and the mechanistic interpretation of dissolution has progressed during OrBiTo (100, 109). The consideration of fluid hydrodynamics for immediate release (38) 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 (46, 110).
- Changes to how we model gastro intestinal transit (111), gastric emptying, pH variability in the stomach and the amount of bile in the small intestine were also progressed during OrBiTo. These changes will allow more patient centric *in silico* approaches, better disease models (112) or evaluation of drug interactions (113) and improved evaluation of human variability.
- The way in which drug solubility is calculated in complex biorelevant media was modified to incorporate the contribution of the ionized and unionized drug species.
- Modelling of gastric emptying and reaction of gallbladder following different type of food ingestion (95, 96).
- Strategy to integrate dissolution in PBPK models in a more mechanistic way than was achieved before (109, 114).
- Modelling specific patient populations such as patients undergoing bariatric surgery (115).
- SOPs and guidance related to inputting data into models and modelling procedures.
- The development of refined Developability Classification system (116) and its application to an industrial database (117).

Impact of OrBiTo deliverables

OrBiTo has developed a broad toolbox for the prediction of oral drug absorption with an emphasis on the impact of drug form and formulation. The future use and further development 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. The industry partners have been strongly involved in the research work throughout the project, which has facilitated implementation of OrBiTo output into industrial practice. At the end of the project, we have gathered more than 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. Furthermore, the strong involvement of industry 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:

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

With respect to 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 repetition 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 the opportunity for patient-centric product design that optimizes 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 the influence of food, concomitant medications, disease state on drug actions and thereby reduce variability in patient response.

OrBiTo has also significantly enriched the scientific knowledge in the oral biopharmaceutics area as reflected by a total output of about 150 papers in peer-reviewed scientific journals. This is expected to raise to a final total of about 170 papers and an updated list of publications can be found on www.orbitoproject.eu.

We can also foresee that the OrBiTo output may have an impact on future regulatory guidelines in the biopharmaceutics area, especially in context of replacing *in vivo* clinical studies with predictive tools. OrBiTo has published a conference paper with participation from leading scientists at EMA and FDA on future use of biopharmaceutics predictive tools in a regulatory context (118). One particular emerging area that has gained regulatory traction during the years of OrBiTo is the use of integrated *in silico* absorption models in combination with high quality biorelevant *in vitro* data to justify product specifications or

formulation/process changes. Pilot case examples of such regulatory applications has been shared by partners in the project (114, 119). Translational modeling strategies to support drug product development, manufacturing changes and controls in a regulatory context have also more recently been addressed in workshops 2017 (120) and 2019 (https://cersi.umd.edu/event/14385/current-state-and-future-expectations-of-translational-modeling-strategies-to-support-drug-product) organized by University of Maryland together with the U.S., Food and Drug Administration.

Future research opportunities

We envisage that a long-term goal in the future, drug product development will use approaches commonly deployed in other industries such as aerospace and automotive, where development is mainly performed in a virtual setting, informed by predictions and only verified by physical tests at critical stages. Future regulatory implementation of the predictive tools will also require more formal scientific qualifications for example to fully implement virtual bioequivalence trials (120). Example of such topics include drug interactions with food and other drugs with influence on absorption factors, establishing drug particle size and dissolution limits, bioequivalence studies for low solubility drugs (BCS II), special population, eg paediatric bioavailability studies. Thus, further research will be needed on the predictive tools to fully realise this potential.

A related aspect is the huge interest in the pharmaceutical industry on utilization of “big data” and artificial intelligence. This is also likely to play a role in future biopharmaceutics *in vivo* predictions. One opportunity brought forward by OrBiTo project in this context is the OrBiTo database with historical *in vivo* data that will be maintained after the end of the project. These data will be accessed for further research and publication purposes to improve our understanding of the biopharmaceutics models. Data content will continue to evolve, and new fields and metadata will be added in the future.

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 patient factors (95), this remains an extensive research field within oral drug absorption 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 of higher molecular weight and not suitable for oral delivery. Historically this has predominantly 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 targeted delivery. There is therefore 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 rudimental compared to the oral area. For these new modalities, fundamental biopharmaceutical 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 via conventional 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 (121).

Conclusion

OrBiTo has significantly increased the understanding of the GI drug absorption process by successful completion of more than 20 mechanistic studies in man and created at least 16 new or refined *in vitro* methods. A similar number of improvements have been developed for *in silico* tools. OrBiTo has also delivered a database framework for biopharmaceutics data storage populated with historical data from industry partners. This has also included significant standardization and validation work. Findings from the project have been the basis for a framework for the optimal use of predictive tools and preclinical models delivered as decision trees or practical guidance papers in all areas covered by OrBiTo.

OrBiTo has thereby contributed to a step change in model informed drug product development in biopharmaceutics area by significantly improving the use of, and confidence in, *in vivo* predictive *in vitro/in silico* tools. *In vivo* predictive *in vitro* tools and modelling in combination are the core tools during development and *in vivo* studies can be limited to verify outcome or validate models at critical stages. Further research on predictive tools will however be needed for modelled based pharmaceutical development to reach its full potential.

Acknowledgments

To Åsa Mannervik for outstanding administrative support.

One of the co-authors, Anders Lindahl is an employee of Swedish Medical Products Agency. The views expressed in this manuscript should however not be understood or quoted as being made on behalf of or reflecting the position of the Swedish Medical Products Agency .

This work has received support from the Innovative Medicines Initiative Joint Undertaking (http://www.imi.europa.eu) under Grant Agreement No. 115369, resources of which are composed of financial contribution from the European Union's Seventh Framework Program and EFPIA companies' in kind contribution.

