# Bimiralizib distribution in human skin biopsies after topical administration obtained by validated quantitative mass spectrometry imaging.

Raphael Legouffe[1], Selinde S. Wind[2, 3], Debora Schmitz-Rohmer[4], Shane Karnik[5], Mathieu Gaudin[1], Corinne Ramos[1], David Bonnel[1], Robert Rissmann[2,3,6]

1: ALIRI, 152 rue du Dr Yersin, 59120 Loos, France
2: Centre for Human Drug Research, 2333 CL Leiden, The Netherlands
3: Department of Dermatology, Leiden University Medical Center, 2333 ZA Leiden, The Netherlands
4: PIQUR Therapeutics AG, 4057 Basel, Switzerland
5: ALIRI, 4720 Forge Rd # 108, Colorado Springs, CO 80907, USA
6: Leiden Academic Centre for Drug Research, Leiden University, 2333 CC Leiden, The Netherlands

## Novel aspects

This study presents a validation of a Quantitative MALDI Mass Spectrometry Imaging (QMSI) method applied to clinical skin biopsies. The method has then been used to determine the drug distribution and contributed to the interpretation of the efficacy results of this clinical trial.

## Introduction

Since the introduction of MALDI mass spectrometry more than three decades ago, this technique was considered as a non-quantitative method or at least semi-quantitative but a turning point was reached in 2010. Indeed, ImaBiotech (now Aliri) developed the first workflow to get quantitative results by MALDI mass spectrometry imaging. This new development was well received because this technique was more and more implemented in the pharma / biotech industry for its numerous advantages such as the detection of a large range of molecules (drugs, metabolites, lipids, peptides and proteins) and the use of a non-radioactive compound. Even if this technique was used in the pharmaceutical industry to support DMPK and safety evaluation, its application in clinical trials remains uncommon. This study shows how validated QMSI can be used to support first-in-human clinical trial to assess drug exposure in skin biopsies.

Mycosis fungoides (MF) is a subtype of Primary cutaneous T-cell lymphomas (CTCL) with a low incidence and high medical need for novel treatments. The objective of this randomized, placebo-controlled, double-blinded, first-in-human study was to evaluate safety, efficacy, cutaneous and systemic pharmacokinetics (PK) of topical bimiralisib in healthy volunteers (HVs) and MF patients. In this trial, a total of 6 HVs and 19 early-stage MF patients were treated with 2.0% bimiralisib gel and/or placebo. Drug efficacy was assessed by scoring of lesion severity while PK blood samples were collected frequently and cutaneous PK was investigated in skin punch biopsies on the last day of treatment.

## MALDI Mass Spectrometry

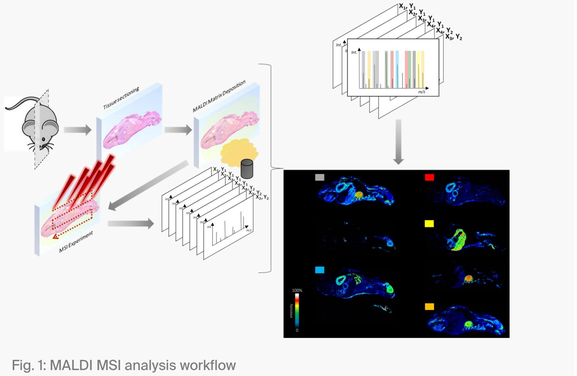


Fig. 1: MALDI MSI analysis workflow

## Validation of MALDI MSI method for clinical study

The validation principles were inspired by the FDA/EMEA guidelines to develop a MALDI mass spectrometry validation workflow for clinical study. The goal was to ensure the robustness of the method.

The criteria are:

### 1- Sensitivity – Calibration curves performance

Goal: To confirm consistency of the working range (i.e. LOD, LLOQ and ULOQ).

Experimental design: Repeated dilution series spotted onto untreated skin tissue analyzed with the same MS method.

