Glycoprotein NMB (gpNMB) Overexpression is Prevalent in Human Cancers: Pancreatic Cancer, Non-Small Cell Lung Cancer, Head and Neck Cancer, and Osteosarcoma

Abdel Halim, Rebecca G. Bagley, Tibor Keler

Celldex Therapeutics, Inc., Hampton, NJ

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INTRODUCTION

Glycoprotein NMB (gpNMB) is an internalizable transmembrane protein overexpressed in 20% of breast cancers, 40% of triple-negative breast cancer (TNBC), and > 80% of melanomas.

Glembatumumab vedotin (GV; CDX-011) is an antibody-drug conjugate (ADC) that delivers the potent cytotoxin monomethyl auristatin E to cancer cells expressing gpNMB. GV is in Phase II clinical trials for TNBC (the pivotal "METRIC" study; NCT01997333) and melanoma (NCT02302339).

We investigated the prevalence of gpNMB overexpression in other human cancers to explore the potential for therapeutic benefit of GV beyond TNBC and melanoma.

MATERIALS & METHODS

An immunohistochemistry (IHC) assay was developed and validated (Mosaic Laboratories, Lake Forest, CA) using the following setup:

- Antibody: R&D goat polyclonal antibody
- Autostainer: DAKO
- Pretreatment: HIER (97°C), High pH (Dako)
- Detection system: Rabbit Anti-Goat (Vector Laboratories) + PowerVision Poly AP Anti-Rabbit IgG (Leica)
- Chromogen: DAB
- Counterstain: Hematoxylin (Dako)

For osteosarcomas, tissue sections were decalcified using Immunocal and Cal-Arrest reagents (American MasterTech)

FFPE tumor and normal (non-malignant) tissues (Mosaic Labs):

- Lung: 20 each squamous cell carcinoma (SCC), adenocarcinoma, and normal tissues
- Pancreas: 20 tumors, 21 normal tissues
- Head & Neck: 20 tumors, 21 normal tissues
- Bone: 21 osteosarcomas, 0 normal bone tissue available.

Slides were scored by 2 pathologists for % of positive tumor epithelial cells and staining intensity (0, 1+, 2+, 3+).

ASSAY DEVELOPMENT & VALIDATION PARAMETERS

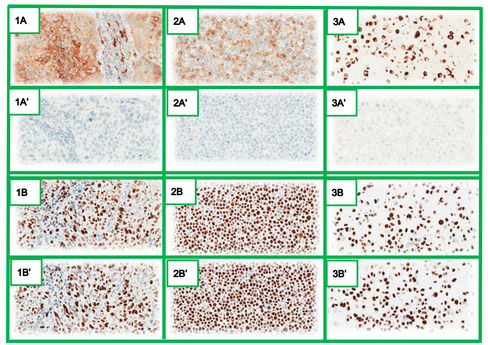
In addition to optimization of antigen retrieval & binding conditions and establishment of the optimal antibody dilution using human tissues and cell lines, the following parameters were addressed:

- Selectivity of binding
- Precision: Intra- and inter-run reproducibility
- Inter-pathologist review variability

SELECTIVITY OF BINDING

- A peptide blocking study was performed to demonstrate specificity of the antibody binding for the target gpNMB by using a gpNMB peptide at excess molar ratio to block the binding.
- 1 NSCLC SCC tissue and 2 cell lines (HeLa and SK-MEL-28) were stained without (images 1A-3A) and with (1A'-3A') the blocking peptide.
- To ensure that lack of staining was due to specific reaction, split slides were stained for Ki67 without (1B-3B) and with (1B'-3B') the gpNMB peptide.
- As shown by the following images, gpNMB but not Ki67 staining was ablated with the peptide which proved the concept.

SPECIFICITY OF STAINING



PRECISION

- An intra-run precision was tested using 4 samples (testis control, HT-1080 cell line, and 2 positive NSCLC) in 3 replicates on a single run and the average CV for % stained cells and H-scores was 0.0% and 3.3%.
- The same samples were stained and scored on 6 separate runs and the average inter-run CV was 3.3% for % stained cells and 8.9% for H-Score.
- Pathologist-to-pathologist score difference in total stained cells and H-score was <25% in 93.3% (56/60) of samples where the remaining 4/60 samples were within 30-50%.

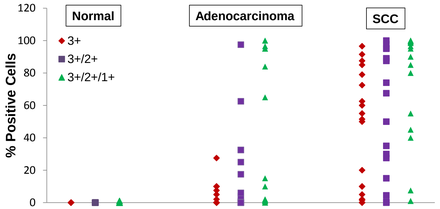
RESULTS

Following assay validation, target prevalence was studied in multiple cancer types and corresponding normal (non-malignant) tissues. Data shown represents expression in tumor epithelial cells.