FIGURES legends

1. OrBiTo partner organisations
2. OrBiTo research strategy
3. Oral drug absorption
4. Overview of WP4 work flow
5. The OrBiTo database structure

Reference list

1) Lennernäs, H., Aarons, L., Augustijnsc, P., Beatod, S., Bolgere, M., Box, K., Brewster, M., Butler, J., Dressman, J., Holm, R., Julia Frank, K., Kendall R., Langguth, P., Sydor, J., Lindahl, A., McAllister, M., Muenster, U., Müllertzr, A., Abrahamsson, B., 2014. Oral biopharmaceutics tools – Time for a new initiative – An introduction to the IMI project OrBiTo. Eur J Pharm Sci. 57:292-9.
2) Rowland, M., Tozer, T. N., 1995. Clinical Pharmacokinetics: Concepts and Applications; 3rd ed., Williams & Wilkins: Philadelphia, chapter 9, pp 119-134.
3) Bønløkke, L., Hovgaard, L., Kristensen, H.G., Knutson, L., Lennernäs, H., 2001. Direct estimation of the in vivo dissolution of spironolactone, in two particle size ranges, using the single-pass perfusion technique (Loc-I-Gut) in humans. Eur. J. Pharm. Sci. 12, 239-50.
4) Flanagan, T., 2019. Potential for pharmaceutical excipients to impact absorption: A mechanistic review for BCS Class 1 and 3 drugs. Eur J Pharm Biopharm. 141:130-138.
5) Maher, S., Ryan, B., Duffy, A., Brayden, D.J., 2014. Formulation strategies to improve oral peptide delivery. Pharm Pat Anal. 3:313-36.
6) Williams H.D., Trevaskis N.L., Charman, S.A., Shanker, R.M., Charman, W.N., Pouton C.W., Porter C.J., 2013. Strategies to address low drug solubility in discovery and development. Pharmacol Rev. 65, 315-499.
7) Hidalgo, I.J., Raub, T.J., Borchardt, R.T. 1989. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology. 96:736-49.
8) Artursson, P., 1990. Epithelial transport of drugs in cell culture. I: A model for studying the passive diffusion of drugs over intestinal absorptive (Caco-2) cells. J. Pharm. Sci. 79:476-482.
9) Dressman, J.B., Amidon, G.L., Reppas, C., Shah, V.P., 1998. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. Pharm. Res. 15, 11-22.
10) Amidon, G. L., Lennernas, H., Shah, V. P., Crison, J. R., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res, 12, 413-420.
11) Agoram, B., Woltosz, W.S., Bolger, M.B., 2001. Predicting the impact of physiological and biochemical processes on oral drug bioavailability. Adv Drug Deliv Rev. 50 Suppl 1, S41-67.
12) Larregieu, C.A., Benet, L.Z., 2013. Drug discovery and regulatory considerations for improving in silico and in vitro predictions that use Caco-2 as a surrogate for human intestinal permeability measurements. AAPS. J., 15, 483-97.
13) Bergström, C.A.S., Holm, R., Jørgensen, S.A., Andersson, S.B., Artursson, P., Beato, S., Borde, A., Box, K., Brewster, M., Dressman, J., Feng, K.I., Halbert, G., Kostewicz, E., McAllister, M., Muenster, U., Thinnes, J., Taylor, R., Mullertz, A., 2014. Early

pharmaceutical profiling to predict oral drug absorption: current status and unmet needs. Eur J Pharm Sci. 57:173-99.

14) Kostewicz, E.S., Abrahamsson, B., Brewster, M., Brouwers, J., Butler, J., Carlert, S., Dickinson, P.A., Dressman, J., Holm, R., Klein, S., Mann, J., McAllister, M., Minekus, M., Muenster, U., Müllertz, A., Verwei, M., Vertzoni, M., Weitschies, W., Augustijns, P., 2014. In vitro models for the prediction of in vivo performance of oral dosage forms. Eur J Pharm Sci. 57:342-66.

15) Sjögren, E., Abrahamsson, B., Augustijns, P., Becker, D., Bolger, M.B., Brewster, M., Brouwers, J., Flanagan, T., Harwood, M., Heinen, C., Holm, R., Juretschke, H.P., Kubbinga, M., Lindahl, A., Lukacova, V., Münster, U., Neuhoff, S., Nguyen, M.A., V., Reppas, C., Hodjegan, A.R., Tannergren, C., Weitschies, W., Wilson, C., Zane, P., Lennernäs, H., Langguth, P., 2014. In vivo methods for drug absorption - comparative physiologies, model selection, correlations with in vitro methods (IVIVC), and applications for formulation/API/excipient characterization including food effects.Eur J Pharm Sci. 57:99-151.

16) Kostewicz E.S., Aarons L., Bergstrand M., Bolger M.B., Galetin A., Hatley O., Jamei M., Lloyd R., Pepin X., Rostami-Hodjegan A., Sjögren E., Tannergren C., Turner D.B., Wagner C., Weitschies W., Dressman J., 2014.PBPK models for the prediction of in vivo performance of oral dosage forms.Eur J Pharm Sci. 57:300-21.

17) Andersson, S.B.E., Alvebratt, C., Bevernage, J., Bonneau, D., da Costa Mathews, C., Dattani, R., Edueng, K., He, Y., Holm, R., Madsen, C., Müller, T., Muenster, U., Müllertz, A., Ojala, K., Rades, T., Sieger, P., Bergström, C.A.S., 2016. Interlaboratory Validation of Small-Scale Solubility and Dissolution Measurements of Poorly Water-Soluble Drugs. J Pharm Sci. 105:2864-2872.

18) Khadra, I., Zhou, Z., Dunn, C., Wilson, C.G., Halbert, G., 2015. Statistical investigation of simulated intestinal fluid composition on the equilibrium solubility of biopharmaceutics classification system class II drugs. Eur J Pharm Sci. 67:65-75.

19) Zhou, Z., Dunn, C., Khadra, I., Wilson, C.G., Halbert, G.W., 2017. Statistical investigation of simulated fed intestinal media composition on the equilibrium solubility of oral drugs. Eur J Pharm Sci. 99:95-104.

20) Perrier, J., Zhou, Z., Dunn, C., Khadra, I., Wilson, C.G., Halbert, G., 2018. Statistical investigation of the full concentration range of fasted and fed simulated intestinal fluid on the equilibrium solubility of oral drugs. Eur J Pharm Sci. 111:247-256.

21) Ainousah, B.E., Perrier, J., Dunn, C., Khadra, I., Wilson, C.G., Halbert, G., 2017. Dual Level Statistical Investigation of Equilibrium Solubility in Simulated Fasted and Fed Intestinal Fluid. Mol Pharm. 14:4170-4180.

22) Madsen, C.M., Feng, K.I., Leithead, A., Canfield, N., Jørgensen, S.A., Müllertz, A., Rades, T., 2018. Effect of composition of simulated intestinal media on the solubility of poorly soluble compounds investigated by design of experiments. Eur J Pharm Sci. 111:311-319.

23) Dunn C, Perrier J, Khadra I, Wilson CG, Halbert GW. 2019. Topography of Simulated Intestinal Equilibrium Solubility. Mol Pharm.16:1890-1905.

