#3294: The GSPT1 molecular glue degrader MRT-2359 is active against prostate cancer

Ralph Tiedt[1], Martin Schillo[1], Arnaud Osmont[1], Débora Bonenfant[1], Rajiv Narayan[2], Owen Wallace[2], Markus Warmuth[2]

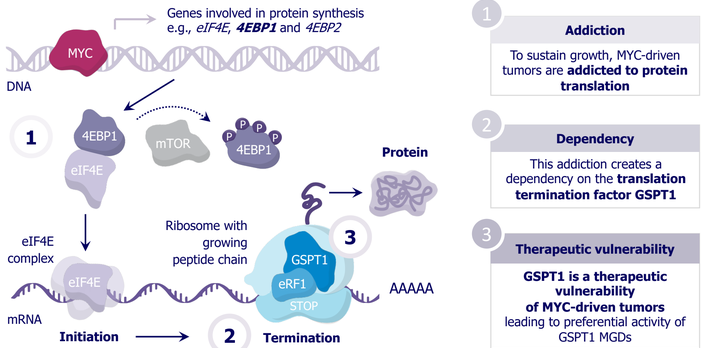
[1]*Monte Rosa Therapeutics AG, WKL-136.3, Klybeckstrasse 191, 4057 Basel, Switzerland*

[2]*Monte Rosa Therapeutics Inc., 321 Harrison Ave, Boston, MA 02118, United States*

American Association for Cancer Research (AACR) Annual Meeting, April 5-10, 2024, San Diego

Introduction

We had previously observed preferential activity of GSPT1 molecular glue degraders (MGD) in MYC-driven breast and lung cancer cell models in vitro and in vivo[1,2]. The schematic depicted below outlines a mechanistic hypothesis that could explain this observation.



1 Addiction

To sustain growth, MYC-driven tumors are **addicted to protein translation**

2 Dependency

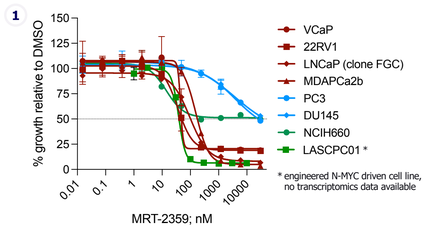
This addiction creates a dependency on the **translation termination factor GSPT1**

3 Therapeutic vulnerability

GSPT1 is a therapeutic vulnerability of MYC-driven tumors leading to preferential activity of GSPT1 MGDs

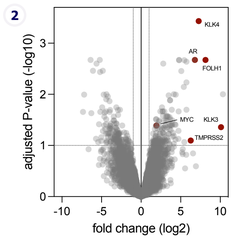
Opportunity for GSPT1 MGD in prostate cancer

MRT-2359 is a GSPT1 MGD, which was optimized to achieve preferential antiproliferative activity in MYC-driven lung cancer. While profiling MRT-2359 across hundreds of cancer cell lines representing multiple cancer types, we noted a clear segregation of prostate cancer cell lines into sensitive and resistant, associated with androgen receptor (AR) and MYC expression as potential biomarkers.

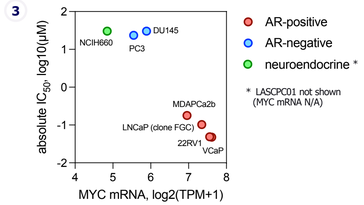


* engineered N-MYC driven cell line, no transcriptomics data available

CellTiger-Glo assays, 72h incubation with MRT-2359



Differential gene expression analysis at baseline between sensitive (n = 4) and resistant (n = 3) cell lines shown in panel 2



- AR-positive
- AR-negative
- neuroendocrine *

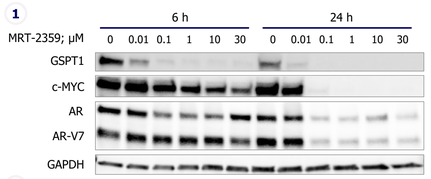
* LASCPC01 not shown (MYC mRNA N/A)

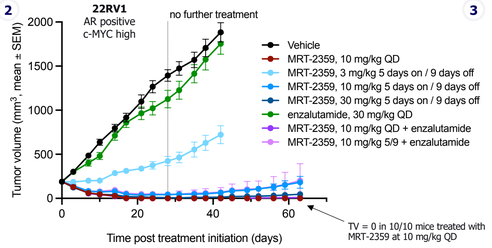
Absolute IC50 (data above) vs MYC mRNA expression (DepMap)

