Drug-Anchored *in vitro* and *in vivo* CRISPR Screens to Identify Targetable Vulnerabilities and Modifiers of Response to MRTX849 in KRAS$^{G12C}$-Mutant Models

Lars Engstrom – Principal Scientist
Mirati Therapeutics – San Diego, CA
Lars Engstrom is an employee and stock holder of Mirati Therapeutics
MRTX849 is a Clinically Active, Irreversible, KRAS$^{G12C}$ Inhibitor

**MRTX849 Covalently Inhibits KRAS G12C**

1. **Targeting KRAS**
   - KRAS $^{G12C}$ Active
   - KRAS $^{G12C}$ Inactive
   - GTP
   - GDP
   - Cysteine-12
   - MRTX849
   - Picomolar Affinity for GTP

2. **KRAS G12C is irreversibly locked in the inactive state**
   - Cysteine-12 is adjacent to a shallow Switch II pocket
   - MRTX849 covalently binds to the cysteine and an induced Switch II pocket

3. **KRAS G12C is irreversibly locked in the inactive state**
   - KRAS $^{G12C}$ Inactive

**Tumor cell death**

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**AACR-NCI-EORTC 10/28/2019**

600 mg BID Dose Patients: Best Tumor Response*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Maximum % Change from Baseline</th>
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<tbody>
<tr>
<td>CRC (N=2)</td>
<td>-47% PR$^1$</td>
</tr>
<tr>
<td>NSCLC (N=5)</td>
<td>-36% PR$^1$</td>
</tr>
<tr>
<td>NSCLC (N=5)</td>
<td>-43% PR$^6$</td>
</tr>
</tbody>
</table>

* Based on local radiographic scans every 6 weeks using RECIST 1.1 criteria
$^1$ Confirmed response (1st scan: -37%, 2nd scan: -47%)
$^2$ Response yet to be confirmed (on study but only 1 scan)
$^6$ Patient had confirmed PR post data cut-off (1st scan: -33%, 2nd scan: -43%)

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Data cut-off date: 11-Oct-2019

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Based on the local radiographic scans every 6 weeks using RECIST 1.1 criteria, the 600 mg BID dose of MRTX849 has shown promising results in patients with KRAS$^{G12C}$ mutations. The best tumor response is observed in CRC patients, with a confirmed response rate of 47% (1st scan: -37%, 2nd scan: -47%). The response is yet to be confirmed in some NSCLC patients, and one patient had confirmed PR post data cut-off. The study is ongoing with data cut-off date set for 11-Oct-2019.
MRTX849 Demonstrates Broad Spectrum Tumor Regression in KRAS$^{G12C}$ Nonclinical Tumor Growth Models

CDX and PDX models were treated with MRTX849 @ 100mg/kg PO, QD in all models shown. % change from baseline control was calculated on ~ day 22 post initiation of dosing.
MRTX849 Demonstrates Broad Spectrum Tumor Regression in KRAS\textsuperscript{G12C} Nonclinical Tumor Growth Models

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Nonclinical Tumor Growth Models

CDX and PDX models were treated with MRTX849 @ 100mg/kg PO, QD in all models shown. % change from baseline control was calculated on ~ day 22 post initiation of dosing.
Drug Anchored CRISPR Screen Reveals Drug MOA, Resistance Biomarkers & Combo Targets

~5000 sgRNA Library
- 20 Negative Controls
  - 20 intronic targets
- 10 Positive Control Targets
  - 10 sgRNAs / gene
- 938 Target Genes
  - 5 sgRNAs / gene

In Vivo Log FC

Vehicle D14 / plasmid

Log FC

MRTX849 D14 / plasmid

LU99 In Vivo

“ENRICHMENT” LOF Promotes Growth / Resistance

“DROP OUT” Dependency Genes MRTX849 Combination Targets

MRTX849 3 replicates

in vitro

DMSO

MRTX849

in vivo

5 replicates

Library (Virus)

