Combining in Vivo Metabolism and Whole-Body Biodistribution for Comprehensive Insight into ERAP2 Inhibitor Pharmacokinetic

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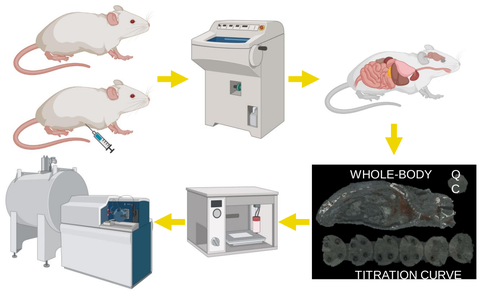
Introduction

This research presents a comparative analysis between conventional liquid assays and state-of-the-art mass spectrometry imaging techniques to assess the stability and pharmacokinetics of a potent and selective ERAP2 inhibitor (IC50=0.019 µM). The study encompasses both in vitro and in vivo assessments, aiming to elucidate the compound's behavior in physiological environments. Classic liquid assays provide foundational insights, while mass spectrometry imaging offers a comprehensive examination of drug and metabolite's biodistribution. This integrative approach facilitates a thorough understanding of the compound's physico-chemical characteristics.

Method

Plasma and liver samples were collected from treated mice at specified time points. After preparation, LC-MS was performed for compound's quantification, employing calibration curves, and pharmacokinetic parameters were calculated. Liver microsomes were incubated with the test compound to simulate in vivo conditions. Samples collected were analyzed to determine the compound's metabolic stability. Whole body sections from treated animals and controls were prepared and subjected to MS imaging acquisition. Spatially resolved mass spectra were acquired, enabling quantitative assessment of the compound and its metabolite's distribution.



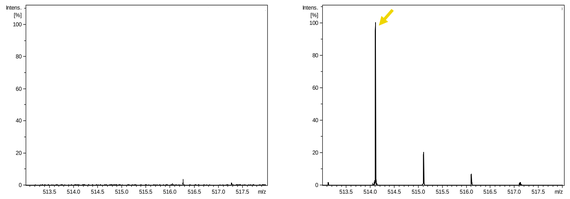
PK and Stability Studies

Pharmacokinetic study identified the time point of maximum concentration (Cmax) at 20 minutes post-administration. This time point was selected to sacrifice the mice for the biodistribution study. Microsomal stability assay allowed the identification of metabolites originating from biochemical metabolism processes, that were further investigated via MSI.

MSI Method Development

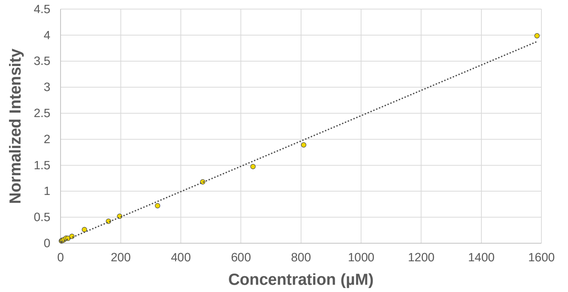
DHB matrix at 40 mg/ml + 0,1% TFA was selected for compound's detection. Isotopically labelled form of the compound was added to the matrix and homogeneously spray coated on whole body tissue sections to be used as internal standard.

ERAP2 Inhibitor Compound [M+H]⁺



Control Treated

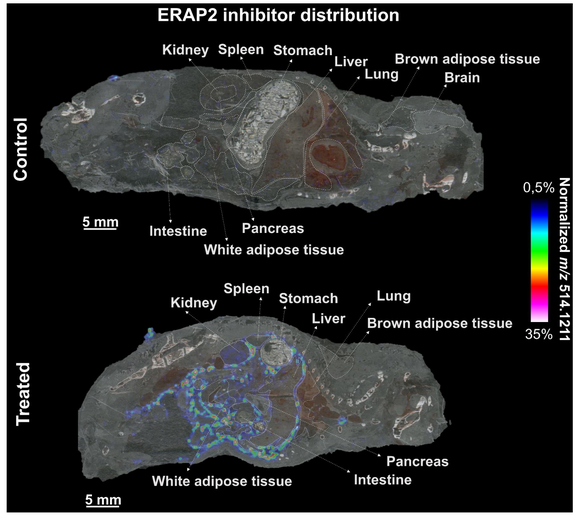
Titration curve was built by spotting increasing amount of compound on surrogate matrix. Linearity, limit of detection and lower limit of quantification were assessed.



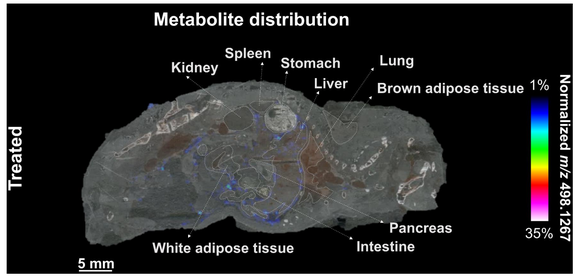
Titration Curve					
Equation	R^2	Weighing	LOD (µM)	LLOQ (µM)	ULOQ (µM)
y= 4.6207x + 0.03585	0.996	Linear 1/X	3.21	3.21	1586.30

Biodistribution Study

Images were acquired in a CASI (Continuous Accumulation of Selected Ions) window of 100 Da centered at m/z 514.12. Intensity was normalized for the internal standard signal. After validating the absence of signal in the control animal, the biodistribution in whole-body section was assessed.

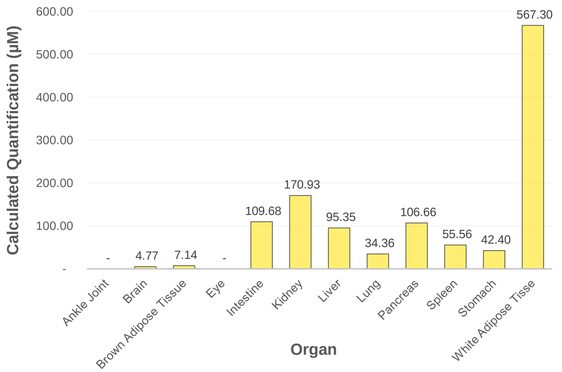


Out of the investigated ones, a metabolite was detected via MSI and its biodistribution was compared to the parent compound one.



ERAP2 Inhibitor Quantification

The compound distributed prevalently in the peritoneum, site of administration. The quantification module on Multimaging™ software (Aliri France SAS) allowed to calculate the concentration of compound concentration in the different organ exposed, based on the titration curve.



Conclusion

ERAP2 inhibitor compound was successfully detected as [M+H]+ adduct, LLOQ was 3.21 µM. The compound exhibited therapeutic concentration in various organs, with the higher concentration measured on kidney. One metabolite was detected and had similar biodistribution compared to the parent compound. This research integrates liquid studies with imaging analysis to comprehensively assess the stability, pharmacokinetics, and biodistribution of an ERAP2 inhibitor.

References

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