Discovery of MRT-2359, an orally bioavailable GSPT1 molecular glue degrader, for MYC-driven cancers

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I have the following relevant financial relationships to disclose:

Employee of Monte Rosa Therapeutics
Stockholder in Monte Rosa Therapeutics
Molecular Glue Degraders are a Clinically Validated Modality

- MGD binds to E3 ligase
- Protein surface is reshaped
- PPI induced with neosubstrate
- Neosubstrate is ubiquitinated
- Ubiquitinated protein shuttled to proteasome
- Protein is degraded

Ubiquitination

Proteasome-mediated degradation of neosubstrate
MYC Family Transcription Factors are Key Cancer Dependence Genes

- **MYC up-regulation** dysregulates key cellular processes (e.g. ribosome biogenesis and protein synthesis)
- **MYC dysregulation** is frequently associated with **poor prognosis** and **unfavorable patient survival**
- **MYC family**: c-MYC, N-MYC, and L-MYC
- **MYCs are considered undruggable** by classic methods

*Cells expressing high MYC are sensitive to MYC CRISPR KO*

*DepMap data, each dot represents a cell line*
Identification of GSPT1 Degraders Active in MYC-driven Solid Tumors

GSPT1 MGDS selectively affect MYC-addicted cells

Viability effects are cereblon-dependent

MYC expression status governs cell sensitivity to primary hit

Proteomics reveals selective degradation of GSPT1

Representative hit from MGD library inducing the degradation of GSPT1
GSPT1 Target and Desired MGD Profile

**Desired MGD Profile:**
- Oral
- Optimal selectivity for GSPT1 vs other neosubstrates
- Maximal preferential effect (MYC-driven vs non MYC-driven cancers)
- Differentiation over other pathway mechanisms/compounds

- To sustain growth, MYC-driven tumors are **addicted to protein translation**
- This addiction creates a **dependency on** the translation termination factor **GSPT1**
MedChem Design was Focused on Degradation Kinetics, Selectivity, Oral Bioavailability

- Kinetic measurements of degradation reveal **novel parameter for optimization**
- GSPT1 degradation **kinetics are linked to its MoA**
- MRT-2359 achieves **a high selective effect** (2.4 U) in NSCLC
- MRT-2359 has been rationally designed to be in the ADMET sweet-spot
- Several compounds with good oral bioavailability discovered (large circles = >40%F po)

* Compounds with reactive metabolite flag
Carbonyl-Switch of MRT-2359 was Critical for Selectivity

- Sidechain dictated binding-mode forces isoindolinone carbonyl in new position
- GSPT1 degron is engaged through extended sidechain interactions
- Alternative ZnF neosubstrates are no longer recruited resulting in high selectivity
MRT-2359 is a Highly Optimized and Potent GSPT1 MGD

CRBN/MRT-2359/GSPT1 ternary complex

<table>
<thead>
<tr>
<th>Biochemical and cellular data</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CRBN binding (HTRF; $K_d$)</td>
<td>113 nM</td>
</tr>
<tr>
<td>Ternary complex (HTRF; EC$_{50}$)</td>
<td>7 nM</td>
</tr>
<tr>
<td>Selectivity (TMT proteomics)</td>
<td>GSPT1 / GSPT2</td>
</tr>
<tr>
<td>DC$_{50}$/Dmax (high Myc lung lines, 6 hr)</td>
<td>1-20 nM / 100%</td>
</tr>
<tr>
<td>High N-Myc NSCLC H1155 / ABC-1 (EC$_{50}$)</td>
<td>25 / 74 nM</td>
</tr>
<tr>
<td>High L-Myc SCLC H82 / H1836 (EC$_{50}$)</td>
<td>31 / 11 nM</td>
</tr>
<tr>
<td>MM/lymphoma panel</td>
<td>broad activity</td>
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</tbody>
</table>
MRT-2359 is a Highly Selective & Oral GSPT1-directed MGD

MRT-2359 is orally bioavailable and has favorable ADMET profile

<table>
<thead>
<tr>
<th>MRT-2359, μM</th>
<th>-</th>
<th>0.3</th>
<th>3</th>
<th>30</th>
<th>30</th>
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<tbody>
<tr>
<td>Bortezomib</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MLN-4924</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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</tbody>
</table>

Proximity – Turbo ID

- CYP DDIs: > 30 μM
- hERG inhibition patch clamp: EC50 > 30 μM
- Oral bioavailability all species: ~50%
  - No activity observed in an in vitro panel of 44 safety targets

6hr post treatment in MM1S and Kelly (SALL4)

1hr post treatment
Preferential Activity of MRT-2359 in MYC-Driven NSCLC Lines

MRT-2359 induces GSPT1 degradation in all cell models, and shows preferential antiproliferative activity in N-MYC high cell lines.