KRAS^{G12C} +Cas9 cells

x 15

3 replicates

in vitro

5 replicates

Vehicle

MRTX849

Combination Targets and Putative Resistance Biomarkers

EGFR

RTKs

FGFR1

Grb2

SOS1

SHP2

GDP-KRAS^{G12C}

GTP-KRAS^{G12C}

cRAF

ERK

AKT

mTOR

PTEN

RAS GAPs

NF1

PI3K

TP53

KEAP1

PTEN

RAS GAPs

PI3K

CDK4

RB1

S6

S6K1

CCND1

ERK

MYC

RSK

CDK4/6

RB1

E2F

EGFR

FGFR1

Grb2

SOS1

SHP2

GDP-KRAS^{G12C}

GTP-KRAS^{G12C}

cRAF

ERK

AKT

mTOR

PTEN

RAS GAPs

NF1

PI3K

TP53

KEAP1

PTEN

RAS GAPs

PI3K

CDK4

RB1

S6

S6K1

CCND1

ERK

MYC

RSK

CDK4/6

RB1

E2F
Functional Contribution of Top MRTX849 Regulated Pro-survival and Apoptosis Genes Elucidated by CRISPR Screen

- MRTX849 treatment regulates expression of selected pro-survival and pro-apoptotic genes which correlates with tumor growth inhibition in multiple KRAS\textsuperscript{G12C} mutant models.
- Functional role for many genes confirmed in CRISPR data
  - Pro-survival genes BIRC5/Survivin, BCL2L1, MCL1: Decreased by MRTX849 & Exhibit Dropout
  - Pro-apoptotic genes BCL2L11: Increased by MRTX849 & Exhibit Enrichment
  - Apoptotic regulators APAF1, CASP3/9 enriched in drug-treated CRISPR data, in particular
- Several tumor suppressors enriched suggesting potential for intrinsic resistance

### CRISPR

<table>
<thead>
<tr>
<th>Model</th>
<th>MRTX849</th>
<th>MYC</th>
<th>KRAS</th>
<th>BIRC5</th>
<th>BCL2L1</th>
<th>MCL1</th>
<th>CASP9</th>
<th>BCL2L11</th>
<th>BAX</th>
<th>CASP3</th>
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### RNAseq

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<th>BCL2L1</th>
<th>MCL1</th>
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<th>BIM</th>
<th>CASP7</th>
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<th>BBC3</th>
<th>BCL2L11</th>
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</table>

- **Pro-Survival**
- **Pro-Apoptosis**

### Tumor Suppressors

<table>
<thead>
<tr>
<th>Model</th>
<th>MRTX849</th>
<th>CBL</th>
<th>TSC2</th>
<th>RB1</th>
<th>TP53</th>
<th>TSC1</th>
<th>TIP39</th>
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</table>

**log2 FC**

- \(< -2.5\)
- \(0\)
- \(2.5 >\)
Increased KRAS\textsuperscript{G12C} Modification Via Upstream Combinations Improves Response

**Model**
- MRTX849
- PTPN11
- FGFR1
- ERBB2
- EGFR
- FGFR3
- SOS1
- NF1

**LU99**
- In Vitro (-)
- In Vivo (+)

**H358**
- In Vitro (-)

**KYSE410**
- In Vitro (+)

**log2 FC**
- < -2.5
- 0
- 2.5 >

**HERi -- H2122**
- KRAS mobility shift
- MRTX849 single agent
- MRTX849 + afatinib (0.2 μmol/L)

**SHP2i -- KYSE-410**
- Normalized Active RAS

**SOS1i -- MiaPaca-2**
- Normalized Active RAS

**Tumor Volume (mm\(^3\))**

- Vehicle
- BI-I-13 50mgkg BID
- MRTX849 10mgkg QD
- Combo
Alternative Pathway Activation Through mTOR May Contribute to Adaptive Resistance

- mTOR pathway genes drop out +/- MRTX849 treatment
- Loss of TSGs PTEN and TSC1/2 provide growth advantage
- mTOR combinations further reduce pS6 activation and leads to increased in vivo efficacy

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**“DROP OUT”**

Dependency Genes
MRTX849
Combination Targets

**“ENRICHMENT”**

LOF Promotes Growth / Resistance

- Vehicle
- MRTX849 100 mg/kg
- Vistusertib 15mg/kg
- MRTX849 + Vistusertib

Model: SW1573, KYSE-410, H368, H2122, LU11692

Tumor Volume (mm^3)

Study Day

Log2 FC

-2.5 0 2.5

GDP-KRAS
GTP-KRAS
PI3K
mTOR
S6K1
S6
RAS GAPs
NF1
G12C
RSK
MEK
ERK
cRAF
AKT
PTEN
PTPN11
GRB2
MTOR
STK11
FGFR1
PIK3CA
TSC2
TSC1
PTEN

