Gut feelings

Addendum I

Impact paragraph

Drug development and safety evaluation is a costly and time-consuming process. In current drug safety research, animal models are predominantly used in pre-clinical testing phases, even though their accuracy to replicate human responses or adverse effects is only 50-70% [1, 2]. Consequently, approval of candidate compounds after clinical trials or regulatory checks by e.g. the United States Food and Drug Administration (USFDA) and the European Medicines Agency (EMA) often fails. This can be unrewarding to both the researchers and investors, including pharmaceutical companies, who spent resources and efforts on drug design and development [3]. In order to establish a more accurate alternative to drug research for the pharmaceutical industry, we evaluated the use of organoids as more advanced *in vitro* models. Intestinal organoids models were derived from healthy tissue samples of small intestine and colon of human subjects. A 3D co-culture system was also designed combining human colon organoids with immune cells. These novel 3D *in vitro* models can potentially replace the standard 2D cultures of Caco-2 or HT-29 cell lines, as these lack tissue complexity, and rodent studies, as these poorly reflect all human risks. Moreover, PBPK modelling was used to improve the experimental design that simulates drug concentrations that reach the intestines *in vivo* [4]. High-throughput transcriptomics combined with other omics and functional endpoints was performed to generate new gene expression data that can be applied to predict intestinal toxicity [5, 6].

The societal relevance of the present thesis lies in the further pharmacotherapies for cancer patients. 5-fluorouracil (5-FU), doxorubicin (DOX) and gefitinib, exerting different mechanisms of action elucidated in chapters 2 and 3, 4, and 5, respectively, can cause intestinal toxicity, which usually culminates in diarrhoea. As a result, anti-cancer therapies need to be adjusted or interrupted, hindering the treatment of cancer. Concerns about intestinal adverse effects, which have been underestimated compared to other tissues, are growing among clinicians. By unveiling the molecular mechanisms underlying drug-induced intestinal toxicity and differentially expressed genes (DEGs), this thesis contributes to the improvement of strategies to assess drug safety and risks, minimizing side effects. Consequently, this can decrease chemotherapeutics withdrawal and improve patients' quality of life. In this context, the 3D organoid models are a noteworthy tool to identify gene markers that might aid the progress of personalized medicine applicable to patients' specific responses to cancer therapies, as explored in chapter 3.

In addition, the societal and scientific impact this thesis brings would be the reduction of animal tests. Rodent studies are still an inevitable step in pharmaceutical and toxicological research, despite the efforts to reduce and substitute animal use [7, 8]. However, translation of research from animals to humans is often not possible. For instance, distinct immune responses or composition of the microbiome in rodents versus humans constitutes a major setback when investigating drug responses [9]. Therefore, a more advanced model that reflects more closely the organization and behavior of the human intestinal tract may be better predictive and, thus reduce incorrect evaluations

of compounds. Furthermore, more accurate *in vitro* models can be faster in evaluating drug toxicity, and generate a larger and quicker turnover, hence delivering new treatment options more efficiently and less costly. This brings us to the economic impact of this thesis. The possibility of cheaper drug design and assessment opens doors to more accessible medicines and reduction of health care costs. This would greatly benefit not only the quality of medical care and all patients receiving pharmacotherapy but also pharmaceutical companies as these are interested in developing more efficient and safer medicines in a faster and less costly way.

Apart from improving the understanding of cellular processes taking place during drug exposure and providing opportunities to study new drugs, the work presented in this thesis also contributes to the progress of *in vitro* systems, particularly co-cultures as established in chapter 6. In turn, this can contribute to the improvement of the translatability from *in vitro* to human conditions. It is of great interest the translation of gene markers into clinical settings as biomarkers to detect and/or prevent intestinal toxicity during disease therapies, which in turn would improve personalized medicine approaches. Both the academic community and pharmaceutical companies can benefit from our results as they provide an opportunity for further research and they form a basis for future risk assessment of new medicines. Effectively, the new transcriptomic data described in this thesis is currently being applied and validated through *in sillico* models as part of the TransQST project [10]. This will be instrumental in establishing a quantitative systems toxicology (QST) model as a novel tool to predict intestinal toxicity induced by new compounds during pre-clinical studies.

Taken all together, in this thesis we showed the potential use of intestinal organoids as the future culture systems to study drug efficacy and toxicity. These models can be implemented in the research of gene expression profiles and biological processes affected by a certain compound. Potentially the organoid models may provide gene markers that facilitate the detection and monitoring of intestinal damage during therapies. The future of toxicity research would benefit from advancing co-culture models [11, 12] as endeavored in this work, in which organoids are combined with other cell types that are part of the *in vivo* tissue complex organization to obtain more accurate outcomes. Although the results described in this thesis are mainly useful for the toxicogenomics field and *in vitro* drug research, they can also be of great interest and value to pharmaceutical companies, clinics/hospitals, and patients in general.

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