GSPT1 degradation

Viability

Doxycycline-inducible N-MYC model

N-MYC overexpression sensitizes NCI-H2023 resistant cells to MRT-2359

GSPT1 western blot at 6 hr (N-Myc high) and 24 hr (low). 72 hr viability assay (CTG)

Incucyte, 96 hr post treatment
MRT-2359 Shows Preferential Activity in MYC High or Neuroendocrine (NE) Cancer Cell Lines

Prostate cell lines (c-MYC)

- High c-MYC: 22RV1, VCaP
- Low c-MYC: PC3, DU-145

SCLC cell lines (L-MYC)

- High L-MYC: NCI-H1836, NCI-H1876
- Low L-MYC: NCI-H2286, NCI-H196

Lung cancer cell lines (NE)

- High NE: NCI-H810, NCI-H1770, NCI-H1693
- Low NE: NCI-H2405

72 hr viability assay (CTG)
MRT-2359 Preferentially Impairs Protein Synthesis in Tumor Cells with High MYC Expression

MRT-2359 induces ribosome stalling at stop codon only in N-MYC high cell line

MRT-2359 completely abrogates protein synthesis only in N-MYC high cell line

Low N-MYC
NCI-H2023

High N-MYC
NCI-H1155

Puromycin incorporation

Metagene plots

Peak density

Average read density

Distance from stop codon (nt)

Ribo-Seq – 24 hr post-treatment
MRT-2359 Affects MYC and MYC Pathway in N-MYC High NSCLC Cell Lines

MRT-2359 induce GSPT1 degradation leading to N-MYC protein downregulation in NCI-H1155

Degradation of GSPT1 leads to downregulation of N-MYC transcriptional output in NCI-H1155

Low N-MYC NCI-H2023

High N-MYC NCI-H1155
MRT-2359 Preferential Activity in MYC High Lung Cancer Lines is Unique

Other therapeutic agents targeting protein translation process or machinery lack preferential activity in the MYC high lung lines

Similarly for agent targeting Myc transcriptional reprogramming

- Initiation inhibitor (eIF4Ai, Zotatifin)
- Elongation inhibitor (Homoharringtonine)
- mTOR inhibitor (Rapamycin)
- Cytotoxic (Doxorubicin)
- Clinical CDK9 inhibitor

EC50 (µM)

- NSCLC
- SCLC

72 hr viability assay (CTG)
Three Mechanisms Driving Preferential Activity in MYC High Cancer Lines

1. **Preferential GSPT1 degradation**
   - MRT-2359 leads to rapid and deeper degradation of GSPT1 in cancer cells with high MYC expression.

2. **Preferential inhibition of translation**
   - MRT-2359 preferentially impairs protein synthesis in tumor cells with high MYC expression.

3. **MYC down modulation**
   - MRT-2359 indirectly affects MYC expression and transcriptional activity.

Mechanism is applicable to c-MYC, N-MYC and L-MYC.
MRT-2359 Mouse-trial in NSCLC, SCLC and Lung NE Patient-derived Xenografts

Collection of PDX models

All models have been characterized by DNA and RNAseq

Models selected across a range of N-MYC and L-MYC mRNA expression levels or NE status were treated with:

- Vehicle
- MRT-2359

3 mice for each treatment group

NSCLC

<table>
<thead>
<tr>
<th>L-MYC mRNA expression (Log2 [TPM+1])</th>
<th>N-MYC mRNA expression (Log2 [TPM+1])</th>
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<tbody>
<tr>
<td>4</td>
<td>5</td>
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<tr>
<td>8</td>
<td>10</td>
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<td>12</td>
<td>16</td>
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Selected 48 models

SCLC

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<tr>
<th>L-MYC mRNA expression (Log2 [TPM+1])</th>
<th>N-MYC mRNA expression (Log2 [TPM+1])</th>
</tr>
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<tbody>
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<td>8</td>
<td>10</td>
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<td>12</td>
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Selected 20 models

Large cell NE carcinoma or NE lung cancer

<table>
<thead>
<tr>
<th>NE genes</th>
<th>Log2 FPKM</th>
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</table>

Selected 10 models
Dose-dependent Anti-tumor Activity Post Treatment with MRT-2359 and Using Different Schedules in PDX Models

**NSCLC N- or L-MYC high**
- 25 PDXs

**NSCLC N- and L-MYC low**
- 23 PDXs

**SCLC N- or L-MYC high**
- 19 PDXs

Study suggests dose dependent activity and similar efficacy of continuous vs on/off schedule.
MRT-2359-001 Clinical Study Design

**Phase 1: Dose Escalation**
Lung cancer (NSCLC & SCLC), DLBCL, high-grade neuroendocrine tumors, and N-/L-MYC amplified solid tumors

- Dose level 1
- Dose level 2
- Dose level 3
- MTD or RP2D
- Backfill slots for additional patients for each dose level

**Phase 2: Expansion Cohorts**

- NSCLC* – high N- or L-MYC expression
  - low N- and L-MYC expression

- SCLC* – high N- or L-MYC expression
  - low N- and L-MYC expression

- Solid tumors – N- or L-MYC amplification

* Efficacy guided stratification per N-/L-MYC expression

Patient dosing initiated in October 2022
Acknowledgments

MRT team

Project team

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