- Four AR-positive / MYC-high prostate cancer cell lines are very sensitive to MRT-2359 (absolute IC_{50} ~50-150 nM)
- Two AR-negative / MYC-low prostate cancer cell lines are resistant to MRT-2359 (absolute IC_{50} > 10 µM)
- Mixed in vitro response in the two tested neuroendocrine (NE) prostate cancer cell lines, but low relative IC_{50} in both cases

Activity of MRT-2359 in an AR(-V7) positive / c-MYC high prostate cancer model

22RV1 prostate cancer cells express high levels of both full-length AR and the AR variant AR-V7 that lacks the ligand binding domain. 22RV1 cells were treated with MRT-2359 in vitro and protein levels of GSPT1, c-MYC and AR were measured **(1)**. Subsequently, subcutaneous 22RV1 xenografts in immunodeficient, castrated mice were treated with MRT-2359, enzalutamide, or a combination thereof **(2)**. Efficacy parameters were calculated on day 28 **(3)**.





TV = 0 in 10/10 mice treated with MRT-2359 at 10 mg/kg QD

3

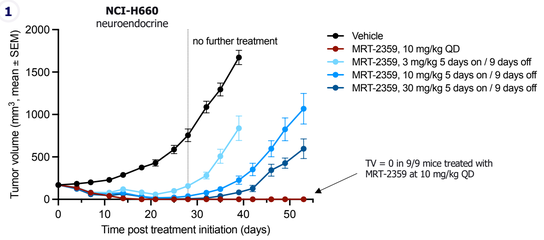
Group	Treatment regimen	Day 0 TV (mm^3, mean)	Day 28 TV (mm^3, mean)	Change in TV (mm^3)	T/C or regression (%)
1	Vehicle	189.5	1392.9	1203.4	100.0
2	MRT-2359, 10 mg/kg QD	189.5	0.0	-189.5	-100
3	MRT-2359, 3 mg/kg 5 days on/9days off	189.7	422.9	233.2	20
4	MRT-2359, 10 mg/kg 5 days on/9days off	189.4	42.3	-147.1	-78
5	MRT-2359, 30 mg/kg 5 days on/9days off	189.5	7.6	-181.9	-96
6	Enzalutamide, 30 mg/kg QD	189.6	1125.8	936.2	78
7	MRT-2359, 10 mg/kg QD + enzalutamide, 30 mg/kg QD	189.5	0.0	-189.5	-100
8	MRT-2359, 10 mg/kg 5 days on/9days off + enzalutamide, 30 mg/kg QD	189.6	44.7	-144.9	-76

Abbreviations: 5 days on / 9 days off, 5 daily doses of MRT-2359 followed by 9 days of drug holiday; AR, androgen receptor; QD, daily (quaque die); regression, % of tumor volume change relative to initial tumor volume of same group on day 0 (only calculated if tumors were shrinking on average); SEM, standard error of the mean; T/C, treated divided by control, relative % tumor volume change of treatment to vehicle group (only calculated if no regression); TV, tumor volume.

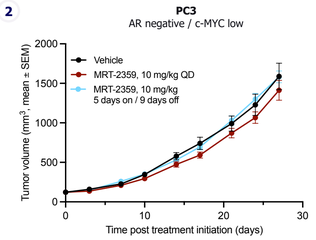
MRT-2359 mediated GSPT1 degradation led to marked reduction of c-MYC and AR / AR-V7 after 24h in vitro. In vivo, MRT-2359 was efficacious upon continuous daily (QD) or intermittent (5 days on / 9 days off) administration. MRT-2359 at 10 mg/kg QD led to full tumor regression (TV = 0), and there was no sign of tumor regrowth 35 days after treatment cessation. As expected, enzalutamide was not active in this model, and it did not contribute to efficacy in combination with MRT-2359.

Activity of MRT-2359 in NE or AR negative /c-MYC low prostate cancer models

In vitro profiling indicated limited MRT-2359 sensitivity in AR negative / c-MYC low prostate cancer cell lines, whereas the sensitivity of a NE prostate cancer cell line was unclear. Therefore, MRT-2359 in vivo activity was assessed in subcutaneous xenograft models of the NE cell line NCI-H660 **(1)** or the AR negative / c-MYC low cell line PC3 **(2)**.



TV = 0 in 9/9 mice treated with MRT-2359 at 10 mg/kg QD

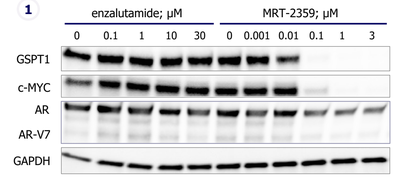


NCI-H660, regression (%) on day 28: MRT-2359, 10 mg/kg QD, -100; 3 mg/kg 5 days on / 9 days off: -7; 10 mg/kg 5 days on / 9 days off, -78. **Abbreviations:** as above; NE, neuroendocrine.

