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

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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. Glumetuzumab 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 in patients with 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
- Detection system: Rabbit Anti-Goat (Vector Laboratories) + PowerVision Poly AP Anti-Rabbit IgG (Leica)
- Counterstain: Hematoxylin (Dako)
- Autostainer: DAKO
- Antibody: R&D goat polyclonal antibody
- PowerVision Poly AP Anti-Rabbit IgG (Leica)
- Counterstain: Hematoxylin (Dako)

For osteosarcomas, tissue sections were decalcified using Immunocal

In addition to optimization of antigen retrieval & binding conditions, the following setup was used:

- Pathologist-to-pathologist score difference in total stained cells
- 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.
- As shown by the following images, gpNMB but not Ki67 staining was ablated with the peptide which proved the concept.

RESULTS

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.

SPECIFICITY OF STAINING

- 1 NSCLC SCC tissue and 2 cell lines (Hela and SK-MEL-29) 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.

PRECISION

- An intra-run precision was tested using 4 samples (testis control, HT-1080 cell line, and 2 positive NSCLC) in 3 runs and the average inter-run CV was 3.3% for % stained cells and 8.9% for H-Score.
- 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% (80/85) of samples where the remaining 12% samples were within 30-50%.

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

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.

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>% Positive Cells</th>
<th>Normal (n=20) Cancer (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (n=21) Cancer (n=22)</td>
<td>Normal (n=20) Cancer (n=20)</td>
</tr>
<tr>
<td>Lung</td>
<td>5% 3+, 10% 2+, 5% 1+</td>
<td>5% 3+, 10% 2+, 5% 1+</td>
</tr>
<tr>
<td>Head &amp; Neck</td>
<td>20% 3+, 70% 2+</td>
<td>20% 3+, 70% 2+</td>
</tr>
<tr>
<td>Pancreas</td>
<td>50% 1+, 50% 2+</td>
<td>50% 1+, 50% 2+</td>
</tr>
<tr>
<td>Bone</td>
<td>25% 1+, 75% 2+</td>
<td>25% 1+, 75% 2+</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>40% 3+, 60% 2+</td>
<td>40% 3+, 60% 2+</td>
</tr>
<tr>
<td>Head &amp; Neck</td>
<td>10% 3+, 25% 2+, 55% 1+</td>
<td>10% 3+, 25% 2+, 55% 1+</td>
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<tr>
<td>Osteosarcoma</td>
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</tr>
</tbody>
</table>

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 glumetuzumab 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.