Mass Spectrometry Imaging Enables Localization of Immuno- and Targeted Therapies Delivered Transarterially for Hepatocellular Carcinoma

Erin H. Seeley[1]; Nina M. Munoz[2]; Rony Avritscher[2]; Bhanu Koppolu[3]; Nivedita Ramkumar[3]; Rhiannon Johnson[3]; Steve Kangas[3]; Rahul A. Sheth[2]

[1]University of Texas at Austin, Austin, TX, [2]University of Texas MD Anderson Cancer Center, Houston, TX, [3]BTG/Boston Scientific, Natick, MA

OVERVIEW

Purpose:
- To determine the spatial distribution of therapeutic agents administered via image-guided transarterial delivery to hepatocellular carcinoma (HCC) tumors as well as endogenous molecules

Methods:
- Image-guided transarterial dosing
- MS imaging of drugs, lipids, and proteins

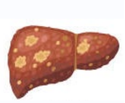
Results:
- Imiquimod was detected at just above the limit of detection in treated tumors, but olaparib was below the LOD
- Several endogenous molecules were found to be affected by drug treatment

INTRODUCTION

Hepatocellular carcinoma (HCC) is increasing globally and is currently the third leading cause of cancer deaths. Transarterial delivery of chemotherapies is a standard of care treatment, but outcomes remain poor. Recently, immunotherapies and targeted therapies have shown tremendous promise. However, little is known about how well the drugs are absorbed into HCC tumors. We have used mass spectrometry imaging (MSI) of transarterially delivered novel therapies into HCC tumors to determine the spatial localization of the drugs and endogenous changes within the tumors allowing for visualization of the drug without the need for labeling that may alter efficacy of treatment.

METHODS

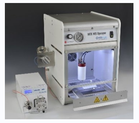
- Orthotopic, syngeneic model of hepatocellular carcinoma
- Image-guided trans-arterial delivery of Imiquimod (50 ug in 1 cc volume) or Olaparib (50 ug in 1 cc volume)



- Tissue (tumors and normal) harvested at 1 and 4 days post dosing
- Snap frozen and sent for MSI

- Thermo NX50 Cryostat
- Sectioned at 12 µm thickness
- Collected on ITO slides
- Serial sections for H&E staining

- Hamamatsu NanoZoomerSQ
- Digital microscopy images acquired at 20X magnification



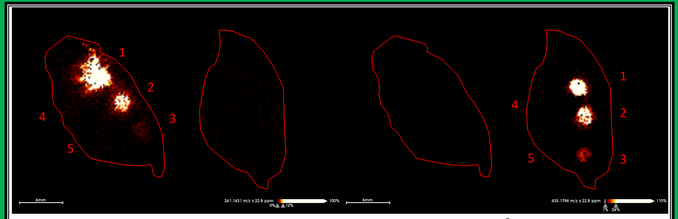
- HTX M5 Sprayer
- 40 mg/mL DHB in 50% ACN, 0.1% TFA for small molecules
- Sections for protein imaging washed with Carnoy's fluid
- 10 mg/mL SA in 90% ACN, 0.1% TFA, rehydration in 22% HOAc at 85°C for 3.5 min

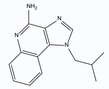
- Bruker timsTOF fleX MALDI QTOF
- Drugs and lipids/metabolites
- Positive ion mode
- 100 µm resolution

- Bruker rapifleX MALDI TOF/TOF
- Proteins
- Positive ion mode
- 150 µm resolution

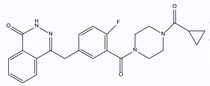
- Images combined for normalization and visualization in SCiLS Lab Pro
- Putative lipid and metabolite identification using MetaboScape and METASPACE