24) Andersson, S.B.E., Alvebratt, C., Bevernage, J., Bonneau, D., da Costa Mathews, C., Dattani, R., Edueng, K., He, Y., Holm, R., Madsen, C., Müller, T., Muenster, U., Müllertz, A., Ojala, K., Rades, T., Sieger, P., Bergström, C.A.S., 2016. Interlaboratory Validation of Small-Scale Solubility and Dissolution Measurements of Poorly Water-Soluble Drugs. J Pharm Sci. 105:2864-2872.
25) Andersson, S.B.E., Alvebratt, C., Bergström, C.A.S., 2017. Controlled Suspensions Enable Rapid Determinations of Intrinsic Dissolution Rate and Apparent Solubility of Poorly Water-Soluble Compounds. Pharm Res. 34:1805-1816.
26) Christfort, J.F., Plum, J., Madsen, C.M., Nielsen, L.H., Sandau, M., Andersen, K., Müllertz, A., Rades, T., 2017. Development of a Video-Microscopic Tool To Evaluate the Precipitation Kinetics of Poorly Water Soluble Drugs: A Case Study with Tadalafil and HPMC. Mol Pharm. 14:4154-4160.
27) Holmboe, M., Larsson, P., Anwar, J., Bergström, C.A., 2016. Partitioning into Colloidal Structures of Fasted State Intestinal Fluid Studied by Molecular Dynamics Simulations. Langmuir. 32:12732-12740.
28) Larsson, P., Alskär, L.C., Bergström, C.A.S., 2017. Molecular Structuring and Phase Transition of Lipid-Based Formulations upon Water Dispersion: A Coarse-Grained Molecular Dynamics Simulation Approach. Mol Pharm 14:4145-4153.
29) Andreas, C.J., Rosenberger, J., Butler, J., Augustijns, P., McAllister, M., Abrahamsson, B., Dressman, J., 2018. Introduction to the OrBiTo decision tree to select the most appropriate in vitro methodology for release testing of solid oral dosage forms during development. Eur J Pharm Biopharm. 130:207-213.
30) Garbacz, G., Rappen, G.M., Koziolek, M., Weitschies, W., 2015. Dissolution of mesalazine modified release tablets under standard and bio-relevant test conditions. J Pharm Pharmacol. 67:199-208.
31) Schneider, F., Beeck, R., Hoppe, M., Koziolek, M., Weitschies, W., 2017. In vitro simulation of realistic gastric pressure profiles. Eur J Pharm Sci. 107:71-77.
32) Sager, M., Schneider, F., Jedamzik, P., Wiedmann, M., Schremmer, E., Koziolek, M., Weitschies, W., 2017. Effect of Coadministered Water on the In Vivo Performance of Oral Formulations Containing N-Acetylcysteine: An In Vitro Approach Using the Dynamic Open Flow-Through Test Apparatus. Mol Pharm. 14:4272-4280.
33) Schneider, F., Hoppe, M., Koziolek, M., Weitschies, W., 2018. Influence of Postprandial Intragastric Pressures on Drug Release from Gastroretentive Dosage Forms. AAPS PharmSciTech. 19:2843-2850.
34) Schick, P., Sager, M., Wegner, F., Wiedmann, M., Schapperer, E., Weitschies, W., Koziolek, M., 2019. Application of the GastroDuo as an in Vitro Dissolution Tool To Simulate the Gastric Emptying of the Postprandial Stomach. Mol Pharm. 16:4651-4660.
35) Sager, M., Jedamzik, P., Mischek, M., Koziolek, M., Weitschies, W., 2019. Comparison of *in vitro* data from the GastroDuo with *in vivo* results obtained with the

salivary tracer technique: Fasted state administration of immediate release dosage forms (article in preparation 2019).

36) Jedamzik, J., Sager, M., Koziolek, M., Weitschies, W. The rapid onset of plasma concentrations after oral administration of a novel Aspirin® formulation in the fed state can be explained by the presence of the Magenstraße (article in preparation 2019).

37) Heinen, C.A., Reuss, S., Amidon, G.L., Langguth, P., 2013. Ion pairing with bile salts modulates intestinal permeability and contributes to food-drug interaction of BCS class III compound trospium chloride. Mol Pharm. 10:3989-96.

38) Cvijić, S., Parojčić, J., Langguth, P., 2014. Viscosity-mediated negative food effect on oral absorption of poorly-permeable drugs with an absorption window in the proximal intestine: In vitro experimental simulation and computational verification. Eur J Pharm Sci. 61:40-53.

39) Cvijić, S., Langguth, P., 2014. Improvement of trospium-specific absorption models for fasted and fed states in humans. Biopharm Drug Dispos. 35:553-8.

40) Jedamzik, J., Sager, M., Koziolek, M., Weitschies, W. The rapid onset of plasma concentrations after oral administration of a novel Aspirin® formulation in the fed state can be explained by the presence of the Magenstraße (article in preparation 2019).

41) Markopoulos, C., Vertzoni, M., Symillides, M., Kesisoglou, F., Reppas, C., 2015. Two-Stage Single-Compartment Models to Evaluate Dissolution in the Lower Intestine. J Pharm Sci. 104: 2986-97.

42) Georgaka, D., Butler, J., Kesisoglou, F., Reppas, C., Vertzoni, M., 2017. Evaluation of Dissolution in the Lower Intestine and Its Impact on the Absorption Process of High Dose Low Solubility Drugs. Mol Pharm. 14:4181-4191.

43) Markopoulos, C., Andreas, C.J., Vertzoni, M., Dressman, J., Reppas, C., 2015. In-vitro simulation of luminal conditions for evaluation of performance of oral drug products: Choosing the appropriate test media. Eur J Pharm Biopharm. 93:173-82.

44) Andreas, C.J., Chen, Y.C., Markopoulos, C., Reppas, C., Dressman, J., 2015. In vitro biorelevant models for evaluating modified release mesalamine products to forecast the effect of formulation and meal intake on drug release. Eur J Pharm Biopharm. 97:39-50.

45) Andreas, C.J., Tomaszewska, I., Muenster, U., van der Mey, D., Mueck, W., Dressman, J.B., 2016. Can dosage form-dependent food effects be predicted using biorelevant dissolution tests? Case example extended release nifedipine. Eur J Pharm Biopharm. 105:193-202.

46) Andreas, C.J., Pepin, X., Markopoulos, C., Vertzoni, M., Reppas, C., Dressman, J.B., 2017. Mechanistic investigation of the negative food effect of modified release zolpidem. Eur J Pharm Sci. 102:284-298.

