Rapid Determination Of The Pharmacokinetics, Metabolism, Elimination And Tissue Distribution Of A PROTACs Drug Using UHPLC-MS/MS, HRMS And DESI Imaging. Abstract ID number: 322535

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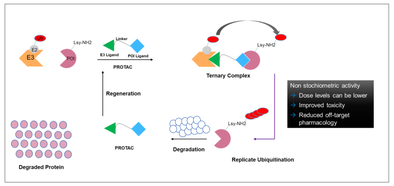
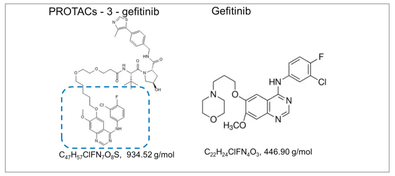
INTRODUCTION

Proteolysis Targeting Chimeras (PROTACs) are an emerging class of drug molecules which work by mobilizing the ubiquitin–proteasome system to achieve proteasome-mediated degradation of the target protein[1]. These PROTACs molecules significantly increase the number of “druggable” proteins in the human proteome, opening the possibility for new safer medicines. PROTACs are “large small molecules”, >800g/mol, as such understanding their DMPK properties presents a new challenges i) monitoring fate and disposition of the dosed molecule and ii) quantifying any cleavage metabolites produced from both the target binding moieties (TBM) and ligand binding moieties (LBM)[2]. Gefitinib is a TKI inhibitor for the treatment of non-small cell lung cancer as an epidermal growth factor inhibitor[3], however, these first generation TKI exhibit resistance via irreversible binding to receptor proteins.



In this study we have evaluated the pharmacokinetics and metabolic biotransformation of PROTACs – 3 – Gefitinib (a PROTAC version of gefitinib) in male Wistar rats following a single subcutaneous administration at 10 mg/kg.



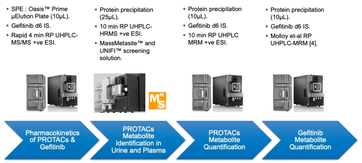
Ethics Statement

This study is compliant with the corresponding projects APAFIS #32640-202110141911946 47 v5. This project was reviewed by the Evotec Management and Ethical Committee (identified as CEPAL: CE 029) and compliant with national (UK) and EU regulations.

METHODS

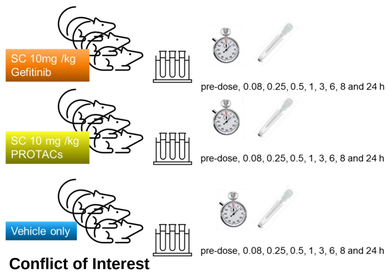
DMPK

Drug and metabolite concentrations were determined by reversed – phase UHPLC-MS/MS using a Waters ACQUITY™ Premier UPLC™ System connected to a Waters Xevo™ TQ Absolute Mass Spectrometer. Metabolite identifications were performed using the identical chromatography system coupled to a Waters Xevo MRT HRMS mass spectrometer. All LC separations were performed on a 2.1 x 100 mm 1.7um HSS T3 C18 Waters MaxPeak™ Premier chromatography column.



Study Design

15 male Sprague Dawley rats (7-9 weeks) were divided into 3 groups and dosed subcutaneously with PROTACs – 3 – gefitinib at either 0 or 10 mg/kg or gefitinib at 10 mg/kg. Blood samples (50 µL) was taken at 0, 0.08, 0.5, 1, 3, 6, 8 and 24 h post dose. Tissue samples were harvested at 1, 3, 8 and 24 h post dose for. Urine samples were collected at 1, 3, 8, & 24 h post dose. Derived plasma and urine samples were prepared by protein precipitation with organic solvent containing SIL gefitinib d6.

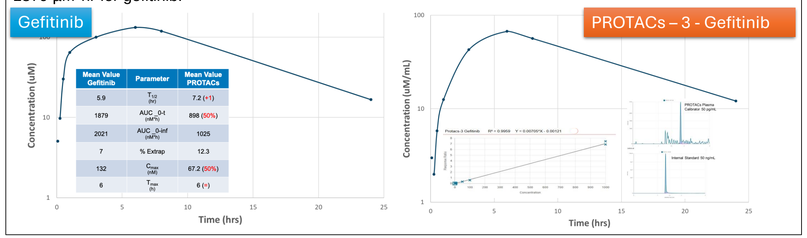


Conflict of Interest

Andrew Leightner, Steven Lai, Anthony Midey and Robert Plumb are employees of Waters Corporation. Prof Ian Wilson is a paid consultant for several companies including Waters Corporation.

