Development of a Novel CDx IHC Assay on the BOND-III to detect NaPi2b in Serous Ovarian Cancer Samples to Identify Patients Eligible for Treatment with Upifitamab Rilsodotin (UpRi)

Nnamdi Okeke[1], Aatish Patel[1], Eric Cowden, Olga Skende[1], Paul Krassnoff[1], Melissa Alexander[1], Rebecca Mosher[2], Ina Menzl[1]

1. Leica Biosystems, Inc., Danvers, MA, U.S 2. Mersana Therapeutics, Inc., Cambridge, MA

Introduction

NaPi2b, a sodium-dependent phosphate transporter protein, is broadly expressed in solid tumors such as serous epithelial ovarian cancer (OC) and non-small cell lung adenocarcinoma with limited expression in healthy tissues. A novel human-rabbit chimeric antibody (Ab) has been developed as a proposed Companion Diagnostic immunohistochemical (IHC) reagent as a fully automated IHC assay system (hereafter referred to as NaPi2b (67) Assay) on the BOND-III immunostainer (BOND-III) for detection of the NaPi2b protein in formalin-fixed, paraffin-embedded (FFPE) OC tissue. The NaPi2b (67) Assay is currently under development with the intent to be indicated as an aid in identifying OC patients who may have increased probability of clinical benefit following treatment with upifitamab rilsodotin (UpRi), a first-in-class Dolaflexin based antibody-drug conjugate (ADC), targeting NaPi2b-expressing tumor cells. The NaPi2b (67) Assay is not an FDA approved device, and the use is restricted only for investigational purposes.

Methods

The NaPi2b (67) Assay System is comprised of the NaPi2b (67) primary antibody (Investigational Use Only), the NaPi2b (67) Cell Line Control (Investigational Use Only), the BOND Polymer Refine Detection kit, the BOND Ready-to-Use Negative Control (Rabbit). Samples used for developing this assay were sectioned at 4 µm thickness, mounted on positively charged glass slides, dried overnight, and baked for 30 minutes at 60°C prior to being placed on the BOND-III for deparaffinization. A ready-to-use NaPi2b (67) Cell Line Control slide was run on each slide staining assembly (SSA) containing a test sample during execution of the runs (**Figure 4**). The BOND-III immunostainer protocol, and the NaPi2b (67) Assay staining, and interpretation workflow are summarized and defined in **Table 1** and **Figure 1,** respectively.

Table 1. BOND Protocol Summary

BOND Protocol Type	Protocol Name	Comments
Preparation	Dewax	72°C for 30 seconds
Pre-treatment	HIER 20 min with ER1	100°C for 20 minutes, pH 6
Staining	IHC Protocol P	15-minute primary antibody incubation (ambient)

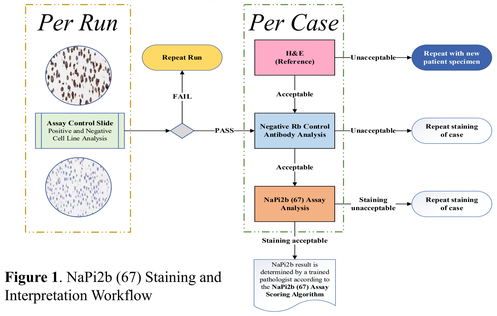

Figure 1. NaPi2b (67) Staining and Interpretation Workflow

NaPi2b (67) Assay sensitivity (prevalence) was performed using whole slide sections from 397 commercially procured OC samples. Additionally, specificity, robustness, and precision were evaluated. Precision of this assay was conducted on a set of 72 OC FFPE samples to verify that sections from the same FFPE sample showed similar staining when run on the same and on different SSA's within a BOND-III instrument (within run and between run), on 3 different BOND-III instruments (between instrument), 5 non-consecutive days over a period of 20 days (between days) and using 3 reagent lots (between antibody and between detection lots). A set of 50 unique OC samples was selected from the precision study sample population and scored by 3 trained pathologists independently. Specificity of this assay was determined by flow cytometry (FC) analysis of well-characterized cell lines and by surveying reactivity in tissue microarrays (TMA) that included various normal and neoplastic tissues.

Results

Data presented are informational only and are not intended to be inferred as claims of safety and/or effectiveness of the device under development. All samples were scored using a ≥75% tumor proportion score (TPS) cut-off to determine frequency of high NaPi2b expressing samples as seen in **Fig 2.** The cut-off was determined by correlating NaPi2b score with clinical outcome. An H-score of 110 was used in previous publications as a cut-off, which has a similar frequency as the TPS, 62.5% and 58.9%, respectively.