Acceptance criteria: RSD of LOD<50%; RSD of LLOQ<50% and RSD of ULOQ<50%

### 2- Accuracy

Goal: To ensure inter-day accuracy of quantitative MSI method

Experimental design: Triplicate spots of Bimiralisib on untreated skin tissue sections measured three times on three consecutive days (two concentrations).

Acceptance criteria: accuracy between 60% and 140%

### 3- Precision

Goal: To ensure precision of QMSI method

Experimental design: Triplicate spots of Bimiralisib on untreated skin tissue sections measured three times on three consecutive days (two concentrations).

Acceptance criteria: RSD below 40%

## Sample preparation for MALDI mass spectrometry imaging

- Sectioning of human skin samples performed at a thickness of 10µm with a cryostat at -23°C. Sections were collected on ITO slides for MSI acquisitions and on Superfrost slides for H&E staining.
- Bimiralizib dilution series were prepared in water/methanol/DMSO 50/49.5/0.5. Each calibrator was spotted on untreated human skin tissues sections.
- DHB MALDI matrix at 40 mg/mL in methanol / water + 0.1% TFA, 7:3 was sprayed over Bimiralizib dilution series and untreated/treated human skin tissue sections with an automatic sprayer system (TM-Sprayer, HTX Imaging).
- Data acquisition with the 7T MALDI-FTICR (Solarix), mode: CASI, polarity: positive, mass range: 412.17 ± 30 Da, laser frequency: 200Hz, spatial resolution: 50µm

## Results of the validation

### Sensitivity

| | LOD | | | LLOQ | | | ULOQ | | |
|---|---|---|---|---|---|---|---|---|---|
| **Day** | **µM (spotted solution)** | **µM tissue** | **µg/g of tissue** | **µM (spotted solution)** | **µM tissue** | **µg/g of tissue** | **µM (spotted solution)** | **µM tissue** | **µg/g of tissue** |
| **Day 1 Calib. Curve Y1** | 0.035 | 0.4 | 0.1 | 0.035 | 0.4 | 0.1 | 35.0 | 328.9 | 135.3 |
| **Day 2 Calib. Curve Y2** | 0.035 | 0.3 | 0.1 | 0.05 | 0.4 | 0.2 | 20.0 | 177.9 | 73.2 |
| **Day 3 Calib. Curve Y3** | 0.035 | 0.3 | 0.1 | 0.035 | 0.3 | 0.1 | 50.0 | 389.4 | 160.2 |
| **RSD** | - | 6% | 6% | - | 17% | 17% | - | 36% | 36% |

The RSD (%) measured reached our acceptance criteria

### Accuracy and Precision

**Concentration (QC low)**

| **Spots** | **Concentration according to curve (Y1) (µg/g)** | **Accuracy of Y1** | **Concentration according to curve (Y2) (µg/g)** | **Accuracy of Y2** | **Concentration according to curve (Y3) (µg/g)** | **Accuracy of Y3** |
|---|---|---|---|---|---|---|
| **Replicate 1** | 0.5 | 67% | 0.4 | 46% | 0.5 | 62% |
| **Replicate 2** | 0.8 | 100% | 0.5 | 72% | 0.8 | 104% |
| **Replicate 3** | 1.0 | 129% | 0.8 | 107% | 1.0 | 127% |
| **Average** | 0.8 | 99% | 0.6 | 75% | 0.7 | 97% |
| **Global Accuracy** | | | | | | 90% |
| **Global Precision** | | | | | | 32% |

**Concentration (QC high)**

| **Spots** | **Concentration according to curve (Y1) (µg/g)** | **Accuracy of Y1** | **Concentration according to curve (Y2) (µg/g)** | **Accuracy of Y2** | **Concentration according to curve (Y3) (µg/g)** | **Accuracy of Y3** |
|---|---|---|---|---|---|---|
| **Replicate 1** | 61.5 | 105% | 37.3 | 64% | 74.9 | 129% |
| **Replicate 2** | 75.7 | 126% | 58.5 | 106% | 46.6 | 81% |
| **Replicate 3** | 61.0 | 102% | 56.1 | 102% | 39.2 | 70% |
| **Average** | 66.1 | 111% | 50.6 | 91% | 53.6 | 93% |
| **Global Accuracy** | | | | | | 98% |
| **Global Precision** | | | | | | 24% |

Accuracy and Precision measured reached our acceptance criteria

## Tissue analysis (Molecular distribution)

Following the validation of the method for sample analysis in clinical trial, tissue sections were analyzed. The following example highlights the detection of Bimiralizib in one non lesional sample. The compound was distributed in the epidermis and in the papillary dermis.

