Nanoliposomal Targeting of Ephrin Receptor A2 (EphA2): Clinical Translation

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Abstract

Ephrin receptor A2 (EphA2) is part of the Ephrin family of cell-cell junction proteins highly overexpressed in several solid tumors, and is associated with poor prognosis. We developed a novel EphA2-targeted docetaxel nanoliposome, leveraging organ specificity through the enhanced permeability and retention effect and cellular specificity through EphA2 targeting. The goal of the study was to develop the diagnostic framework enabling the clinical implementation of EphA2-based exclusion criteria in future MM-310 trials.

We used qFACS and an *in vitro* assay for liposome (Ls)-cell interaction to identify the minimum number of EphA2 receptors to enable antibody-mediated internalization of Ls. We developed an IHC assay able to differentiate EphA2 - vs + cell lines. We characterized EphA2 staining pattern in tumor samples of various indications and developed a scoring algorithm that allows selection of patients in early clinical trials.

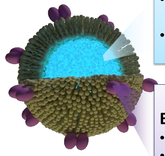
While non-targeted Ls do not associate with cells *in vitro*, there is a strong correlation between EphA2 expression and EphA2-Ls cell association independent of the cell line origin. We used the non-targeted Ls to determine the extent of non-specific binding that can be achieved (~340 Ls/cell) and used partitioning to determine the minimum number of EphA2 receptors necessary to mediate targeting (~3000 receptors/cell). We have developed and validated a qIHC assay for EphA2 (precision ~90%, linearity 0.8 and reproducibility CV<5%). We stained a set of ~200 tumor samples from various indications. EphA2 was found to be expressed in tumor cells, tumor-associated myofibroblasts, and tumor-associated blood vessels. Using an inclusive cutoff of 10%, EphA2 prevalence was found to range from 50% to 100% in the tumor types evaluated. No significant difference in staining was seen between metastasis and primary tumors in matched samples.

In summary, we developed a diagnostic framework for prospective selection of EphA2+ patients for MM-310 trials based on a mechanistic single cell cut-off and a clinical-grade IHC assay.

	Cancer Cells	Tumor associated myofibroblasts	Tumor associated blood vessels	EphA2 Overall Score
Bladder	19/20 (95%)	0/20 (0%)	16/20 (80%)	19/20 (95%)
Gastric	18/20 (90%)	3/20 (15%)	17/20 (85%)	20/20 (100%)
Head & Neck	16/19 (84%)	0/19 (0%)	9/19 (47%)	19/19 (100%)
Lung	24/41 (58%)	1/41 (2.4%)	24/41 (58%)	28/41 (68%)
Lung-FNA	7/9 (78%)	--	--	7/9 (78%)
Ovarian	10/18 (55%)	7/18 (39%)	17/18 (95%)	17/18 (95%)
Pancreatic	15/19 (79%)	0/19 (0%)	11/19 (58%)	17/19 (89%)
Prostate	7/23 (27%)	7/23 (27%)	9/23 (28%)	12/23 (52%)
TNBC	6/77 (7%)	0/77 (0%)	34/77 (44%)	37/77 (48%)

Introduction

MM-310



Docetaxel Prodrug Nanoliposome
- Formulation extends drug circulation time in pre-clinical models with reduction in hematological toxicities compared to docetaxel.
- Liposome deposition leads to sustained release at the tumor site.

EphA2 Targeting
- Targets EphA2-expressing cancer cells in tumors.
- Targets is expressed in tumor-associated blood vessels.
- Leads to more pronounced and sustained tumor regression *in vivo*.

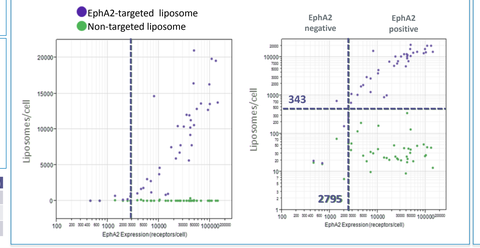
Determination of number of receptors required for targeted liposome uptake

Panel of 78 cell lines including tumor types reported in literature to be high in EphA2 (NSCLC n=23, BrCA n=23, OvCA n=19)

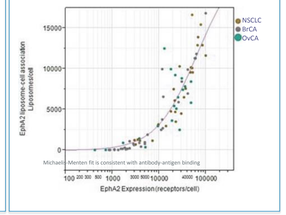
- Quantify EphA2/cell by flow cytometry with calibration to labeled beads (BD Biosciences Quantibrite™ PE kit).
- Measure target-specific liposome uptake by incubating cells with fluorescently-conjugated liposomes (EphA2-targeted and non-targeted).
- Preparation of FFPE cell pellets for IHC reference array.