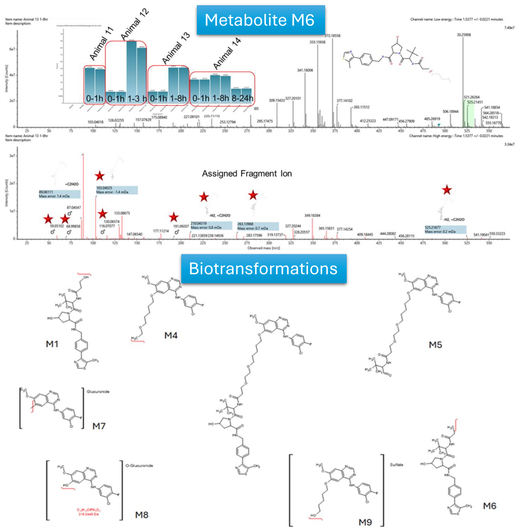
PHARMACOKINETICS

A rapid 5 min RPLC-MS/MS assay was validated for the quantification of PROTAC-3-gefitinib & gefitinib over the range of 0.02 – 500 ng/mL in plasma and urine. PROTAC-3-gefitinib had a observed half-life 7.2 h, T_{max} 6 h,C_{max} 67 ng/mL and AUC0-t 898 µM*hr, compared to a half-life 5.9 h, T_{max} 6 h, C_{max} 132 ng/mL and AUC0-t 1879 µM*hr for gefitinib.



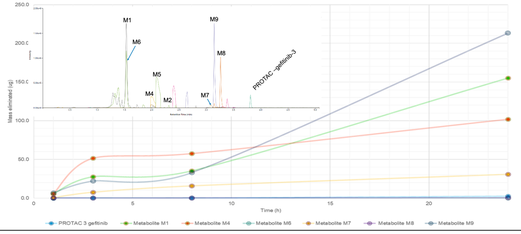
DRUG METABOLISM

At total of 9 PROTACs biotransformations were detected in the plasma and urine samples via RPLC-HRMS analysis. Metabolite detection and identification was carried out using the waters-connect™ metabolite Application manager and MassMetaSite software. PROTACs metabolism was characterized by N-dealkylation and ester hydrolysis of the aliphatic linker followed by sulphation and glucuronidation.



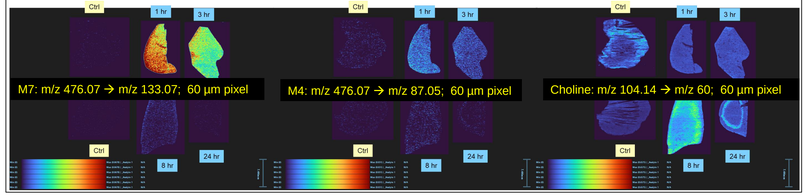
METABOLITE ELIMINATION

The detected PROTACs metabolites were quantified using RPLC-MS/MS. Approximately 22% equivalent of dosed PROTACs was recovered in the urine. Metabolites M1, M4, M7 and M9 accounted for the majority of drug related material eliminated in the urine over the 0 – 24 h time period.



LIVER TISSUE DESI MS IMAGING

The presence of the PROTACs drug & related metabolites were screened for in the liver tissues via DESI-MS imaging using Waters Xevo TQ Absolute operating in MRM mode. The PROTACs drug was weakly observed in the 8 h sample only, with the M4 and M7 metabolites clearly observed in the 1 and 3 h samples. There was also a noticeable increase in endogenous Choline at the 8 h time point.



CONCLUSION

- **Pharmacokinetics and Metabolism of PROTAC-3-gefitinib and gefitinib were determined in male rats following subcutaneous dosing and microsampling with UHPLC MS/MS and HRMS analysis.**
- **PROTAC-3-gefitinib had a observed half-life 7.2 h, Tmax 6 h,Cmax 67 ng/mL and AUC0-t 898 µM*hr, compared to a half-life 5.9 h, Tmax 6 h, Cmax 132 ng/mL and AUC0-t 1879 µM*hr for gefitinib.**
- **9 major PROTAC-3-gefitinib metabolites were identified, these mainly involving cleavage of the linker followed by glucuronide and sulphate conjugation of the TBM and EBM.**
- **The majority of the PROTACs dose was eliminated as M1, M4, M9 and M7, two cleavage and two glucuronide metabolites.**
- **Unchanged PROTACs drug and metabolites M4 and M7 were detected by QqQ MS DESI in liver samples at the 1 and 3 h time points.**

References

1. Békés M, Langley D.R, Crews C.M. PROTAC targeted protein degraders: the past is prologue. Nat Rev Drug Discov 21, 181–200 (2022). https://doi.org/10.1038/s41573-021-00371-6
2. Goracci L, Desantis J, Valeri A, Castellani B, Eleuteri M, Cruciani G. Understanding the Metabolism of Proteolysis Targeting Chimeras (PROTACs): The Next Step toward Pharmaceutical Applications. J Med Chem. 2020 22;63(20):11615-11638. doi: 10.1021/acs.jmedchem.0c00793
3. McKillop, D., et al. 2004a. Pharmacokinetics of gefitinib, an epidermal growth factor receptor tyrosine kinase inhibitor, in rat and dog. Xenobiotica; the fate of foreign compounds in biological systems, 34 (10), 901–915.
4. Molloy BJ, King A, Mullin LG, Gethings LA, Riley R, Plumb RS, Wilson ID. Rapid determination of the pharmacokinetics and metabolic fate of gefitinib in the mouse using a combination of UPLC/MS/MS, UPLC/QToF/MS, and ion mobility (IM)-enabled UPLC/QToF/MS. Xenobiotica. 2021 51(4):434-446. doi: 10.1080/00498254.2020.

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