A shift in signal >3 orders was observed by flow cytometry when OVCAR3 cells were stained by NaPi2b (67) Ab, as compared with a non-expressing cell line as seen in **Figure 3**. Immunoreactivity was observed in normal tissue and non-ovarian tumor specimens (data not shown) consistent with previously published data[2,6,7]. For each precision study, the TPS value from each section of a unique sample was used to determine a majority score which was used as the reference for agreement analysis. The between reader study employed a similar approach, where the majority score from 3 pathologists was used to determine whether the unique sample was positive or negative for use as the reference for agreement analysis (see **Tables 2** and **3**).

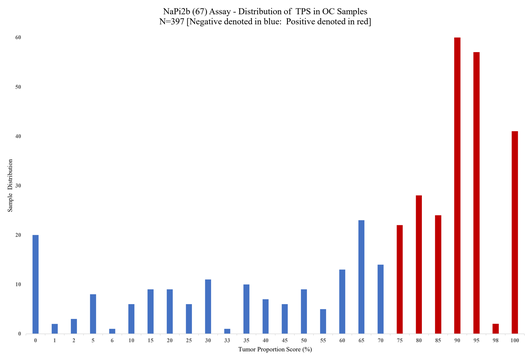

Figure 2. Distribution of staining 397 unique serous ovarian cancer samples (sensitivity/prevalence).

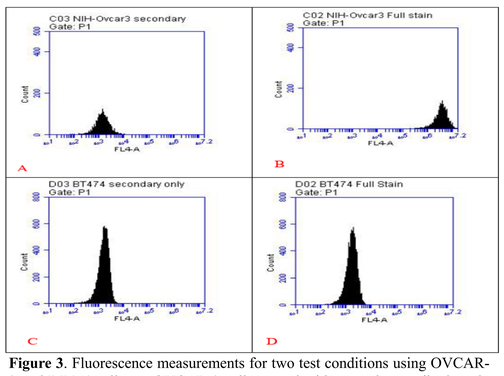

Figure 3. Fluorescence measurements for two test conditions using OVCAR-3 and BT474 cells: **A**. OVCAR-3 cells treated with secondary antibody only **B.** OVCAR-3 cells treated with both NaPi2b (67) and secondary antibody **C.** BT474 cells treated with secondary antibody only **D.** BT474 cells treated with both NaPi2b (67) and secondary antibody.

Results (cont'd)

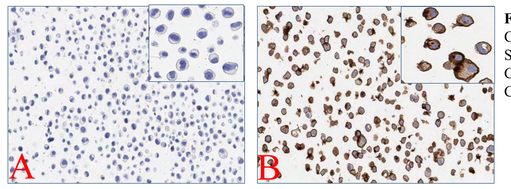

Figure 4. NaPi2b (67) Cell Line Control slide Acceptable Staining **A**. Negative Cell Line Control **B.** Positive Cell Line Control

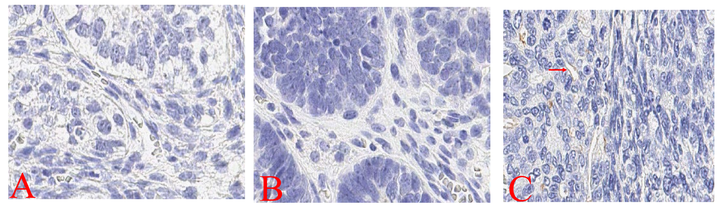

Figure 5. Absence of NaPi2b (67) staining in tumor cells **A.** and **B.** No detectable staining in membrane of tumor cells **C**. Weak, faint luminal staining (red arrow). All images scanned at 20x magnification using Aperio AT2 scanner from Leica Biosystems.

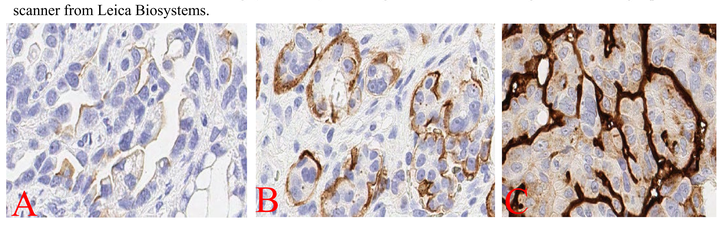

Figure 6. NaPi2b (67) staining pattern in tumor cells **A**. Weak plasma membrane staining not readily visible at low scanning magnification **B**. Moderate plasma membrane staining visible at low/scanning magnification **C**. Strong membrane staining easily visible at low/scanning magnification. All images scanned at 20x magnification using Aperio AT2 scanner from Leica Biosystems.