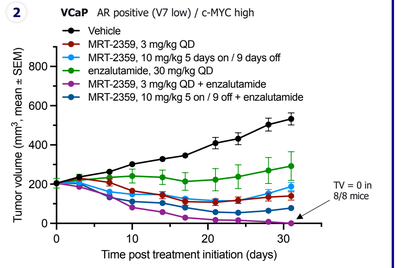
Despite apparent moderate maximal effect in a 3-day in vitro assay, MRT-2359 led to profound regression of NCI-H660 xenografts in vivo. This observation is reminiscent of the activity of MRT-2359 in neuroendocrine lung cancer models[1]. In line with in vitro data, no significant MRT-2359 anti-tumor activity was detected in the AR negative / c-MYC low xenograft model PC3.

MRT-2359 + enzalutamide in an AR positive (V7 low) / c-MYC high model

VCaP prostate cancer cells express mostly full-length AR and only minimal amounts of AR-V7. VCaP cells were treated with MRT-2359 or enzalutamide in vitro and protein levels of GSPT1, c-MYC and AR were measured **(1)**. Subsequently, subcutaneous VCaP xenografts in immunodeficient castrated mice were treated with MRT-2359, enzalutamide, or a combination thereof **(2)**.



In vitro treatment (panel 1): 24h. **VCaP efficacy parameters** (panel 2) on day 31: enzalutamide, 30 mg/kg QD, 27% T/C; MRT-2359, 3 mg/kg QD, -32% regression; MRT-2359, 10 mg/kg 5 days on / 9 days off, -8% regression; MRT-2359, 3 mg/kg QD + enzalutamide, 30 mg/kg QD, -100% regression; MRT-2359, 10 mg/kg 5 on / 9 off + enzalutamide, 30 mg/kg QD, -62% regression. **Abbreviations:** as before.



TV = 0 in 8/8 mice

MRT-2359-mediated GSPT1 degradation led to marked reduction of c-MYC and AR after 24h in vitro. In vivo, MRT-2359 was efficacious upon continuous daily (QD) or intermittent (5 days on / 9 days off) administration. Combination with enzalutamide led to deeper regression. Daily administrations of 3 mg/kg MRT-2359 + 30 mg/kg enzalutamide caused full tumor regression (TV = 0) in all mice after 31 days of treatment.

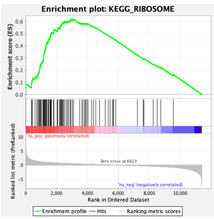
Higher expression of translation-related genes in sensitive prostate cell lines

GSEA (sensitive vs resistant)

Rank	Gene set	Size	NES
1	HALLMARK_ANDROGEN_RESPONSE	96	2.64
2	KEGG_RIBOSOME	82	2.35

GSEA was run on a pre-ranked list of genes differentially expressed at baseline between sensitive and resistant cell lines using n=205 MSigDB Hallmark and KEGG pathway gene sets with Broad GSEA software. The top 2 positively enriched sets are shown above. An enrichment plot for the KEGG ribosome gene set is shown on the right. Similar enrichment was observed for "mTOR dependent" genes (Hsieh et al, Nature 2012 and Park et al, Cell Reports 2017; gene sets are not part of MSigDB).

Abbreviations: GSEA, gene set enrichment analysis; NES, normalized enrichment score.



- Besides androgen receptor expression and activity, sensitive prostate cancer cell lines are characterized by high expression of ribosomal genes.
- This observation suggests an altered state of global translation (see "Introduction"), potentially linked to high c-MYC, leading to increased sensitivity to translation blockade via GSPT1 degradation.

Summary and Future Development

- In vitro cancer cell line profiling of the GSPT1 MGD MRT-2359 revealed marked sensitivity in a subset of prostate cancer cell lines associated with readily measurable biomarker candidates (AR + c-MYC or NE).
- Treatment of MRT-2359 sensitive prostate cancer cell lines in vitro led to a loss of c-MYC and, to a lesser extent, AR (including AR-V7) proteins.
- Marked tumor regression upon treatment with MRT-2359 as single agent was observed in three cell line derived xenograft models that were positive for the biomarker candidates discovered in vitro, but no efficacy was seen in a biomarker negative xenograft model.
- In an AR positive model with minimal expression of the variant AR-V7, combinations of MRT-2359 and enzalutamide were more efficacious than the respective single agent treatments.
- These data warrant clinical investigation of MRT-2359 as single agent or in combination with an AR antagonist in patients with prostate cancer.

References: **1.** Gavory et al. AACR Annual Meeting 2023; **2.** Gavory et al. AACR-NCI-EORTC 2021

All authors are employees of Monte Rosa Therapeutics