Application of AP-MALDI-Orbitrap-Mass Spectrometric Imaging (AP-MALDI-OT-MSI) for the localization of small molecular drugs in different types of mouse tissues

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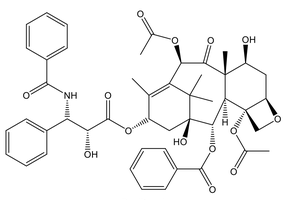
Overview

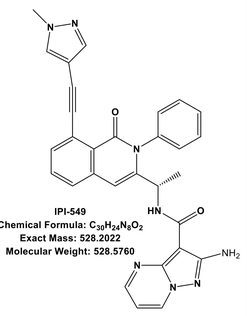
- Here, we successfully demonstrated the application of AP-MALDI (MassTech) coupled to Orbitrap IDX (Thermo) system for localization of small molecular drugs in different mouse tissues.
- Different instrument parameters and matrices were tested and optimized to achieve the best conditions for the detection of Paclitaxel (PTX), IPI-549, and Avapritinib.
- Based on AP-MALDI-MSI images of Nano-PI (PTX and IPI-549) dosed mouse tissues, nano-formulations may co-deliver two drugs into same regions of tumors and lymph nodes.
- Avapritinib was found to be localized in all over the mouse brain and it has coursed the changes in endogenous molecular profiles in the mouse brain semi-quantitatively in different locations.

Introduction

- AP-MALDI-MSI is a label-free technique which is widely used in the field of tissue-based research by providing spatial localization information.
- AP-MALDI is applicable for wide range of analytes including pharmaceutical drugs and their metabolites.
- Here, we studied the localization of small molecular drugs, Paclitaxel, IPI-549, and Avapritinib in different mouse tissues by AP-MALDI-OT-MSI.
- Paclitaxel and IPI-549 are anti-cancer drugs while Avapritinib is specifically used for the treatment of advanced systemic mastocytosis.
- **The main goals of this study are**
  - **Localization of nano-formulated Paclitaxel and IPI-549 in mouse tumor and Lymph nodes.**
  - **Investigation of the changes to endogenous molecular profiles in mouse brain upon administration of Avapritinib.**
- These goals were achieved by performing AP-MALDI-OT-MSI on Paclitaxel, IPI-549, and Avapritinib in different mouse tissues.

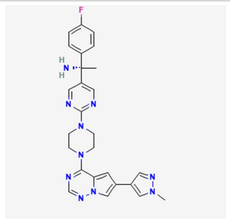


Paclitaxel
Chemical Formula: $C_{47}H_{51}NO_{14}$
Exact Mass: 853.3310
Molecular Weight: 853.9180



IPI-549
Chemical Formula: $C_{30}H_{24}N_8O_2$
Exact Mass: 528.2022
Molecular Weight: 528.5760

Introduction



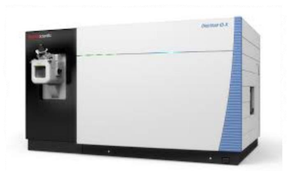
Avapritinib
Chemical Formula: C26H27FN10
Exact Mass: 498.2404
Molecular Weight: 498.5704

Structures of Paclitaxel, IPI-549, and Avapritinib

Methods

Instrumentation

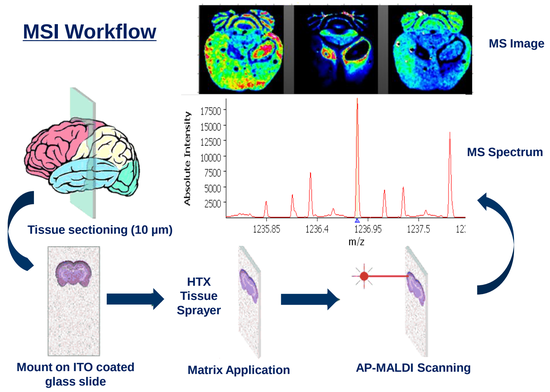
- AP-MALDI Source (MassTech Inc) equipped with 10 kHz Nd:YAG laser (355 nm) coupled to Orbitrap IDX MS (Thermo Fisher)
- Image visualization: SCiLS Lab (Bruker)



Thermo Orbitrap IDX Tribrid MS

AP-MALDI (ng) source (MassTech Inc)

MSI Workflow



- Nano formulated Paclitaxel (PTX) and IPI-549 were administered (IV) at a dosage of Paclitaxel: 100 mg/kg and IPI-549: 50 mg/kg to PyMT mice and, tumors and lymph nodes were collected 4 hrs later.
- Avapritinib was administered orally at a dosage of 60 mg/kg to CD-1 mice and brains were collected 2 hrs later.
- All organs were snap frozen in liquid nitrogen and stored in -20 ^{0}C until sectioning.
- Matrixes:10 mg/mL CHCA for Avapritinib, 10 mg/mL 2′,5′-dihydroxyacetophenone for Paclitaxel and IPI-549.