- Alternative Pathway Activation Through mTOR May Contribute to Adaptive Resistance
Cell Cycle Strongly Implicated in CRISPR Screens and CDK4/6 Inhibitors Augment MRTX849 In Vivo Efficacy

GTP-KRAS\textsuperscript{G12C} → MEK → ERK → cRAF → AKT → PI3K → PTEN → GTP-KRAS\textsuperscript{G12C} → SOS1 → Grb2 → RTKs

EGFR → FGFR1

SHP2 → GDP-KRAS\textsuperscript{G12C} → NF1 → RAS GAPS

SOS1 → SOS1 → SOS1 → SOS1 → SOS1

CDK4/6 → CCND1 → MYC → RSK → S6K1 → S6 → E2F → RB1

Model

<table>
<thead>
<tr>
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<tbody>
<tr>
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<td>+</td>
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<td>KYSE410</td>
<td>(-)</td>
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“DROP OUT” Dependency Genes
MRTX849 Combination Targets

“ENRICHMENT” LOF Promotes Growth / Resistance

H358

LU99

KYSE410

H2030

LU6406

LU11692

Vehicle
MRTX849 100 mg/kg
Palbociclib 130mg/kg
MRTX849 + Palbo
MRTX849 Demonstrates Broad Spectrum Tumor Regression in KRAS\textsuperscript{G12C} Nonclinical Tumor Growth Models
Single Agent Activity of MRTX849 in Selected Nonclinical KRAS\textsuperscript{G12C} Tumor Models that Exhibit Intrinsic or Adaptive Resistance

CDX and PDX models were treated with MRTX849 @ 100mg/kg PO, QD in all models shown. % change from baseline control was calculated on ~ day 22 post initiation of dosing.
MRTX849 Demonstrates Broad Spectrum Tumor Regression in KRAS<sup>G12C</sup> Nonclinical Tumor Growth Models

Pan-HERi Combination – Afatinib

SHP2i Combination – RMC-4550

MRTX849 in Combination with HERi or SHP2i Further Inhibit KRAS<sup>G12C</sup> Resulting in Dramatic Regression in Models Partially Resistant to Single Agent MRTX849
MRTX849 Demonstrates Broad Spectrum Tumor Regression in KRAS\textsubscript{G12C} Nonclinical Tumor Growth Models

- Esophagus
- Lung
- Lung PDX

MRTX849 in Combination with Vistusertib or Palbociclib, Block Downstream Pathway Activation and Induce Dramatic Regression in Models Partially Resistant to Single Agent MRTX849

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<th>Model</th>
<th>Lung</th>
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mTORi Combination – Vistusertib

CDK4/6i Combination – Palbociclib
KEAP1 KO Modifies Response to KRAS$^{G12C}$ Inhibition and May Contribute to Adaptive Resistance

LU99 CRISPR Clones

![Graph showing tumor volume over study days for different groups. The graph includes lines for LU99 sgNT Vehicle, LU99 sgNT MRTX849, LU99 sgKEAP1 3-1 Vehicle, LU99 sgKEAP1 3-5 Vehicle, LU99 sgKEAP1 3-1 MRTX849, LU99 sgKEAP1 3-5 MRTX849, LU99 sgNRF2 Vehicle, and LU99 sgNRF2 MRTX849. The x-axis represents study days (0-30), and the y-axis represents tumor volume (mm$^3$).]
Conclusions

- MRTX849 is broadly active as a single agent across a panel of KRAS$^{G12C}$-mutant xenograft models.
- Executed MRTX849-anchored CRISPR screens targeting ~1,000 genes in 3 KRAS$^{G12C}$ cell lines, *in vitro* & *in vivo*.
- Tumor suppressor genes that promoted tumor growth also conferred partial drug resistance including KEAP1, NF1, Rb1, TSC1/2, and PTEN.
- Screened ~70 rational compounds in combination with MRTX849 across 8 lung cell lines *in vitro* that were partially MRTX849-resistant *in vivo*.
- Top combination targets validated with *in vivo* combinations are EGFR family, SHP2, SOS1, mTOR, and CDK4/6.
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Pasi Janne (DFCI)
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