47) Schilderink, R., Protopappa, M., Fleth-James, J., Vertzoni, M., Frank, K.J., Havenaar, R., Wobst, I., Metzger, M., Reppas, C. Usefulness of compendial setups (USP apparatus II and IV) and tiny-TIM system in the evaluation of the performance of

orally administered modified release drug products: Case example sodium salt of A6197 *(article in preparation 2019*).

48) Hens, B., Brouwers, J., Anneveld, B., Corsetti, M., Symillides, M., Vertzoni, M., Reppas, C., Turner, D.B., Augustijns, P., 2014. Gastrointestinal transfer: In vivo evaluation and implementation in in vitro and in silico predictive tools. Eur. J. Pharm. Sci. 63:233-42.

49) Kourentas, A., Vertzoni, M., Stavrinoudakis, N., Symillidis, A., Brouwers J., Augustijns P., Reppas, C., Symillides, M., 2016. An in vitro biorelevant gastrointestinal transfer (BioGIT) system for forecasting concentrations in the fasted upper small intestine: Design, implementation, and evaluation. Eur. J. Pharm. Sci. 82:106-14.

50) Kourentas, A., Vertzoni, M., Khadra, I., Symillides, M., Clark, H., Halbert, G., Butler, J., Reppas, C., 2016. Evaluation of the impact of excipients and an albendazole salt on albendazole concentrations in upper small intestine using an in vitro biorelevant gastrointestinal transfer (BioGIT) System. J. Pharm. Sci. 105:2896-2903.

51) Kourentas, A., Vertzoni, M., Symillides, M., Hens, B., Brouwers, J., Augustijns, P., Reppas, C, 2016. In vitro evaluation of the impact of gastrointestinal transfer on luminal performance of commercially available products of posaconazole and itraconazole using BioGIT. Int. J. Pharm. 515:352-358.

52) Kourentas, A., Vertzoni, M., Barmpatsalou, V., Augustijns, P., Beato, S., Butler, J., Holm, R., Ouwerkerk, N., Rosenberg, J., Tajiri, T., Tannergren, C., Symillides, M., Reppas, C., 2018. The BioGIT System: a valuable in vitro tool to assess the impact of dose and formulation on early exposure to low solubility drugs after oral administration. AAPS J. 20:71.

53) Kesisoglou, F., Vertzoni, M., Reppas, C., 2018. Physiologically based absorption modeling of salts of weak bases based on data in hypochlorhydric and achlorhydric biorelevant media. AAPS PharmSciTech. 19: 2851-2858.

54) Ruff, A., Fiolka, T., Kostewicz, E., 2017. Prediction of Ketoconazole absorption using an updated in vitro transfer model coupled to physiologically based pharmacokinetic modelling. European Journal of Pharmaceutical Sciences. 100: 42-55.

55) Ruff, A., Holm, R., Kostewicz, E., 2017. Evaluating the predictability of the in vitro transfer model and in vivo rat studies as a surrogate to investigate the supersaturation and precipitation behaviour of different Albendazole formulations for humans. European Journal of Pharmaceutical Sciences. 105:108-118.

56) Pathak, S., Ruff, A., Kostewicz, E., Patel, N., Turner, D., Jamei, M., 2017. Model-based Analysis of Biopharmaceutical Experiments to Improve Mechanistic Oral Absorption Modelling - An Integrated in vitro in Vivo Extrapolation (IVIV_E) Perspective using Ketoconazole as a Model Drug. Molecular Pharmaceutics. 14:4305-4320.

57) Forner, K., Holm, R., Morakul, B., Junyprasert, V., Ackermann, M., Mazur, J., Langguth, P., 2017a. Dissolution and dissolution/permeation experiments for

predicting systemic exposure following oral administration of the BCS class II drug clarithromycin. Eur. J. Pharm Sci 101:211-219.

58) Forner, K., Hidalgo, I., Lin, J., Ackermann, M., Langguth, P., 2017b. Dissolution/permeation: The importance of the experimental setup for the prediction of formulation effects on fenofibrate in vivo performance. Pharmazie 72:581-586.

59) Berben, P., Brouwers, J. P., Augustijns, P., 2018. Assessment of Passive Intestinal Permeability Using an Artificial Membrane Insert System. J Pharm Sci. 107:250-256.

60) Berben, P., Brouwers, J.P., Augustijns, P., 2018. The artificial membrane insert system as predictive tool for formulation performance evaluation. Int J Pharm. 537:22-29

61) Sassene, P.J., Fanø, Mu. H, Rades, T., Aquistapace, S., Schmitt, B., Cruz-Hernandez, C., Wooster, T.J., Müllertz, A., 2016. Comparison of lipases for in vitro models of gastric digestion: Lipolysis using two infant formulas as model substrates. Food Funct. 7:3989-3998.

62) Khoshakhlagh, P., Johnson, R., Langguth, P., Nawroth, T., Schmueser, L., Hellmann, N., Decker, H., Szekely, N.K., 2015. Fasted-state simulated intestinal fluid "FaSSIF-C", a cholesterol containing intestinal model medium for in vitro drug delivery development. J Pharm Sci. 104:2213-24.

63) Nawroth, T., Buch, P., Buch, K., Langguth, P., Schweins, R., 2011. Liposome formation from bile salt-lipid micelles in the digestion and drug delivery model FaSSIF(mod) estimated by combined time-resolved neutron and dynamic light scattering. Mol Pharm. 8:2162-72.

64) Verwei, M., Minekus, E., Zeijdner, R., Schilderink, R., Havenaar, 2016. Evaluation of two dynamic in vitro models simulating fasted and fed state conditions in the upper gastrointestinal tract (TIM-1 and tiny-TIM) for investigating the bioaccessibility of pharmaceutical compounds from oral dosage forms. Int J Pharm. 498:178-86.

65) Van Den Abeele, J., Brouwers, J., Tack, J., Augustijns, P., 2017a. Exploring the link between gastric motility and intragastric drug distribution in man. Eur J Pharm Biopharm. 112:75-84.

66) Al-Gousous, J., Amidon, G.L., Langguth, P., 2016. Toward Biopredictive Dissolution for Enteric Coated Dosage Forms. Mol. Pharm. 13:1927-36.

67) Al-Gousous, J., Tsume, Y., Fu, M., Salem, I.I., Langguth, P., 2017. Unpredictable Performance of pH-Dependent Coatings Accentuates the Need for Improved Predictive in Vitro Test Systems. Mol. Pharm. 14:4209-4219.