LIMIT OF DETECTION DETERMINATION





Imiquimod
$C_{14}H_{16}N_4$
Exact Mass: 240.1375

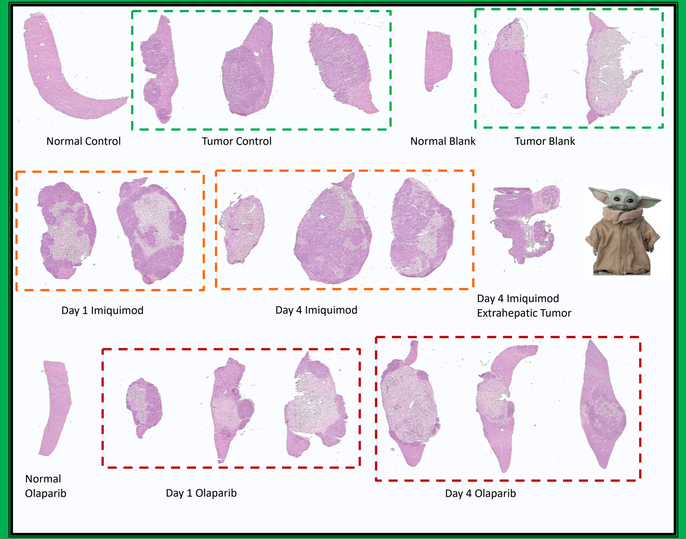


Olaparib
$C_{24}H_{23}FN_4O_3$
Exact Mass: 434.1754

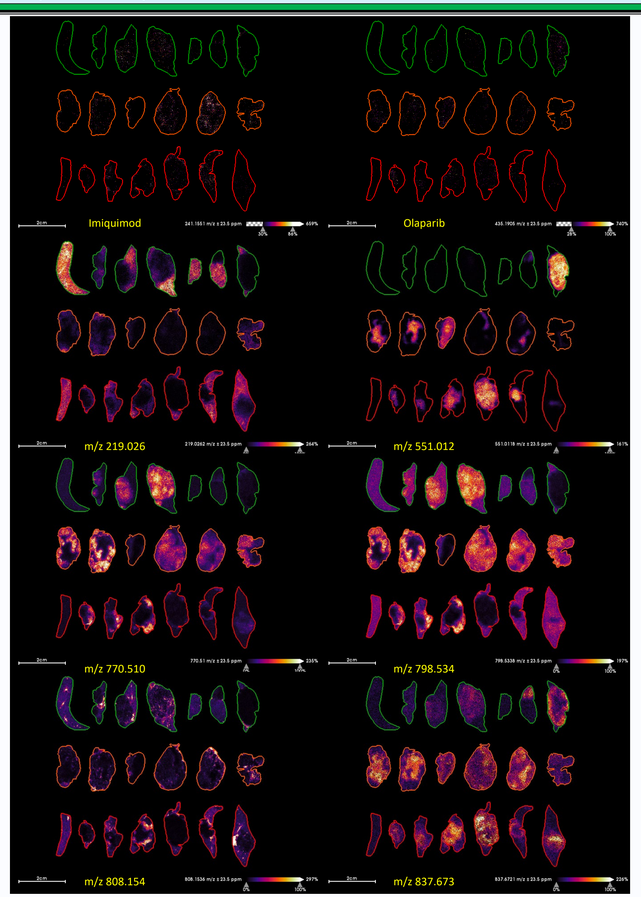
1 – 1 mg/mL; 2 – 0.1 mg/mL; 3 – 0.01 mg/mL; 4 – 0.001 mg/mL; 5 – 0.0001 mg/mL

Determination of Detections Limits. Imiquimod and Olaparib were diluted in 50% methanol and 0.5 µL of each dilution was spiked onto sections of control liver tumors and allowed to dry. DHB matrix was applied and images were acquired in positive ion mode using a Bruker timsTOF fleX mass spectrometer. Both drugs were detected at 0.01 mg/mL from tissue sections with slightly better sensitivity for Olaparib.

HISTOLOGICAL STAINING

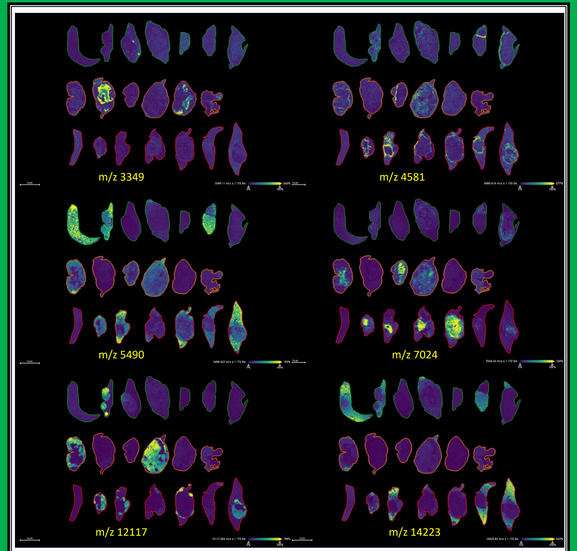


DRUG, METABOLITE, AND LIPID IMAGING



Ion Images from timsTOF. Imiquimod was detected in tissues at just above the LOD, with higher signal in the Day 4 tumors. Olaparib was below the limit of detection. A signal at m/z 219.026 (likely [hexose + K]$^+$) was detected in normal liver, but at lower abundance in Imiquimod treated samples. A signal at m/z 551.012 was localized to necrotic regions. Signals at m/z 770.510 (likely [PE(38:6) + Na]$^+$) and m/z 798.534 (likely [PC(34:1) + Na]$^+$) were localized to viable tumor. The peak at m/z 808.154 (likely flavin adenine dinucleotide [FAD + H]$^+$) colocalized with blood and m/z 837.673 (likely [20:3-Glc-cholesterol + H]$^+$) was mostly in necrotic areas with higher intensity in treated tumors.

PROTEIN IMAGING



Selected Protein Images from the rapifleX. An ion at m/z 3349 was almost exclusively detected in tumors treated with Imiquimod while one at m/z 14223 was nearly absent from Imiquimod treated tumors. A protein at m/z 5490 was found in histologically normal-appearing regions of most samples. A protein at m/z 12117 was detected in viable tumor regions while m/z 4581 was found in the advancing edge of tumors. A protein at m/z 7024 was found in the necrotic areas of treated tumors, but was nearly absent from untreated tumors.

CONCLUSIONS AND FUTURE DIRECTIONS

- Both Imiquimod and Olaparib had LODs in tissue of about 0.01 mg/mL which corresponded to approximately 37 µg/g of tissue
- Imiquimod was detected just above the limit of detection in treated tumors, but Olaparib was below the LOD in this study
- Numerous endogenous molecules were detected that displayed localization that correlated with treatment or histology
- Image-guided, transarterial delivery of chemotherapeutics can be monitored by MSI without the need for labeling that could affect the efficacy of the treatment
- Work is ongoing to increase the sample number per treatment group and validate the results observed in this study

ACKNOWLEDGEMENTS

- The authors are grateful to Malea Williams for help with tumor treatment and sample collections
- The authors acknowledge funding from Cancer Prevention and Research Institute of Texas RP190617 and Boston Scientific