The MDM2 inhibitor milademetan induces synthetic lethality in GATA3 mutant, ER positive breast cancer

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Speaker disclosures

- Employee of Rain Therapeutics
GATA3 mutations associated with shorter survival and WT TP53 in ER+/HER2- breast cancer

Clinical outcomes by GATA3 status (ctDNA, n = 101)¹

- Progression-free survival (PFS) for GATA3 wild vs GATA3 mutant: 4.2 vs 6.7 months, p = 0.023
- Overall survival (OS) for GATA3 wild vs GATA3 mutant: 14.1 vs 27.1 months, p = 0.00028

Mutual exclusivity of GATA3 and TP53 mutations²

- GATA3 mutations account for 12-18% of HR+ mBC¹ with predominantly frameshift (fs) and splice site mutations
  - Mutually exclusive of TP53 mutations²
  - Standard of care is hormone therapy + CDK4/6
- ER+HR- breast cancer patients with GATA3 frameshift mutations show significantly shorter PFS with endocrine therapy and OS¹

1. Velimirovic et al., Ab#1065(338605) ASCO 2021
2. Bianco et al., BioRxiv, May 20, 2020
GATA3 mutations and MDM2 loss induce synthetic lethality in ER+ breast cancer

(A) Schematic representation of the project DRIVE shRNA screen data used to identify synthetic lethal interactors of GATA3. (B) SLIdR-derived statistical significance (-log10(P)) plotted against the difference in the mean viability scores between GATA3-mutant and GATA3-wild type breast cancer cell lines for each gene knocked-down in the shRNA screen. (C) Viability scores of MDM2 knock-down in GATA3-mutant and GATA3-wild type cell lines. Middle lines of the boxplots indicate medians. Box limits are first and third quartiles. The whiskers extend to the range.
MDM2 knockdown induces synthetical lethality with GATA3 knockdown or GATA3fs mutation

Proliferation kinetics of breast cancer cell lines transfected with siRNA targeting MDM2 or control

MCF7, ER+ GATA3 G335fs

BT-474 (GATA3 WT)

MDA-MB-134 (GATA3 WT)
GATA3 and MDM2 synthetic lethality is p53-dependent

TP53 knockdown abolishes the effect of MDM2 knockdown

TP53 knockdown abolishes the effect of MDM2 inhibition

Re-introduction of WT GATA3 rescues the effect of milademetan

MCF7
(ER+, GATA3 G335fs, TP53 WT)

\[ TP53 \text{ knockdown} \] abolishes the effect of \[ MDM2 \text{ knockdown} \]

\[ TP53 \text{ knockdown} \] abolishes the effect of \[ MDM2 \text{ inhibition} \]

Re-introduction of WT GATA3 rescues the effect of \[ \text{milademetan} \]

\[ p < 0.003 \]

\[ p < 0.005 \]

\[ p < 0.003 \]

\[ p < 0.005 \]
Milademetan active in GATA3 fs mutant cell lines

Milademetan sensitivity in GATA3 fs mutant cell lines with WT TP53

<table>
<thead>
<tr>
<th>Tumor</th>
<th>CRC</th>
<th>Breast</th>
<th>Endometrial</th>
<th>CRC</th>
<th>Endometrial</th>
<th>Gastric</th>
<th>Gastric</th>
<th>Lung</th>
<th>Esophageal</th>
</tr>
</thead>
</table>

†Predicted neutral by FATTMM algorithm; may retain WT TP53 activity
§p.A395T is not a GATA3fs mutation

Milademetan active in GATA3fs mutant patient-derived organoids

Milademetan IC50 (nM)

LS180  MCF7  HEC151  RKO  SNGM  KATOIII  NCIN87  NCIH2126  KYSE70

Milademetan active in GATA3fs mutant patient-derived organoids

DMSO  0.3  1  3

% of viable cells (normalized to DMSO)

NS  p<0.019  p<0.001  p<0.001

PDO Mutant  PDO WT
GATA3 fs mutations in ER+ breast cancer associated with altered gene expression

Expression changes indicative of gain of function by GATA3 fs mutations with increased expression of genes/proteins that may induce MDM2 dependence

**Tumor Samples, Breast Invasive Carcinoma**
(TCGA, n = 974)

- **GATA3**
  - GATA3 fs: 2.81E-45
  - No GATA3 fs: 5.36E-22

- **ESR1**

- **MDM2**
  - GATA3 fs: 5.35E-6
  - No GATA3 fs: 1.87E-8

**Breast cancer models**
- BR5496, ER+

**GATA3**

**ER**

**PR**

**MDM2**

**WIP1 (PPM1D)**

**Beta-actin**

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Milademetan antitumor activity in GATA3fs mutant xenograft

**MCF7 breast xenograft**

- Vehicle
- Milademetan, 50mg/kg
- Milademetan, 100mg/kg
- Fulvestrant, 200mg/kg
- Milademetan, 100mg/kg + Fulvestrant, 200mg/kg

**p53 activation in MCF7 xenograft**

- No effect on body weight
- *p < 0.05

Samples: 2 h post dosing on day 17
Milademetan antitumor activity in GATA3fs mutant PDX

Breast PDX (ER+ GATA3 D336GfsTer17 mutant)

Tumor volume (mm\(^3\))

- Vehicle
- Fulvestrant, 200 mg/kg
- Milademetan, 50 mg/kg
- Milademetan, 100 mg/kg
- Milademetan, 100 mg/kg + Fulvestrant, 200 mg/kg

Days

0 10 20 30

0 300 600 900 1200 1500 1800 2100

MIC-1 induction

- Fulvestrant, 200 mg/kg
- Milademetan, 100 mg/kg
- Milademetan, 100 mg/kg + Fulvestrant, 200 mg/kg

MIC-1 fold change vs vehicle

0 5 10 15 20

0 1 2 3 4 5 6 7

Time point (h)

MIC-1 normalized to tumor weight. MIC-1 Fold change: Treated/vehicle

*p < 0.05

p53 activation in tumor samples
Conclusions

- *GATA3fs* mutations are associated with poor outcomes in ER+ breast cancer and are mutually exclusive of *TP53* mutations

- shRNA screening identified *GATA3* loss synthetic lethal with *MDM2* knockdown in ER+ breast cancer
  - *GATA3* and *MDM2* synthetic lethality is p53 dependent

- Depletion or inhibition of MDM2 reduces proliferation in *GATA3* deficient models *in vitro*

- MDM2 inhibition leads to anticancer activity *in vitro* in cell lines and in patient-derived organoids as well as *in vivo*
  - Milademetan induces p53 target gene induction (p21, PUMA, MIC-1)

- A potential clinical trial of milademetan in *GATA3fs* mutant, ER+ breast cancer is being explored