68) Kindgen, S., Wachtel, H., Abrahamsson, B., Langguth, P., 2015. Computational Fluid Dynamics Simulation of Hydrodynamics and Stresses in the PhEur/USP Disintegration Tester Under Fed and Fasted Fluid Characteristics. J Pharm Sci. 104:2956-68.

69) Kindgen, S., Rach, R., Nawroth, T., Abrahamsson, B., Langguth, P., 2016. A Novel Disintegration Tester for Solid Dosage Forms Enabling Adjustable Hydrodynamics. J Pharm Sci. 105:2402-9.

70) Mann, J., Dressman, J., Rosenblatt, K., Ashworth, L., Muenster, U., Frank, K., Hutchins, P., Williams, J., Klumpp, L., Wielockx, K., Berben, P., Augustijns, P., Holm, R., Hofmann, M., Patel, S., Beato, S., Ojala, K., Tomaszewska, I., Bruel, J.L., Butler, J., 2017. Validation of dissolution testing with biorelevant media: an OrBiTo study. Mol Pharm. 14:4192-4201.

71) Berben P, Ashworth L, Beato S, Bevernage J, Bruel JL, Butler J, Dressman J, Schäfer K, Hutchins P, Klumpp L, Mann J, Nicolai J, Ojala K, Patel S, Powell S, Rosenblatt K, Tomaszewska I, Williams J, Augustijns P. 2019. Biorelevant dissolution testing of a weak base: Interlaboratory reproducibility and investigation of parameters controlling in vitro precipitation. Eur J Pharm Biopharm. 140:141-148.

72) Butler, J., Hens, B., Vertzoni, M., Brouwers, J., Berben, P., Dressman, J., Andreas, C.J., Schaefer, K.J., Mann, J., McAllister, M., Jamei, M., Kostewicz, E., Kesisoglou, F., Langguth P., Minekus, M., Müllertz, A., Schilderink, R., Koziolek, M., Jedamzik, P., Weitschies, W., Reppas, C., Augustijns, P., 2019. In vitro models for the prediction of in vivo performance of oral dosage forms: Recent progress from partnership through the IMI OrBiTo collaboration. Eur J Pharm Biopharm. 136:70-83.

73) Zane, P., Gieschen, H., Kersten, E., Mathias, N., Ollier, C., Johansson, P., Van den Bergh, A., Van Hemelryck, S., Reichel, A., Rotgeri, A., Schäfer, K., Müllertz, A., Langguth, P., 2019. In vivo models and decision trees for formulation development in early drug development: A review of current practices and recommendations for biopharmaceutical development. Eur J Pharm Biopharm. 142:222-231.

74) Reppas, C., Karatza, E., Goumas, C., Markopoulos, C., Vertzoni, M., 2015. Characterization of Contents of Distal Ileum and Cecum to Which Drugs/Drug Products are Exposed During Bioavailability/Bioequivalence Studies in Healthy Adults. Pharm Res. 32:3338-49.

75) Guidance for Industry. Food-Effect Bioavailability and Fed Bioequivalence Studies. US Food and Drug Administration, December 2002

76) Koziolek, M., Grimm, M., Garbacz, G., Kühn, J.P., Weitschies, W., 2014. Intragastric volume changes after intake of a high-caloric, high-fat standard breakfast in healthy human subjects investigated by MRI. Mol Pharm. 11:1632-9.

77) Van Den Abeele, J., Brouwers, J., Mattheus, R., Tack, J., Augustijns, P. J., 2016. Gastrointestinal Behavior of Weakly Acidic BCS Class II Drugs in Man--Case Study of Diclofenac Potassium. Pharm Sci. 105:687-696.

78) Hofmann, M., Thieringer, F., Nguyen. M.A., Månsson, W., Galle, P.R., Langguth, P., 2019. A novel technique for intraduodenal administration of drug suspensions/solutions with concurrent pH monitoring applied to ibuprofen formulations. Eur J Pharm Biopharm. 136:192-202.

79) Brouwers, J., Augustijns, P., 2014. Resolving intraluminal drug and formulation behavior: Gastrointestinal concentration profiling in humans. Eur J Pharm Sci. 61:2-10.

80) Hens, B., Brouwers, J., Corsetti, M., Augustijns, P., 2015. Gastrointestinal behavior of nano- and microsized fenofibrate: In vivo evaluation in man and in vitro simulation by assessment of the permeation potential. Eur J Pharm Sci. 77:40-7.

81) Hens, B., Brouwers, J., Corsetti, M., Augustijns, P., 2016. Supersaturation and Precipitation of Posaconazole Upon Entry in the Upper Small Intestine in Humans. J Pharm Sci. 105:2677-2684.

82) Rubbens, J., Brouwers, J., Tack, J., Augustijns, P., 2016. Gastrointestinal dissolution, supersaturation and precipitation of the weak base indinavir in healthy volunteers. Eur J Pharm Biopharm. 109:122-129.

83) Brouwers, J., Geboers, S., Mols, R., Tack, J., Augustijns, P., 2017. Gastrointestinal behaviour of itraconazole in humans - Part 1: Supersaturation from a solid dispersion and a cyclodextrin-based solution. Int J Pharm. 525:211-217.

84) Van den Abeele, J., Schilderink, R., Schneider, F., Mols, R., Minekus, M., Weitschies, W., Brouwers, J., Tack, J., Augustijns, P., 2017. Gastrointestinal and systemic disposition of diclofenac under fasted and fed state conditions supporting the evaluation of in vitro predictive tools. Mol Pharm. 14:4220-4232.

85) Berben, P., Stappaerts, J., Vink, M.J.A., Domínguez-Vega, E., Somsen, G.W., Brouwers, J., Augustijns, P., 2018. Linking the concentrations of itraconazole and 2-hydroxypropyl-β-cyclodextrin in human intestinal fluids after oral intake of Sporanox®. Eur J Pharm Biopharm. 132:231-236.

86) Rubbens, J., Brouwers, J., Tack, J., Augustijns, P., 2019. Gastric and Duodenal Diclofenac Concentrations in Healthy Volunteers after Intake of the FDA Standard Meal: In Vivo Observations and in Vitro Explorations. Mol Pharm. 16:573-582.

87) Rubbens, J., Riethorst, D., Brouwers, J., Wolfs, K., Adams, E., Tack, J., Augustijns, P., 2017. Gastric and Duodenal Ethanol Concentrations after Intake of Alcoholic Beverages in Postprandial Conditions. Mol Pharm. 14:4202-4208.

