The Role of Checkpoint Inhibitor PD-1H/VISTA in Multiple Myeloma Bone Disease

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**Disclosure**

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The remaining authors declare no competing financial interests.
MMP-13 induces osteoclast fusion

MMP-13 KD inhibits MM induced lytic bone lesion
MMP-13 is highly expressed in MM and correlates with bone disease

A

Healthy donors

MM patients

B

MMP-13 serum concentration (pg/ml)

Healthy Donors

MMBD Patients

No MMBD Patients

### Screening of MMP-13 binding protein

#### A

**MMP-13 Pull-down Mass Spectrometry Assay**

1. Bone Marrow
2. Non-adherent Mononuclear cells
3. Cell Lysate + MMP-13-his6 + Ni-NTA Beads
4. Pull-down
5. Elution

#### B

<table>
<thead>
<tr>
<th>UniProt Accession</th>
<th>Uniprot ID</th>
<th>Protein</th>
<th>Peptide Count</th>
<th>Ratio [MMP-13 / Control]</th>
<th>P-Value</th>
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<tbody>
<tr>
<td>CUSTOMSEQ001</td>
<td>CUSTOMSEQ001</td>
<td>Recombinant Protein Human pro-MMP-13</td>
<td>28</td>
<td>24.0</td>
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<tr>
<td>Q9D659</td>
<td>VISTA_MOUSE</td>
<td>PD-1H / VISTA</td>
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</tbody>
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Lentzsch Lab, unpublished data
PD-1H/VISTA checkpoint inhibitor

PD-1H/VISTA as ligand

PD-1H/VISTA as receptor

Adapted from Nowak, E, et al, Immunol Rev. 2017
PD-1H mediates MMP-13 cellular binding

A

<table>
<thead>
<tr>
<th>+</th>
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<tbody>
<tr>
<td>MMP-13-myc Flag-PD-1H</td>
<td>IP: anti-Flag IB: anti-MMP-13</td>
<td></td>
</tr>
<tr>
<td>IP: anti-Flag IB: anti-Flag</td>
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<tr>
<td>Input (WCL) IB: anti-MMP-13</td>
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</tbody>
</table>

B

C

A

MMP-13-GFP Flag-PD-1H Nuclei Merge

EVD

Input (WCL)

IB: anti-MMP-13

EVFlag-PD-1H

Flag-PD-1H

MMP-13 FL ECD flag-PD-1H

IP: anti-Flag IB: anti-MMP-13

IP: anti-Flag IB: anti-Flag

Input (WCL) IB: anti-MMP-13

Extracellular Domain (ECD) Full Length (FL)

Lentzsch Lab, unpublished data
*Pd-1h*<sup>−/−</sup> impairs MMP-13 induced OCL activation

**A**

WT | *Pd-1h*<sup>−/−</sup>
--- | ---
CT | ![Imagery](image1)
MMP-13 | ![Imagery](image2)

**B**

WT | *Pd-1h*<sup>−/−</sup>
--- | ---
CT | ![Imagery](image3)
MMP-13 | ![Imagery](image4)

**C**

WT | *Pd-1h*<sup>−/−</sup> | *Pd-1h*<sup>−/−</sup> OCL | MMP-13
--- | --- | --- | ---
--- | --- | --- | ---
DC-STAMP | ![Image](image5) | ![Image](image6) | ![Image](image7)
NFATc1 | ![Image](image8) | ![Image](image9) | ![Image](image10)
P-ERK1/2 | ![Image](image11) | ![Image](image12) | ![Image](image13)
ERK1/2 | ![Image](image14) | ![Image](image15) | ![Image](image16)
PD-1H | ![Image](image17) | ![Image](image18) | ![Image](image19)
actin | ![Image](image20) | ![Image](image21) | ![Image](image22)

Lentzsch Lab, unpublished data
Pd-1h⁻/⁻ impairs MM induced bone lesion in mice

Lentzsch Lab, unpublished data
$Pd-1h^{-/-}$ impairs MM induced bone lesion in mice

5TGM1 MM induced relative change

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<tbody>
<tr>
<td>$Pd-1h^{+/+}$Rag2$^{+/+}$</td>
<td>-40.0%</td>
<td>+22.2%</td>
<td>-7.3%</td>
<td>-12.1%</td>
<td>+8.1%</td>
<td>-18.9%</td>
<td>-7.3%</td>
<td>-8.2%</td>
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<tr>
<td>$Pd-1h^{+/+}$Rag2$^{-/-}$</td>
<td>-12.5%</td>
<td>+5.5%</td>
<td>-0.6%</td>
<td>-4.2%</td>
<td>+0.6%</td>
<td>-1.6%</td>
<td>-2.7%</td>
<td>-1.7%</td>
</tr>
</tbody>
</table>

$P-$Value | 0.0098 | 0.001 | 0.045 | 0.03 | 0.04 | 7.54E-06 | 0.035 | 0.0018 |
Conclusion

- MMP-13 is highly expressed by myeloma cells and induces osteoclast fusion and activation independently of its enzymatic activity

- PD-1H is highly expressed in osteoclast

- PD-1H binds to MMP-13, mediates MMP-13 cellular binding, and MMP-13 induced osteoclast activation

- *Pd-1h*−/− mice are largely resistant to MM induced bone lesion
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