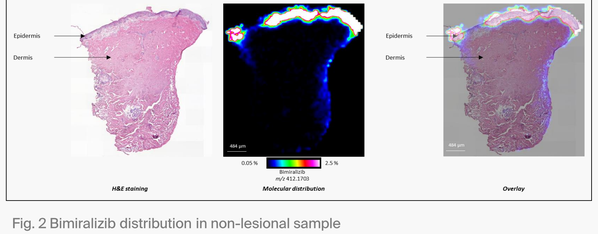


Fig. 2 Bimiralizib distribution in non-lesional sample

Note: Blue signal on the edges is a contamination by punching the skin; it does not reflect the penetration of Bimiralizib from the epidermis to the dermis.

## Tissue analysis (Quantification)

### Accuracy and Precision of low QCs for the tested samples

| Patient analysis related to the calibration curve | Spots | Concentration of spots according to curve (µg/g) | Accuracy of spots according to curve | Average of accuracy of spots according to curve | Precision of spots |
|---|---|---|---|---|---|
| Patient 1 | Replicate 1 | 0.88 | 109% | 122% | 25% |
| | Replicate 2 | 1.21 | 145% | | |
| | Replicate 3 | 0.76 | 110% | | |
| Patient 2 and Patient 3 | Replicate 1 | 0.78 | 114% | 120% | 6% |
| | Replicate 2 | 0.87 | 130% | | |
| | Replicate 3 | 0.8 | 115% | | |
| Patient 5 | Replicate 1 | 0.84 | 110% | 94% | 25% |
| | Replicate 2 | 0.87 | 117% | | |
| | Replicate 3 | 0.53 | 54% | | |
| Patient 6 | Replicate 1 | 0.89 | 124% | 106% | 16% |
| | Replicate 2 | 0.65 | 92% | | |
| | Replicate 3 | 0.72 | 101% | | |
| Patient 7 and Patient 8 | Replicate 1 | 0.81 | 113% | 105% | 5% |
| | Replicate 2 | 0.73 | 97% | | |
| | Replicate 3 | 0.8 | 105% | | |
| Patient 9 and Patient 10 | Replicate 1 | 0.52 | 71% | 89% | 18% |
| | Replicate 2 | 0.74 | 100% | | |
| | Replicate 3 | 0.71 | 96% | | |

### Concentrations measured in the tested samples and per region of interest

| Patient ID | Bimiralizib concentration (µg/g of tissue) | | |
|---|---|---|---|
| | Epidermis | Dermis | Entire |
| 1 | 1.4 | 2.8 | 3.1 |
| 2 | 4.3 | 3.2 | 4 |
| 3 | 3.3 | 0.5 | 1.2 |
| 5 | 0.6 | 0.8 | 0.9 |
| 6 | 0.4 | 0.4 | 0.5 |
| 7 | 3.2 | 10.6 | 9.7 |
| 8 | 2.2 | 0.3 | 1.7 |
| 9 | 0.3 | 0.5 | 0.9 |
| 10 | 0.2 | 0.2 | 0.2 |

## Conclusion

- Demonstration of MALDI MSI method robustness (sensitivity, accuracy and precision) for clinical assay.
- Approach not limited to dermatology; already applied in oncology clinical assay.
- Absolute quantification allowed to determine if the compound reached the site of action at a concentration above the IC50.
- In combination with systemic exposure measurements, this method allows to characterize small compound PK in clinical trial.
- In combination with spatial biology (immunostaining, transcriptomic), this method allows the characterization of the target engagement.