Targeting GCK in RAS-mutant multiple myeloma offer a promising therapeutic approach

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RAS mutation in Multiple Myeloma

A. Multiple Myeloma

- 57.0% RAS\textsuperscript{WT}
- 23.7% K-RAS\textsuperscript{Mut}
- 19.3% N-RAS\textsuperscript{Mut}

B. Kaplan-Meier Plot

CoMMpass IA15

C. Abnormal proliferation, differentiation
GCK is a novel therapeutic target in MM with RAS mutation

- Germinal Center Kinase (GCK), also named MAP4K2, is an upstream activator in the MAPK pathway
- GCK is predominantly and highly expressed in the germinal center of B cells
- GCK participates in B cell differentiation into plasma cells
- GCK is a potential therapeutic target in colon cancer, DLBCL, AML and ALL
GCK is critical for proliferation and survival of RAS\textsuperscript{Mut} MM cells

A. Tet-on shCNTL Tet-on shGCK

RAS Mut

Dox - + - +

GCK β-Actin

MM.1S (K-RAS\textsuperscript{G12A})

B. Proliferation relative to control %

Tet-on shCNTL Tet-on shGCK

MM.1S (K-RAS\textsuperscript{G12A})

C. Apoptotic cells %

Tet-on shCNTL Tet-on shGCK

MM.1S (K-RAS\textsuperscript{G12A})

D. Tet-on shCNTL Tet-on shGCK

RAS WT

Dox - + - +

GCK β-Actin

LP-1 (RAS\textsuperscript{WT})

E. Proliferation relative to control %

Tet-on shCNTL Tet-on shGCK

LP-1 (RAS\textsuperscript{WT})

F. Apoptotic cells %

Tet-on shCNTL Tet-on shGCK

LP-1 (RAS\textsuperscript{WT})
Knockdown of GCK decreases c-MYC, IKZF1, IKZF3 and BCL6 expression in RAS<sup>Mut</sup> MM cells

**A.**

- Tet-on shCNTL - + - +
- Tet-on shGCK - + - +

- GCK
- IKZF-1
- IKZF-3
- c-MYC
- BCL-6
- β-Actin

**B.**

- Tet-on shCNTL - + - +
- Tet-on shGCK - + - +

- GCK
- IKZF-1
- IKZF-3
- MYC
- BCL-6
- β-Actin

**C.**

The mRNA level relative to control

- MM.1S (K-RAS<sup>G12A</sup>)

- RPMI-8226 (K-RAS<sup>G12A</sup>)
- H929 (N-RAS<sup>G13D</sup>)
- U266 (RAS<sup>WT</sup>)

* indicates significant difference compared to control.
Rescue experiments exclude possible off-target effects of GCK shRNA

A

GCK

Q F H Q V K F

WT M5

GAG TTT CAC GAG TTT CAC GAG TTT CAC GAG TTT CAC GAG TTT CAC GAG TTT CAC

B

Dox

EV+ tet-on-shGCK GCK+ tet-on-shGCK GCK(M5)+ tet-on-shGCK

Exogenous GCK Endogenous GCK

IKZF1 c-MYC β-Actin

C

Vehicle Dox

Proliferation relative to CT %

D

Vehicle Dox

EV+ GCK GCK(M5)

Exogenous GCK Endogenous GCK

7AAD Annexin V

E

Vehicle Dox

Counts PI
Pharmacological blockage of GCK activity inhibits the growth of RAS$^{\text{Mut}}$ multiple myeloma
GCK is critical for MM tumor growth in vivo

A. 

B. 

C. 

Vehicle 

Dox 

Tet-on-shCNTL 

Tet-on-shGCK 

GCK Staining 

IKZF1 Staining 

10 μM
GCK is critical for MM tumor growth in vivo

**

**

Total Fluc
1 week 2 weeks 3 weeks
0
1.0\times10^7
2.0\times10^7
3.0\times10^7
4.0\times10^7
5.0\times10^7
GCK Inhibition overcomes resistance to lenalidomide in MM

A. 

<table>
<thead>
<tr>
<th>LEN (μM)</th>
<th>shCNTL</th>
<th>shCRBN</th>
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<tbody>
<tr>
<td>0</td>
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</tr>
<tr>
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<tr>
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CRBN, IKZF1, β-actin

H929 (N-RAS<sup>G13D</sup>)

B. 

<table>
<thead>
<tr>
<th>TL4-12 (μM)</th>
<th>shCNTL</th>
<th>shCRBN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
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<tr>
<td>1</td>
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<tr>
<td>3</td>
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</tbody>
</table>

CRBN, IKZF1, β-actin

H929 (N-RAS<sup>G13D</sup>)

Proliferation relative to CT %

![Graph showing proliferation relative to CT % for LEN at different concentrations](image)

![Graph showing proliferation relative to CT % for TL4-12 at different concentrations](image)
Conclusion
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