LUNG

% of Tumor Epithelial Cells Expressing at Different Cut-Offs from Total

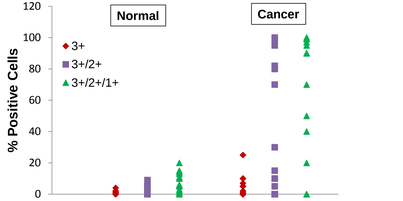
	Normal (n=20)			Adenocarcinoma (n=20)			SCC (n=20)		
Cut-off	3+	3/2+	3/2/1+	3+	3/2+	3/2/1+	3+	3/2+	3/2/1+
≥ 1%	0.0	0.0	10.0	35.0	45.0	85.0	85.0	85.0	100.0
≥ 10%	0.0	0.0	0.0	20.0	30.0	45.0	70.0	80.0	85.0
≥ 25%	0.0	0.0	0.0	5.0	25.0	35.0	55.0	75.0	85.0
≥ 50%	0.0	0.0	0.0	0.0	10.0	35.0	55.0	60.0	75.0



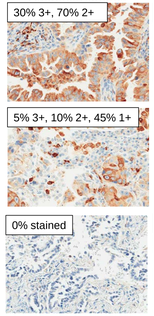
PANCREAS

% of Tumor Epithelial Cells Expressing at Different Cut-Offs from Total

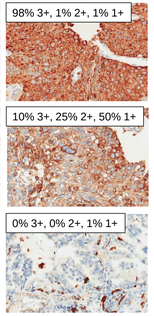
	Normal (n=21)			Cancer (n=20)		
Cut-off	3+	3/2+	3/2/1+	3+	3/2+	3/2/1+
≥ 1%	42.9	66.7	90.5	45.0	60.0	75.0
≥ 10%	0.0	0.0	33.3	15.0	55.0	75.0
≥ 25%	0.0	0.0	0.0	5.0	40.0	70.0
≥ 50%	0.0	0.0	0.0	0.0	35.0	65.0



Adenocarcinoma



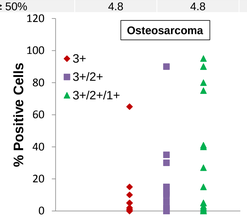
SCC

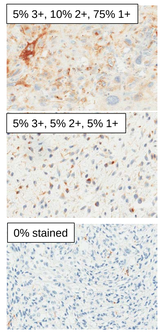


OSTEOSARCOMA

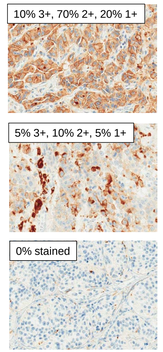
% of Tumor Epithelial Cells Expressing at Different Cut-Offs from Total

	Osteosarcoma (n=21)		
Cut-off	3+	3/2+	3/2/1+
≥ 1%	47.6	61.9	76.2
≥ 10%	14.3	38.1	47.6
≥ 25%	4.8	14.3	42.9
≥ 50%	4.8	4.8	23.8





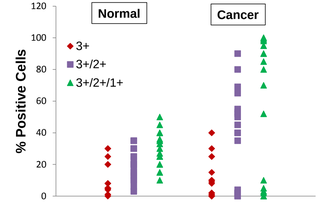
Adenocarcinoma

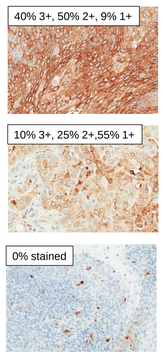


HEAD & NECK

% of Tumor Epithelial Cells Expressing at Different Cut-Offs from Total

	Normal (n=21)			Cancer (n=20)		
Cut-off	3+	3/2+	3/2/1+	3+	3/2+	3/2/1+
≥ 1%	9.52	100.0	100.0	75.0	90.0	90.0
≥ 10%	23.8	90.5	100.0	45.0	60.0	70.0
≥ 25%	14.3	38.1	76.2	25.0	60.0	65.0
≥ 50%	0.0	0.0	4.8	0.0	40.0	65.0





CONCLUSIONS

- The gpNMB IHC assay is validated for use in clinical trials for patients with breast cancer, melanoma, lung, pancreatic, head and neck cancer, or osteosarcoma.
- Over-expression of gpNMB in human pancreatic, lung, H&N cancers and osteosarcoma samples suggests that these indications are appropriate for evaluating the clinical activity of glembatumumab vedotin.
- In addition to TNBC and melanoma, studies have been initiated in osteosarcoma and uveal melanoma; a study is planned in SCC of the lung.
- If early phase clinical trials show a predictive value in these new indications, then the IHC test can be used as a companion diagnostics for further development.