Results

Localization of nano-formulated Paclitaxel and IPI-549 in mouse tumor and Lymph nodes

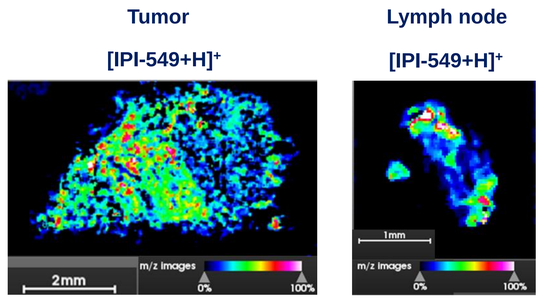


Fig. 1. AP-MALDI images of IPI-549 at 529.21±5 ppm in tumor and lymph node tissues

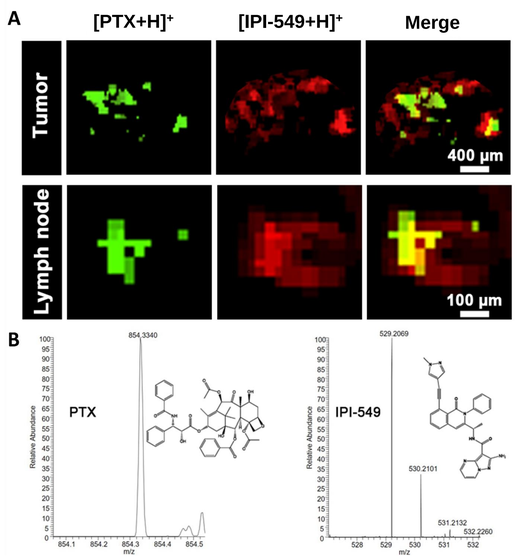
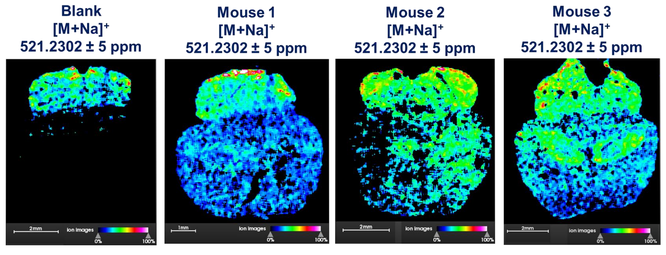
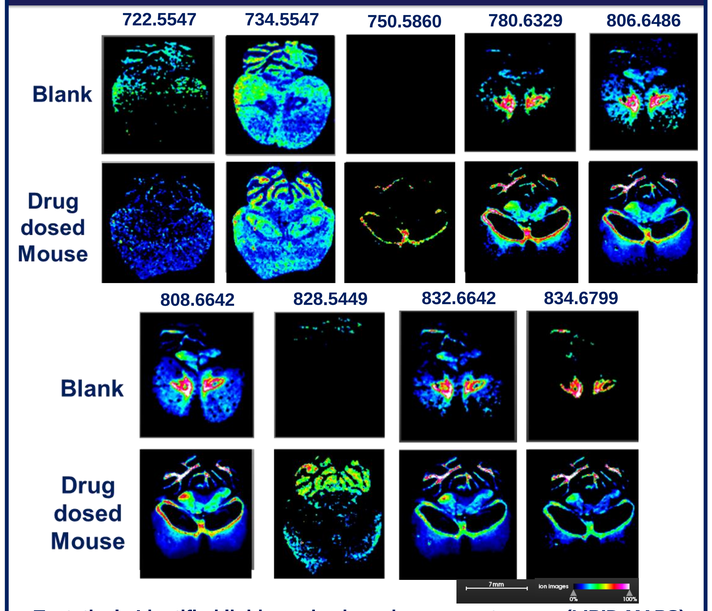


Fig. 2. (A) AP-MALDI-MSI shows the drug localization in tumor and lymph nodes that was delivered by Nano-PI. PTX is shown in green, IPI-549 is shown in red, and Overlay image (yellow) suggests nano-formulations may co-deliver two drugs into same regions of tumors and lymph nodes. (B) Mass spectra of PTX and IPI-549

Investigation of the changes to endogenous molecular profiles in mouse brain upon administration of Avapritinib



Results



Tentatively Identified lipid species based on accurate mass (LIPID MAPS)

Identified Lipid Species	Actual *m/z* ([M+Na]+)	Experimental *m/z* ([M+Na]+)	Mass Accuracy (ppm)
HexCer 34:1	722.5547	722.5580	4.6
HexCer (9Me) 34:2	734.5547	734.5581	4.6
HexCer 36:1	750.5860	750.5883	3.1
HexCer 38:0	780.6329	780.6354	3.2
HexCer 40:1	806.6486	806.6508	2.7
HexCer 40:0	808.6642	808.6667	3.1
Hex2Cer 30:1	828.5449	828.5484	4.2
HexCer 42:2	832.6642	832.6630	1.4
HexCer 42:1	834.6799	834.6783	1.9

Discussion and Conclusion

- AP-MALDI-OT-MSI was successfully utilized to localize Paclitaxel and IPI-549 in mouse Tumor and Lymph node tissues, and Avapritinib on mouse brain tissue.
- Several lipid species were tentatively identified based on accurate mass (within 5 ppm). Their relative intensity and localization have been significantly changed in avapritinib dosed mouse brain in comparison to normal mouse brain.
- These endogenous lipid species changes may be related to brain toxicity course by Avapritinib.
- Validation and Identification of more lipid species are under progress.

References

1. Song, Y., Bugada, L., Li, R., Hu, H., Zhang, L., Li, C., Yuan, H., **Rajanayake, K.K.**, Truchan, N.A., Wen, F. and Gao, W., 2022. Albumin nanoparticle containing a PI3Kγ inhibitor and paclitaxel in combination with α-PD1 induces tumor remission of breast cancer in mice. Science Translational Medicine, 14(643).

Acknowledgements

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