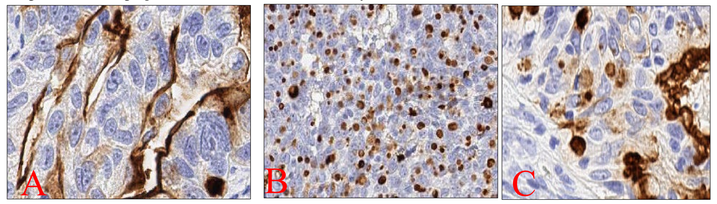

Figure 7. Three unique cases stained using NaPi2b (67) Assay **A.** Exhibits apical membrane staining **B.** Depicts aggregate staining **C**. Exhibits mix of aggregate and membrane staining. All images scanned at 20x magnification using Aperio AT2 scanner from Leica Biosystems.

Table 2. Agreement analysis for NaPi2b (67) staining precision studies (2x2 table)

Within run		Majority Score		Agreement, % (95% CI)	
		NEG	POS		
NaPi2b Status	NEG	109	0	NPA	98.2 (93.7-99.5)
	POS	2	105	PPA	100.0 (96.5-100.0)
	Total	111	105	OPA	99.1 (96.7-99.7)
Between-run		**Majority Score**		**Agreement, % (95% CI)**	
		NEG	POS		
NaPi2b Status	NEG	107	2	NPA	99.1 (94.9-99.8)
	POS	1	106	PPA	98.1 (93.5-99.5)
	Total	108	108	OPA	98.6 (96.0-99.5)
Between days (5 days)		**Majority Score**		**Agreement, % (95% CI)**	
		NEG	POS		
NaPi2b Status	NEG	253	20	NPA	93.7 (90.1-96.0)
	POS	17	250	PPA	92.6 (88.8-95.2)
	Total	270	270	OPA	93.1 (90.7-95.0)

Results (cont'd)

Table 3. Agreement analysis for NaPi2b (67) staining precision studies contd. (2x2 table)

Between Instruments (3 Instruments)		Majority Score		Agreement, % (95% CI)	
		NEG	POS		
NaPi2b Status	NEG	137	10	NPA	95.1 (90.3-97.6)
	POS	7	170	PPA	94.4 (90.1-97.0)
	Total	144	180	OPA	94.8 (91.8-96.7)
Between Antibody Lots (3 Ab Lots)		**Majority Score**		**Agreement, % (95% CI)**	
		NEG	POS		
NaPi2b Status	NEG	144	10	NPA	95.1 (90.3-97.6)
	POS	0	170	PPA	94.4 (90.1-97.0)
	Total	144	180	OPA	94.8 (91.8-96.7)
Between Detection Lots (3 Det Kit Lots)		**Majority Score**		**Agreement, % (95% CI)**	
		NEG	POS		
NaPi2b Status	NEG	177	0	NPA	98.3 (95.2-99.4)
	POS	3	144	PPA	100.0 (97.4- 100.0)
	Total	180	144	OPA	99.1 (97.3-99.7)
Between reader (3 Pathologists)		**Majority Score**		**Agreement, % (95% CI)**	
		NEG	POS		
NaPi2b Status	NEG	70	2	NPA	97.2 (90.4-99.2)
	POS	2	76	PPA	97.4 (91.1-99.3)
	Total	72	78	OPA	97.3 (93.3-99.0)

The results of agreement analysis among sections from one sample for within run and between run (**Table 2**), between days, between instruments, and between reagent lots (**Table 3**) show high agreement rates. Furthermore, the reader precision results show high agreement between readers when evaluating the same sample independently (**Table 3**, bottom).

Conclusion

The NaPi2b (67) Assay system stains OC tissues exhibiting membrane staining, apical membrane staining, focal aggregate staining, and a mix of apical membrane and aggregate staining (see **Figures 6 and 7**). The NaPi2b (67) Assay has a dynamic range from 0 – 100% TPS NaPi2b expression in OC samples (**Fig 2**). The high agreement rates resulting from the precision studies shown in **Tables 3** and **4** suggest that OC samples can be reproducibly stained on the BOND-III using the NaPi2b (67) Assay. Furthermore, the results suggest that readers can evaluate samples stained with the NaPi2b (67) Assay with high precision. This investigational assay is currently being evaluated as a potential CDx in the UPLIFT Study Cohort of UpRi in OC (NCT03319628) clinical trial. The NaPi2b (67) Assay is not an FDA approved device, and the use is restricted only for investigational purposes.

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