A.35
Defining The Pharmacometabodynamics of Gefitinib after Intravenous Administration to the Mouse by UHPLC/MS and UPLC-IM-MS Study
Ian D. Wilson¹, Lee A. Gethings², Robert S. Plumb³
¹Imperial College London, ²Waters Corporation, Wilmslow, UK, ³Waters Corporation, Milford, USA

Gefitinib, an anilinoquinazoline inhibitor of thymidylate kinase (selective for the epidermal growth factor receptor (EGFR)), was originally developed as a treatment for non-small cell lung cancer. In this study Male C57 BL6 mice were dosed IV with gefitinib (10 mg/kg) and then microsampling combined with a rapid (sub 5 min) UHPLC/qqqMS used to determine the pharmacokinetics of the drug and its major circulating metabolites. In addition, 10 circulating metabolites of the drug and 15 present in the urine were characterized using UHPLC/IM/HRMS. The addition of an IM separation gave rise to much improved MS data thereby aiding the identification of several novel glucuronide metabolites. As well as the drug and its metabolites untargeted metabolic phenotyping (metabonomics/metabolomics) enabled a range of time-related effects of the drug on endogenous metabolism to be detected. Changes in endogenous metabolite profiles, both increases and decreases in amounts, appeared shortly after dosing and had largely returned to their predose values by 24hrs. The changes in the amounts of endogenous metabolites excreted in the urine mirrored to some extent the plasma pharmacokinetics of the drug demonstrating a possible pharmacometabonomic effect. This type of combined drug and endogenous metabolite profiling may represent a method for better understanding the pharmacology of drugs in terms of the way that their effects modulate the metabolic pathways of the organisms exposed to them, including patients.

100321, https://doi.org/10.1016/j.mcpro.2022.100356

A.36
Chemoproteomic identification and functional characterization of colibactin-modified proteins that drive colorectal cancer progression
Nan Qiu¹, ², Daniel Abegg², Dany Pechalrieu², Kevin M. Wernke³, Seth B. Herzon², Alexander Adibekian¹, ²
¹The Scripps Research Institute, Jupiter, FL 33458, ²Department of Chemistry, UF Scripps Biomedical Research, Jupiter, FL 33458, ³Department of Chemistry, Yale University, New Haven, CT 06520

Colibactin is a toxic bacterial polypeptide produced by intestinal Escherichia coli and other members of Enterobacteriaceae harboring the clb (also known as pks) gene cluster. The presence of Clb+ E. coli and colibactin is implicated in colorectal cancer (CRC) onset and progression by arresting epithelial renewal, accelerating tumor growth, and creating distinct mutational signatures in CRC patients. Although colibactin can crosslink DNA and induce double strand breaks in mammalian cells, the full molecular mechanism underlying the malignant transformations and pro-cancer phenotypes remains elusive. To access DNA in the nucleus, it is conceivable that colibactin must travel through the cytoplasm that is crowded with reactive protein nucleophiles. Herein, we showed that colibactin and its synthetic analogues can covalently modify host proteins through the electrophilic aminocyclopropane moieties by direct detection of mass adducts. Using an LC-MS/MS-based chemoproteomic approach, we identified proteome-wide colibactin targets in colon cancer cells by incorporating transient bacterial infection and stable colibactin-inspired analogues. Biochemical validation confirmed the binding between colibactin and the LIM domain on LIM and SH3 domain protein 1 (LASP1), the commonly competed target upon E. coli infection and model colibactin treatment. Altogether, our results shed light on the protein-reactivity of the elusive colibactin for the first time and illuminate the possible role of colibactin-modified LASP1 in promoting CRC progression. Future effort will be devoted to detailing the biological consequences of colibactin-modification LASP1 in cytoskeleton remodeling, migration, and invasion behaviors of colorectal cancer cells.

100321, https://doi.org/10.1016/j.mcpro.2022.100357