Milademetan is a potent MDM2 inhibitor, highly active in TP53 wild-type (p53\textsuperscript{WT}) Merkel cell carcinoma (MCC) cells

**Varsha Ananthapadmanabhan\textsuperscript{1,2}*\textsuperscript{,} Aine Knott\textsuperscript{3}, Kara M. Soroko\textsuperscript{3}, Prafulla C. Gokhale\textsuperscript{3}, Vijaya Tirunagaru\textsuperscript{4}, Robert Doebele\textsuperscript{4}, James A. DeCaprio\textsuperscript{1,2}.

\textsuperscript{1} Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts.

\textsuperscript{2} Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts.

\textsuperscript{3} Experimental Therapeutics Core, Dana-Farber Cancer Institute, Boston, Massachusetts.

\textsuperscript{4} Rain Therapeutics, Newark, California.

* Presenter/ Speaker
Speaker: Varsha Ananthapadmanabhan
I have no financial relationships to disclose
Grant/Research support from: NCI
and-I will not discuss off label use and/or investigational use in my presentation
Merkel cell carcinoma (MCC) is a highly aggressive neuroendocrine carcinoma of the skin. Current treatment includes surgery and radiation therapy for localized tumor and checkpoint blockade therapy for advanced disease. Primary and acquired resistance can reduce response to therapy. Around 80% of all MCCs have integrated copies of Merkel cell polyomavirus (MCV). Most MCV positive MCC (MCCP) tumors contain few somatic mutations and express wild-type (WT) p53 (TP53). MCV Small T antigen recruits the MYC homolog MYCL to the EP400 chromatin remodeling complex to form the SLaP (ST, L-MYC, and p400) complex to activate transcription of target genes. MDM2 is a SLaP target gene. MDM2 protein is an E3-ubiquitin ligase that degrades p53. MDM2 inhibitors block p53 degradation. Milademetan (RAIN-32) is a highly potent, orally administered MDM2 inhibitor.
Effect of milademetan on MCC cell lines with p53\textsuperscript{WT}, mutant p53, or p53\textsuperscript{KO}

### Table: Cell Line Sensitivity to AMG232 and Milademetan

<table>
<thead>
<tr>
<th>Established cell line</th>
<th>AMG232 (Absolute IC\textsubscript{50} µM)</th>
<th>Milademetan (Absolute IC\textsubscript{50} µM)</th>
<th>p53 status</th>
</tr>
</thead>
<tbody>
<tr>
<td>MKL-1</td>
<td>3.074</td>
<td>0.1704</td>
<td>Wild-type</td>
</tr>
<tr>
<td>WaGa</td>
<td>0.3953</td>
<td>0.003394</td>
<td>Wild-type</td>
</tr>
<tr>
<td>PeTa</td>
<td>1.189</td>
<td>0.01497</td>
<td>Wild-type</td>
</tr>
<tr>
<td>MS-1</td>
<td>-</td>
<td>-</td>
<td>Mutant</td>
</tr>
</tbody>
</table>

### Diagram A: Effect of AMG232 and Milademetan on Cell Viability

- **AMG232 (µM)**: Graph showing cell viability relative to DMSO for different concentrations of AMG232 for MS-1, MKL-1, WaGa, and PeTa cell lines.
- **Milademetan (µM)**: Graph showing cell viability relative to DMSO for different concentrations of milademetan for MS-1, MKL-1, WaGa, and PeTa cell lines.

### Diagram B: Control vs. p53 KO

- **Control**: Graph showing cell viability relative to DMSO for parental, SCR, AAVS1, P53 KO 1-1, P53 KO 1-2, and P53 KO 1-3 cell lines.
- **p53 KO**: Graph showing protein expression levels for p53 and Vinculin in parental and P53 KO cell lines.
Milademetan activates p53 response in p53<sup>WT</sup> MCC cell lines

Milademetan activates p53 response in both MKL-1 and WaGa cell lines.

Milademetan is more potent than AMG-232 at the same concentration.

<table>
<thead>
<tr>
<th></th>
<th>MKL-1</th>
<th>WaGa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DMSO</td>
<td>AMG-232</td>
</tr>
<tr>
<td>Time (hours)</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>p53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUMA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PARP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cleaved PARP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vinculin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Anti-tumor activity of milademetan in MKL-1 xenograft model

Representative Western blot from tumors collected from 16 mice euthanized at the indicated time points after the last dose of drug was administered.
Anti-tumor activity of milademetan in three MCC p53WT PDX models (pilot study)

- Milademetan efficacy study in PDX #48396 is currently underway.

- PDX #33043
  - Tumor Volume (mm³)
  - Study Days
  - 33043: Vehicle
  - 33043 Milademetan, 100 mg/kg qd
  - End of treatment

- PDX #48396
  - Tumor Volume (mm³)
  - Study Days
  - 33043: Vehicle
  - 33043 Milademetan, 100 mg/kg qd
  - End of treatment

- PDX #96712
  - Tumor Volume (mm³)
  - Study Days
  - 33043: Vehicle
  - 33043 Milademetan, 100 mg/kg qd
  - End of treatment
Summary

- MCC cell lines with WT p53 are highly sensitive to milademetan
- MCC cell lines with mutant p53 or knockout are resistant to milademetan
- Milademetan activates a robust p53 response in MCC cell lines
- In the MKL-1 subcutaneous xenograft model, treatment with milademetan at 50 mg/kg/d and 100 mg/kg/d resulted in significant tumor growth inhibition compared with vehicle control
- In a pilot study with three MCC WT p53 PDX models, treatment with milademetan at 100 mg/kg/d resulted in tumor growth inhibition with regressions
Acknowledgments

https://decapriolab.dana-farber.org/

DeCaprio Lab
James A. DeCaprio, MD
Mona Ahmed
Tim Branigan, PhD
Camille Cushman
Ariel de Botton
Thomas Frost
Ashley Gartin
Jason Nomburg
Hembly Rivas
Joana Rodrigues, PhD
Julia Schnabel

Experimental Therapeutics Core- DFCI
Prafulla C. Gokhale, PhD
Aine Knott
Kara M Soroko

Rain Therapeutics
Robert C. Doebele, MD, PhD
Vijaya Tirunagaru, PhD