88) Rubbens J., Riethorst D., Brouwers J., Wolfs K., Adams E., Tack J., Augustijns P., 2017.Gastric and Duodenal Ethanol Concentrations after Intake of Alcoholic Beverages in Postprandial Conditions. Mol Pharm.14:4202-4208.

89) Sjögren, E., Dahlgren, D., Roos, C., Lennernäs, H., 2015. Human in vivo regional intestinal permeability: quantitation using site-specific drug absorption data. Mol Pharm. 12:2026-39.

90) Dahlgren, D., Roos, C., Sjögren, E., Lennernäs, H., 2015. Direct In Vivo Human Intestinal Permeability (Peff) Determined with Different Clinical Perfusion and Intubation Methods. J Pharm Sci. 104:2702-26.

91) Dahlgren, D., Roos, C., Lundqvist, A., Abrahamsson, B., Tannergren, C., Hellström, P.M., Sjögren, E., Lennernäs, H., 2016. Regional Intestinal Permeability of Three Model Drugs in Human. Mol Pharm. 13:3013-21.

92) Dahlgren, D., Roos, C., Johansson, P., Lundqvist, A., Tannergren, C., Abrahamsson, B., Sjögren, E., Lennernäs, H., 2016. Regional Intestinal Permeability in Dogs: Biopharmaceutical Aspects for Development of Oral Modified-Release Dosage Forms. Mol Pharm. 13:3022-33.

93) Roos, C., Dahlgren, D., Sjögren, E., Tannergren, C., Abrahamsson, B., Lennernäs, H., 2017. Regional Intestinal Permeability in Rats: A Comparison of Methods. Mol Pharm. 14:4252-4261.

94) Forner, K., Roos, C., Dahlgren, D., Kesisoglou, F., Konerding, M.A., Mazur, J., Lennernäs, H., Langguth, P., 2017. Optimization of the Using chamber setup with excised rat intestinal segments for dissolution/permeation experiments of poorly soluble drugs. Drug Dev Ind Pharm. 43:338-346.

95) Guiastrennec, B., Sonne, D.P., Hansen, M., Bagger, J.I., Lund, A., Rehfeld, J.F., Alskär, O., Karlsson, M.O., Vilsbøll, T., Knop, F.K., Bergstrand, M., 2016. Mechanism-Based Modeling of Gastric Emptying Rate and Gallbladder Emptying in Response to Caloric Intake. CPT Pharmacometrics Syst Pharmacol. 5:692-700.

96) Guiastrennec, B., Sonne, D.P., Bergstrand, M., Vilsbøll, T., Knop, F.K., Karlsson, M.O., 2018. Model-Based Prediction of Plasma Concentration and Enterohepatic Circulation of Total Bile Acids in Humans. CPT Pharmacometrics Syst Pharmacol. 7:603-612.

97) Van Den Abeele, J., Brouwers, J., Deloose, E., Tack, J., Augustijns, P., 2017. The Effect of Sparkling Water on Intraluminal Formulation Behavior and Systemic Drug Performance. J Pharm Sci. 106:2472-2482.

98) Roos, C., Dahlgren, D., Sjögren, E., Sjöblom, M., Hedeland, M., Lennernäs, H., 2019. Effects of absorption-modifying excipients on jejunal drug absorption in simulated fasted and fed luminal conditions. Eur J Pharm Biopharm. 142:387-395.

99) Dahlgren, D., Sjöblom, M., Lennernäs, H., 2019. Intestinal absorption-modifying excipients: A current update on preclinical in vivo evaluations. Eur J Pharm Biopharm. 142:411-420.

100) Nguyen, M.A., Flanagan, T., Brewster, M., Kesisoglou, F., Beato, S., Biewenga, J., Crison, J., Holm, R., Li, R., Mannaert, E., McAllister, M., Mueller-Zsigmondy, M., Muenster, U., Ojala, K., Page, S., Parr, A., Rossenu, S., Timmins, P., Van Peer, A., Vermeulen, A., Langguth, P., 2017. A survey on IVIVC/IVIVR development in the pharmaceutical industry – Past experience and current perspectives. Eur J Pharm Sci. 102:1-13.

101) Lacy-Jones, K., Hayward, P., Andrews, S., Gledhill, I., McAllister, M., Abrahamsson, B., Rostami-Hodjegan, A., Pepin, X., 2017. Biopharmaceutics data management system for anonymised data sharing and curation: First application with orbito IMI project. Comput Methods Programs Biomed. 140:29-44.

102) Margolskee, A., Darwich, A.S., Pepin, X., Pathak, S.M., Bolger, M.B., Aarons, L., Rostami-Hodjegan, A., Angstenberger, J., Graf, F., Laplanche, L., Müller, T., Carlert, S., Daga, P., Murphy, D., Tannergren, C., Yasin, M., Greschat-Schade, S., Mück, W., Muenster, U., van der Mey, D., Frank, K.J., Lloyd, R., Adriaenssen, L.,

Bevernage, J., De Zwart, L., Swerts, D., Tistaert, C., Van Den Bergh, A., Van Peer, A., Beato, S., Nguyen-Trung, A.T., Bennett, J., McAllister, M., Wong, M., Zane, P., Ollier, C., Vicat, P., Kolhmann, M., Marker, A., Brun, P., Mazuir, F., Beilles, S., Venczel, M., Boulenc, X., Loos, P., Lennernäs, H., Abrahamsson, B., 2017. IMI - oral biopharmaceutics tools project - evaluation of bottom-up PBPK prediction success part 1: Characterisation of the OrBiTo database of compounds.. Eur J Pharm Sci. 96:598-609.

103) Wilkinson, M.D., Dumontier, M., Aalbersberg, I.J., Appleton, G., Axton, M., Baak, A., Blomberg, N., Boiten, J.W., da Silva Santos, L.B., Bourne, P.E., Bouwman, J., Brookes, A.J., Clark, T., Crosas, M., Dillo, I., Dumon, O., Edmunds, S.., Evelo, C.T., Finkers, R., Gonzalez-Beltran, A., Gray, A.J., Groth, P., Goble, C., Grethe, J.S., Heringa, J., 't Hoen, P.A., Hooft, R., Kuhn, T., Kok, R., Kok, J., Lusher, S.J., Martone, M.E., Mons, A., Packer, A.L., Persson, B., Rocca-Serra, P., Roos, M., van Schaik, R., Sansone, S.A., Schultes, E., Sengstag, T., Slater, T., Strawn, G., Swertz, M.A., Thompson, M., van der Lei, J., van Mulligen, E., Velterop, J., Waagmeester, A., Wittenburg, P., Wolstencroft, K., Zhao, J., Mons, B., 2016. The FAIR Guiding Principles for scientific data management and stewardship.www.nature.com/sdata/. DOI: 10.1038/sdata.2016.18.

