MERS67 is a Novel anti-NaPi2b Antibody and Demonstrates Differential Expression Patterns in Lung Cancer Histologic Subtypes

Hui Yu[(1)], Rebecca Mosher[(2)], Kim Ellison[(1)], Pamela Shaw [(2)], Rafal Dziadziuszk[(3)], Eric Hailman[(2)], Christopher J. Rivard[(1)] and Fred R. Hirsch[(1)]

1. Division of Medical Oncology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, United States of America 2.Mersana Therapeutics Inc., Cambridge, Massachusetts, United States of America 3. Department of Oncology and Radiotherapy, Medical University, Gdansk, Poland.

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Introduction

NaPi2b (SLC34A2) is a sodium-dependent phosphate transporter expressed in lung, ovarian, and thyroid cancers. Prior studies and publicly available expression data have suggested an enrichment of expression in lung adenocarcinoma (1, 2, Figure 1).
XMT-1536 is a NaPi2b targeting ADC (Antibody Drug Conjugate) comprised of a humanized antibody (XMT-1535) conjugated with 10-15 auristatin F-hydroxypropylamide (AF-HPA) payload molecules via the Dolaflexin platform (Figure 2). AF-HPA is capable of controlled bystander-effect killing, resulting in efficacy in models with heterogeneous antigen expression, and is metabolized intra-tumorally to an active non-permeable metabolite to enable greater systemic tolerability.
Previously, we demonstrated pre-clinical activity of XMT-1536 in human primary xenograft models of non-small cell lung cancer (NSCLC). (IASLC, 2016)
MERS67 is a human-rabbit chimeric antibody derived from XMT-1535. MERS67 has been formatted for use as an immunohistochemical (IHC) reagent by multiple methods and expression has been shown to correlate with response in an unselected series of primary ovarian cancer xenografts. (AACR-EORTC, 2017)
We evaluated MERS67 to see if it would preferentially stain lung adenocarcinoma, as has been demonstrated using other NaPi2b antibodies.

Figure 1 RNAseq data was extracted from cBioPortal (3,4; data of June 27, 2018) and demonstrates differential expression of SLC34A2/NaPi2b between Lung Squamous and Adenocarcinoma. Data from ovarian cancer samples is also shown as a comparator.

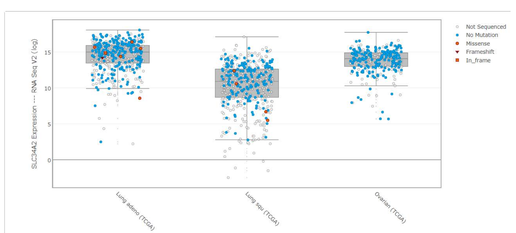
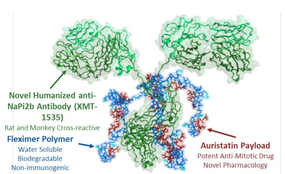


Figure 2 Graphic Rendering of XMT-1536, a NaPi2b Targeting Antibody Drug Conjugate. MERS67 is a human/rabbit chimeric antibody derived from XMT-1535.



Methods

An IHC assay for MERS67 was established on a Leica BondRx Instrument. The assay was performed on a tissue microarray (TMA) of NSCLC tumor tissues. Tumors in the NSCLC array had previously been classified based on morphologic features only. All arrays were scored based on the H-score method. (H= [1 × (% cells 1+) + 2 × (% cells 2+) + 3 × (% cells 3+)])

To characterize the primary tumors further, the tumor TMA was stained with TTF-1 and p40, markers of adenocarcinoma (ACA) and squamous cell carcinoma (SqCC), respectively. Results of this staining were compared to the MERS67 staining patterns.

An additional IHC experiment was performed on cell line TMAs representing NSCLC and small cell lung cancer. To explore the effect of media composition on Napi2b expression two cell lines, H1975 and HCC78, representing high and low levels of NaPi2b were cultured in RPMI (5.63 mM PO_4^{2-})(5) with 10% FBS supplement and MEM, (1.01 mM PO_4^{2-})(5) with no FBS supplement, and RNA expression of NaPi2b was evaluated. For this experiment triplicate wells of the two cell lines were grown in RPMI, described above and then continued in RPMI or switched to MEM. Cells were collected at a 0, 24, or 48 hour time-point and RNA was extracted and quantitated using an SLC34A2 ABI assay relative to a housekeeping gene. RNA values were expressed as RQ=$2^{-\text{delta cT value}}$. 25 ng of cDNA input was used for HCC78, and 200 ng of cDNA was used for H1975 due to the low baseline level of expression for this line.