	EphA2/Cell	Liposomes/cell	Docetaxel Load (n/cell)
EphA2 Negative	1438 ± 147	149 ± 57	$5.9 \times 10^{-6} \pm 2 \times 10^{-6}$
EphA2 Low	4099 ± 336	946 ± 104	$37 \times 10^{-6} \pm 4 \times 10^{-6}$
EphA2 High	36744 ± 3386	8065 ± 551	$321 \times 10^{-6} \pm 21 \times 10^{-6}$

EphA2-dependent liposome uptake requires >~3000 receptors/cell

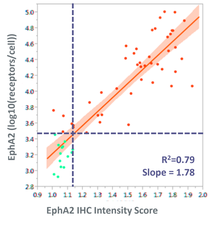


Relationship between *in vitro* targeted liposome uptake and receptor expression is similar in lung, breast, and ovarian cancer cells



Optimized IHC assay for EphA2 shows consistent visible staining at minimum receptor level for targeted liposome uptake

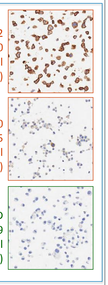
Repeated assays on cell pellet array show consistent correlation to qFACS data for matched cell lines and slope



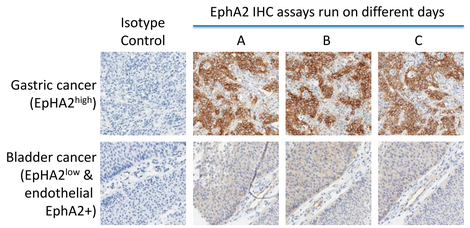
	n	R-Squared	Mean Slope	Intra-assay CV%
Run1	3	0.79	1.80	9.3
Run2	3	0.80	1.70	4.4
Run3	3	0.79	1.70	2.3
			Mean Slope	Inter-Assay CV%
		InterRun	1.76	3.3

Rabbit monoclonal anti-EphA2 (Cell Signaling Technology, #6997)

Visible EphA2 staining in cells with >3000 receptors/cell



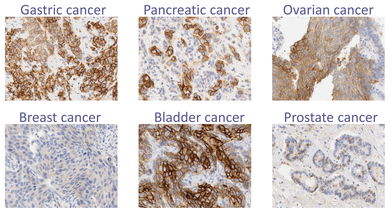
EphA2 IHC assay is reproducible in tumor tissues



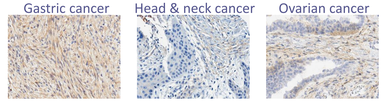
Pathologists consistently scored samples with high or low EphA2 signal from assays run on different days by different operators.

Human tumor survey: EphA2 expression in tumors, stroma, and tumor-associated blood vessels

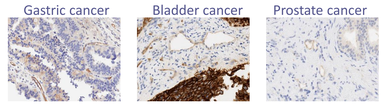
EphA2 expression on tumor cells is usually observed on the cell membranes, with dim cytoplasmic signal also scored for intensity



EphA2 expression in tumor-associated myofibroblasts



EphA2 expression in blood vessels

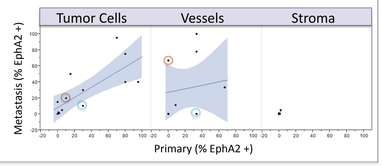


Prevalence of EphA2 in primary tumors and metastases (Ovarian cancer)

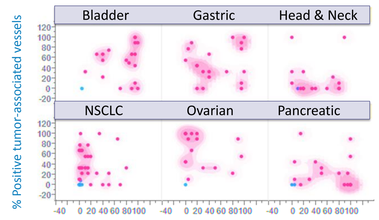
EphA2-positive primary tumors usually also showed EphA2 expression in metastases from same patient.

Metastases \ Primary tumors	EphA2-	EphA2+
EphA2-	2 (16%)	0
EphA2+	1 (8%)	9 (75%)

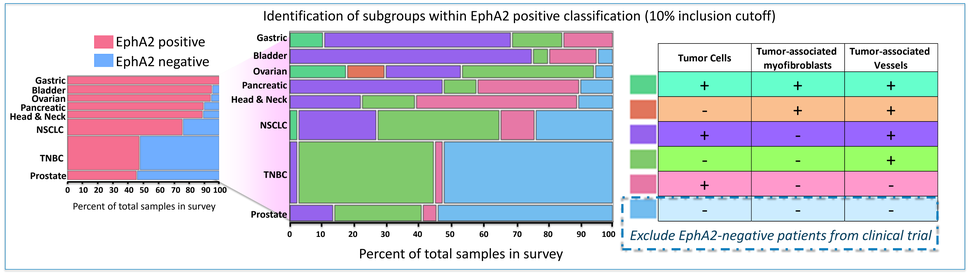
Observed agreement between primary tumor and metastasis is based on tumor cell EphA2 expression in this sample set.



EphA2 prevalence and plan for scoring clinical samples



Identification of subgroups within EphA2 positive classification (10% inclusion cutoff)



Summary

- *In vitro* cell binding data was used to identify minimum number of EphA2/cell to allow targeted liposome uptake.
- Immunohistochemistry assay for EphA2 in formalin-fixed, paraffin-embedded tissues was analytically validated and used to survey human tumors from several indications.
- EphA2 was observed in tumor cells, stroma, and in tumor-associated blood vessels, and was consistently expressed in matched primary tumors and metastases.
- EphA2 negative patients will be excluded from clinical trials based upon prospective screening results. Retrospective analysis of EphA2 compartment contributions when patient outcome data is available will be used to refine inclusion criteria to best serve patients who would benefit from MM-310.