104) Darwich, A.S., Margolskee, A., Pepin, X., Aarons, L., Galetin, A., Rostami-Hodjegan, A., Carlert, S., Hammarberg, M., Hilgendorf, C., Johansson, P., Karlsson, E., Murphy, D., Tannergren, C., Thörn, H., Yasin, M., Mazuir, F., Nicolas, O., Ramusovic, S., Xu, C., Pathak, S.M., Korjamo, T., Laru, J., Malkki, J., Pappinen, S., Tuunainen, J., Dressman, J., Hansmann, S., Kostewicz, E., He, H., Heimbach, T., Wu, F., Hoft, C., Pang, Y., Bolger, M.B., Huehn, E., Lukacova, V., Mullin, J.M., Szeto, K.X., Costales, C., Lin, J., McAllister, M., Modi, S., Rotter, C., Varma, M., Wong, M., Mitra, A., Bevernage, J., Biewenga, J., Van Peer, A., Lloyd, R., Shardlow, C., Langguth, P., Mishenzon, I., Nguyen, M.A., Brown, J., Lennernäs, H., Abrahamsson, B., 2017. IMI - Oral biopharmaceutics tools project - Evaluation of bottom-up PBPK prediction success part 3: Identifying gaps in system parameters by analysing In Silico performance across different compound classes. Eur J Pharm Sci. 96:626-642.

105) Margolskee, A., Darwich, A.S., Pepin, X., Aarons, L., Galetin, A., Rostami-Hodjegan, A., Carlert, S., Hammarberg, M., Hilgendorf, C., Johansson, P., Karlsson, E., Murphy, D., Tannergren, C., Thörn, H., Yasin, M., Mazuir, F., Nicolas, O., Ramusovic, S., Xu, C., Pathak, S.M., Korjamo, T., Laru, J., Malkki, J., Pappinen, S., Tuunainen, J., Dressman, J., Hansmann, S., Kostewicz, E., He, H., Heimbach, T., Wu, F., Hoft, C., Laplanche, L., Pang, Y., Bolger, M.B., Huehn, E., Lukacova, V., Mullin, J.M., Szeto, K.X., Costales, C., Lin, J., McAllister, M., Modi, S., Rotter, C., Varma, M., Wong, M., Mitra, A., Bevernage, J., Biewenga, J., Van Peer, A., Lloyd, R., Shardlow, C., Langguth, P., Mishenzon, I., Nguyen, M.A., Brown, J., Lennernäs, H., Abrahamsson, B., 2017. IMI - Oral biopharmaceutics tools project - Evaluation of bottom-up PBPK prediction success part 2: An introduction to the simulation exercise and overview of results. Eur J Pharm Sci. 96:610.

106) Rosenberger, J., Schmid, K., Schäfer, K. J., Dressman, J., 2019. Surface Activity Profiling (SAP): A potential means of predicting intestinal membrane permeability. Eur J Pharm Biopharm. 142: 291-299.

107) Roos, C., Westergren, J., Dahlgren, D., Lennernäs, H., Sjögren, E., 2018. Mechanistic modelling of intestinal drug absorption – The in vivo effects of nanoparticles, hydrodynamics, and colloidal structures. Eur J Pharm Biopharm 133: 70-76.

108) Hens, B., Pathak, S. M., Mitra, A., Patel, N., Liu, B., Patel, S., Jamei, M., Brouwers, J., Augustijns, P., Turner, D. B., 2017. In Silico Modeling Approach for the Evaluation of Gastrointestinal Dissolution, Supersaturation, and Precipitation of Posaconazole. Molecular Pharmaceutics 14: 4321-4333.

109) Jamei, M., Abrahamsson, B., Brown, J., Bevernage, J., Bolger, M.B., Heimbach, T., Karlsson, E., Kotzagiorgis, E., Lindahl, A., McAllister, M., Mullin, J.M., Pepin, X., Tistaert, C., Turner, D.B., Kesisoglou, F., 2020. Current status and future opportunities for incorporation of dissolution data in PBPK modeling for pharmaceutical development and regulatory applications: OrBiTo consortium commentary, Submitted to Eur J Pharm Biopharm

110) Guiastrennec, B., Söderlind, E., Richardson, S., Peric, A., Bergstrand, M., 2017. In Vitro and In Vivo Modeling of Hydroxypropyl Methylcellulose (HPMC) Matrix Tablet Erosion Under Fasting and Postprandial Status. Pharmaceutical Research 34: 847-859.

111) Hénin, E., Bergstrand, M., Weitschies, W., Karlsson, M. O., 2016. Meta-analysis of Magnetic Marker Monitoring Data to Characterize the Movement of Single Unit Dosage Forms Though the Gastrointestinal Tract Under Fed and Fasting Conditions. Pharmaceutical Research 33(3): 751-762.

112) Doki, K., Darwich, A. S., Patel, N., Rostami-Hodjegan, A., 2017. Virtual bioequivalence for achlorhydric subjects: The use of PBPK modelling to assess the formulation-dependent effect of achlorhydria. Eur J Pharm Sci 109: 111-120.

113) Cristofoletti, R., Patel, N., Dressman, J. B., 2017. Assessment of Bioequivalence of Weak Base Formulations Under Various Dosing Conditions Using Physiologically Based Pharmacokinetic Simulations in Virtual Populations. Case Examples: Ketoconazole and Posaconazole. J Pharm Sci 106(2): 560-569.

114) Pepin, X.J., Sanderson, N.J., Blanazs, A., Grover, S., Ingallinera, T.G., Mann, J.C., 2019. Bridging in vitro dissolution and in vivo exposure for acalabrutinib. Part I. Mechanistic modelling of drug product dissolution to derive a P-PSD for PBPK model input. Eur J Pharm Biopharm. Eur J Pharm Biopharm. 142:421-434.