NaPi2b is Differentially Expressed in Human NSCLC Tumors

Figure 3A NaPi2b H-scores entire set

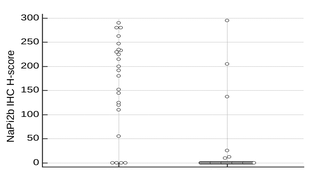
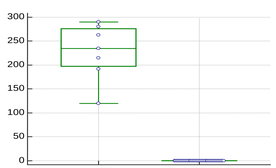


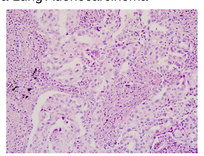
Figure 3B NaPI2b H scores in samples with tumor differentiation confirmed

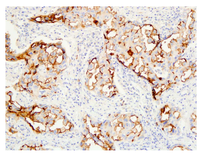


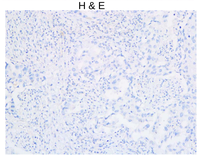
Within the tumor tissue microarray, 99 individual cases were evaluable. By morphologic classification 63 cases were SqCC, and 23 cases were ACA. Using an arbitrary cut point of H=50, there was a statistically significant difference in the number of NaPi2b positive ACA cases (19/23) vs SqCC (3/63) (Figure 3A). Among 43 cases where p40 and TTF-1 were evaluable and were in agreement with morphologic diagnosis, 7/7 cases of ACA were positive for NaPi2b, while 0/36 SqCC were positive (Figures 3B, 4A, and 4B).

MERS67 IHC in Human Lung Tumors

Figure 4a Lung Adenocarcinoma







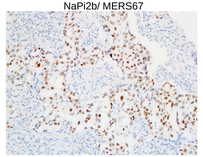
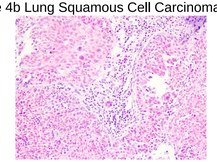
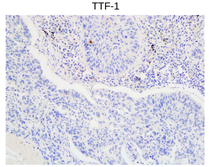
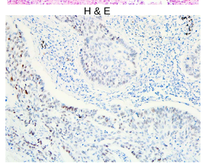
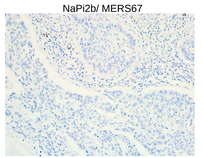


Figure 4b Lung Squamous Cell Carcinoma









Immunoreactivity with NaPi2b/MERS67 was noted in ACA (p40 negative/TTF-1 positive) samples (Figure 4a)
In comparison lung SCC (p40 positive/TTF-1 negative) were not immunoreactive for NaPi2b/MERS67 (Figure 4b)

NaPi2b expression in cell lines may be impacted by media phosphate levels

Figure 5a NaPi2b H-scores in a cell line TMA (lines used in experiment below are highlighted)

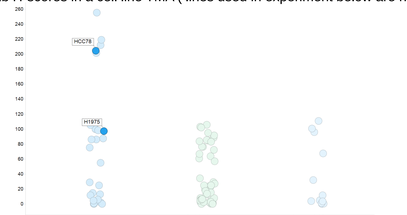


Figure 5b NaPi2b RNA in a high expressing cell line, HCC78, following media change

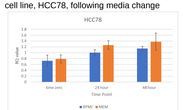
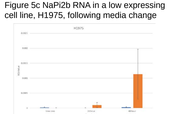


Figure 5c NaPi2b RNA in a low expressing cell line, H1975, following media change



Unlike in tumor tissue samples, where NaPi2b IHC appeared higher in ACA vs SqCC, in cell lines the distinction was not as apparent, and expression of NaPi2b in ACA cell lines was generally lower (Figure 5a)
In HCC78, a shift from a high phosphate media to a low phosphate media did not cause a significant increase in NaPi2b RNA expression (Figure 5b)
In H1975, a cell line with barely detectable NaPi2b RNA at baseline, shift to a low phosphate media resulted in an modest increase in NaPi2b RNA expression, although overall values were low (Figure 5c)

Summary

- MERS67 is an IHC reagent that is a rabbit chimera of XMT-1535, the humanized antibody component of the ADC XMT-1536
- MERS67 shows differential expression between squamous cell and adenocarcinoma tumor tissues and the effect is accentuated when the tumors are classified by TTF-1 and p40 IHC, rather than by morphology alone.
- NaPi2b expression in cell lines tends to be low and expression may be impacted by media composition although additional experiments, including analysis at the protein level, are needed to confirm this hypothesis.

1) D'Arcangelo, et al, ESMO 2014

2) Lin et al, Clinical Cancer Research, 2015

3) Cerami et al, Cancer Discov, 2012

4) Gao et al., Sci Signal, 2013

5) McKee and Komarova, Am J Physiol Cell Physiol, 2017

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