Validation of anti-ADC assays with ADA Characterization to support Micvotabart Pelidotin Clinical Studies

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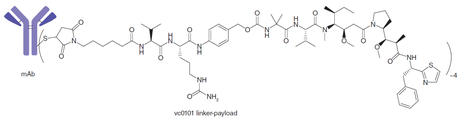
INTRODUCTION

Micvotabart pelidotin (MICVO, aka PYX-201) is an antibody–drug conjugate (ADC) that targets the non-cellular extradomain B of fibronectin (EDB+FN), an extracellular matrix protein highly expressed in tumors compared to normal adult tissues. MICVO consists of a fully human IgG1 monoclonal antibody with site-specific conjugation to a cleavable mcValCitPABC linker and optimized auristatin payload Aur0101, with a homogeneous drug-to-antibody ratio of four. MICVO is designed to bind the EDB splice variant of fibronectin in the tumor extracellular matrix where extracellular proteases cleave the linker and release the cytotoxic payload to diffuse into and kill tumor cells.



MICVO structure

Clinical evaluation of MICVO as a monotherapy (NCT05720117) and in combination with pembrolizumab for difficult-to-treat cancers (NCT06795412) is currently underway. Immune responses to MICVO by the generation of anti-drug antibodies (ADA) could impact the efficacy of the molecule. ADA assays were developed and validated to determine the presence of anti-MICVO in human plasma. The method is a bridging electrochemilumiescent (ECL) immunoassay that follows a tiered approach: screening → confirmation → titration. To prevent potential interference from soluble EDB+FN levels in cancer patients, a scavenger antibody was used in the ADA assay to displace the interferent.

ADAs may bind to different domains of MICVO, affecting its therapeutic function. Targeting the antibody portion can block MICVO from binding to EDB+FN. Binding to the linker-payload region may interfere with the release of the payload. Domain characterization assays were developed and validated to identify the ADA specificity. These method were successfully validated and are being used to support the on-going clinical trials.

METHOD

Matrix: K_2EDTA Human Plasma

Anti-MICVO Screening:

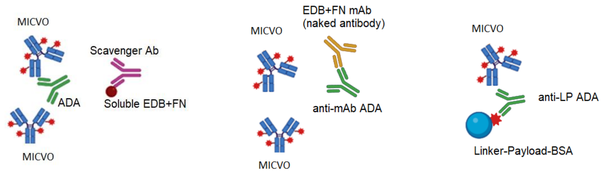
Samples, negative control, and positive controls were treated with 1.2M acetic acid to dissociate any complexes. After a 10 min incubation, sample/controls were added to a Master Mix solution containing Biotin-MICVO, sulfo-tag-MICVO, and anti-fibronectin 1 scavenger antibody in a neutralizing Tris buffer solution. After incubation, the mixture was transferred to a streptavidin coated MSD plate and incubated for 1 hour to allow for binding of the complexes to the plate. After washing, MSD GOLD Read Buffer A was added to the wells before reading the plate on the MSD Sector S 600 or Imager 6000. The ADAs present in plasma will form a complex with the labeled MICVO and produce a signal.

Anti-mAb Domain Specific Assay:

This method follows the screening assay but with the addition of 9.65 µg/mL EDB+FN mAb (naked antibody) to the Master Mix solution to compete out the anti-mAb specific ADAs.

Anti-Linker-Payload Domain Specific Assay:

Samples and controls were treated with 300mM acetic acid to dissociate any complexes. After neutralization with 0.2M Tris, samples were transferred to an MSD plate coated with 2ug/mL Linker-Payload-BSA and incubated, followed by the addition of sulfo-tagged MICVO. The plate was then washed and MSD GOLD Read Buffer A was added to the wells before reading on the MSD Sector S 600 or Imager 6000.



Anti-MICVO Screening Assay | Anti-mAb Specific Assay | Anti-LP Specific Assay

RESULTS

Assay Cut Point Summary

Tier	Cut Point
Screening	1.11
Anti-mAb	1.11
Anti-Linker-Payload	1.08

Sensitivity for anti-MICVO

Tier	Concentration
Screening	2.25 ng/mL
Anti-mAb	7.70 ng/mL
Anti-Linker-Payload	20.0 ng/mL

Tolerated MICVO Concentration

Tier	ADA Positive Control	MICVO Concentration
Screening	5000 ng/mL	> 200 ng/mL
	100 ng/mL	>200 ng/mL
	14 ng/mL	100 ng/mL

Summary of Validation Results

Validation Parameter	Criteria	Results	Status
Control Intra-Assay Precision	%CV ≤ 20% for Screening	Screening= 2.8 - 4.5 %CV Confirmation= 2.21 - 3.8%CV Anti-Linker-Payload= 1.69 – 7.51%CV	Pass
Control Inter-Assay Precision	%CV ≤ 20% for Screening	Screening= 11.1 - 14.9%CV Confirmation= 6.0 - 22.7%CV Anti-Linker-Payload= 10.8 – 14.3%CV	Pass
Soluble Target Interference (up to 200 ng/mL Human EDB + FN)	%CV ≤ 20%; positive controls will screen positive	1.1 - 12.3%CV; 100% positive control screened positive	Pass
Selectivity	≥ 80% of positive control will screen positive	100% positive control screened positive	Pass
Matrix Interference	%CV ≤ 20%; positive controls will screen positive	0.6 - 8.4%CV; 100% positive control screened positive	Pass

CONCLUSION

An ECL immunoassay method to evaluate the presence of anti-MICVO in human plasma was successfully validated. All validation parameters met acceptance based on the regulatory requirements for immunogenicity method validation[1,2]. The method is precise, sensitive, and was not impacted by soluble target interference or the sample matrix. The assay has been successfully applied in the immunogenicity evaluation of anti-MICVO in Pyxis Oncology sponsored clinical trial: phase I monotherapy study NCT05720117 and a Phase 1&2 combination trial with pembrolizumab NCT06795412.

REFERENCES

1. US FDA. Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection (2019).
2. EMEA. Guideline on Immunogenicity Assessment of Therapeutic Proteins (2017).