115) Darwich, A. S., Pade, D., Rowland-Yeo, K., Jamei, M., Asberg, A., Christensen, H., Ashcroft, D. M., Rostami-Hodjegan, A., 2013. Evaluation of an In Silico PBPK Post-Bariatric Surgery Model through Simulating Oral Drug Bioavailability of Atorvastatin and Cyclosporine. CPT: pharmacometrics & systems pharmacology. 2, e47: 1-9.

116) Rosenberger, J., Butler, J., Dressman, J., 2018. A refined Developability Classification system (rDCS). Journal of Pharmaceutical Sciences. 107: 2020-2

117) Rosenberger, J., Butler, J., Muenster, U., Dressman, J. 2019b. Application of a Refined Developability Classification System. Journal of Pharmaceutical Sciences 108(3): 1090-1100.
118) Lennernäs H., Lindahl A., Van Peer A., Ollier C., Flanagan T., Lionberger R., Nordmark A., Yamashita S., Yu L., Amidon G.L., Fischer V., Sjögren E, Zane P., McAllister M., Abrahamsson B., 2017.In Vivo Predictive Dissolution (IPD) and Biopharmaceutical Modeling and Simulation: Future Use of Modern Approaches and Methodologies in a Regulatory Context. Mol Pharm. 14: 1307-1314
119) Pepin, X.J., Flanagan, T.R., Holt, D.J., Eidelman, A., Treacy, D., Rowlings, C.E., 2016. Justification of Drug Product Dissolution Rate and Drug Substance Particle Size Specifications Based on Absorption PBPK Modeling for Lesinurad Immediate Release Tablets. Mol Pharm. 13:3256-69.
120) Heimbach T., Suarez-Sharp S., Kakhi M., Holmstock N., Olivares-Morales A., Pepin X., Sjögren E., Tsakalozou E., Seo P., Li M., Zhang X., Lin H.P., Montague T., Mitra A., Morris D., Patel N., Kesisoglou F., 2019. Dissolution and Translational Modeling Strategies Toward Establishing an In Vitro-In Vivo Link-a Workshop Summary Report. AAPS J. 21:29.
121) Maher, S., Brayden, D.J., Casettari, L., Illum, L., 2019. Application of Permeation Enhancers in Oral Delivery of Macromolecules: An Update. Pharmaceutics. 11:41.

.

Figure 1


gsk
GlaxoSmithKline
NOVARTIS
sanofi aventis
MSD
Boehringer Ingelheim
Pfizer
janssen
AstraZeneca
Bristol-Myers Squibb
Lundbeck
BAYER
ORION PHARMA
abbvie
JOHANN WOLFGANG GOETHE
UNIVERSITÄT FRANKFURT AM MAIN
National and Kapodistrian UNIVERSITY OF ATHENS
JG|U
JOHANNES GUTENBERG UNIVERSITÄT MAINZ
MANCHESTER 1824
The University of Manchester
KU LEUVEN
UPPSALA UNIVERSITET
University of Strathclyde Glasgow
ERNST MORITZ ARNDT UNIVERSITÄT GREIFSWALD
simCYP
sirius
Simulations Plus, Inc.
LÄKEMEDELSVERKET
MEDICAL PRODUCTS AGENCY
TNO innovation for life

Figure 2

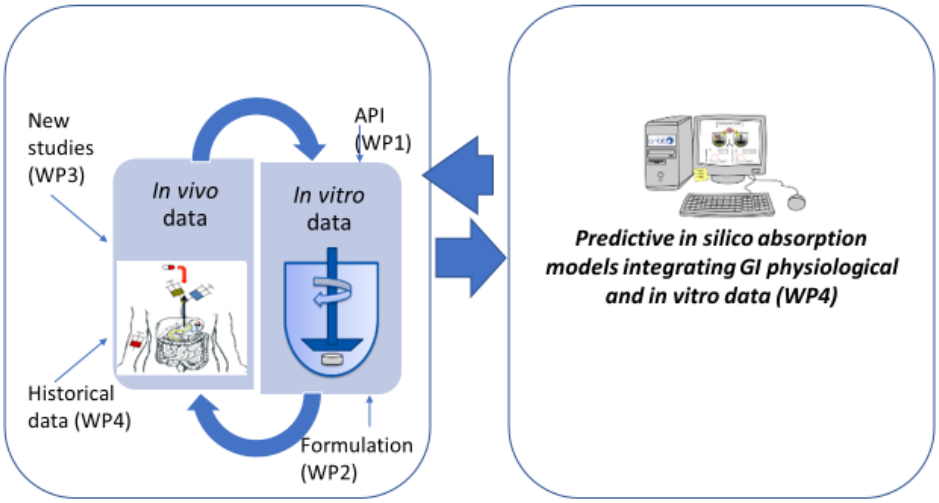
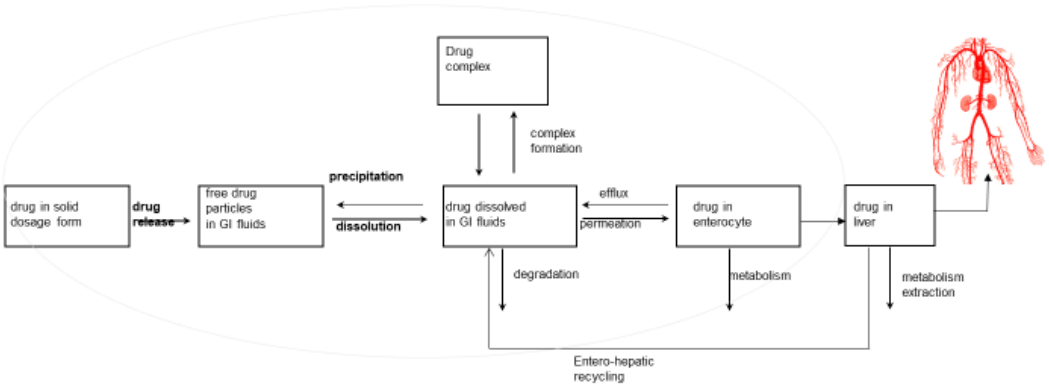

New studies (WP3)
In vivo data
In vitro data
API (WP1)
Historical data (WP4)
Formulation (WP2)
Predictive in silico absorption models integrating GI physiological and in vitro data (WP4)

Figure 3


Drug complex
complex formation
drug in solid dosage form
drug release
free drug particles in GI fluids
precipitation
dissolution
drug dissolved in GI fluids
efflux
permeation
drug in enterocyte
drug in liver
degradation
metabolism
metabolism extraction
Entero-hepatic recycling