Targeting RAS pathways in myeloma

Dr Martin Kaiser, The Institute of Cancer Research

UKMF Spring Day 2015

Making the discoveries that defeat cancer
1) What is RAS and what are the RAS pathways?

2) How can RAS be targeted?

3) Anatomy of RAS in myeloma

4) Targeting RAS pathways in myeloma
1) What is RAS and what are the RAS pathways?
Simplified timeline of RAS research

- **1981**: Mammalian RAS genes identified
- **1982**: RAS mutated in human tumours
- **1983**: N-Ras identified
- **1987**: First Ras mouse
- **1988**: Ras crystal structure
- **1989**: Ras mutations in myeloma
- **1993**: Raf as a Ras effector
- **1994**: PI3K as a Ras effector
- **1997**: K-Ras required for embryo-genesis
- **2001**: Active endogenous K-Ras causes lung cancer
- **2004**: RalGDS is required for Ras tumourigenesis
- **2007**: PIK3CA is required for Ras oncogenesis
- **2001**: Active endogenous K-Ras causes lung cancer
- **2004**: RalGDS is required for Ras tumourigenesis
- **2007**: PIK3CA is required for Ras oncogenesis
- **2011**: Trametinib approval for Melanoma
- **2011**: Verumafenib approval for Melanoma
- **2011**: WGS reveals BRAF V600E mutation in myeloma

Adapted from Karnoub et al., Nat Rev Mol Cell Biol 2008
RAS – a essential molecular switch

Constitutively active

RAS GDP “off”

RAS GTP “on”

RAS GEF

GTP

GDP

Outside signals (RTK)

P

RAS GAP

Effector

Activity
Proliferation,
Transcription,
Motility
RAS – stead y state switch position

Constitutively active

Outside signals (RTK)

RAS GDP “off”

RAS GTP “on”

Effector

Activity
Proliferation,
Transcription,
Motility

RAS GEF

GTP

GDP

P

RAS GAP
RAS – activation by outside signals

Outside signals (RTK)

Constitutively active

RAS GAP

RAS GDP “off”

GTP

RAS GEF

GDP

RAS GTP “on”

Effector

Activity

Proliferation,
Transcription,
Motility
RAS – the “beating heart” of cellular activity

\textit{NCI: The Ras Initiative}

http://www.cancer.gov/researchandfunding/priorities/ras
Ras protein family
High similarity, different functions

Prior et al, Clin Canc Res 2012
Ras signalling made simple

Ras signalling made complex

Ras binds and activates multiple effectors

The most important effector pathway?
Mutant RAS – a highly potent oncogene

NCI: The Ras Initiative

MORE THAN 30% OF ALL HUMAN CANCERS ARE DRIVEN BY MUTATIONS OF RAS GENES

RAS MUTATIONS IN HUMAN CANCERS

- Pancreas – KRAS 95%
- Colorectal – KRAS 45%
- Lung – KRAS 35%
- AML – NRAS 30%
- Melanoma – KRAS 15%
- Bladder cancer – NRAS 15%

“RAS ONCOGENES ARE THE WORST ONCOGENES.”
— Dr. Frank McCormick, RAS National Program Advisor

Table 1. Frequency of Ras Isoform Mutations in Selected Human Cancers

<table>
<thead>
<tr>
<th>Primary Tissue</th>
<th>KRAS (%)</th>
<th>HRAS (%)</th>
<th>NRAS (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>71</td>
<td>0</td>
<td>&lt;1</td>
<td>71</td>
</tr>
<tr>
<td>Colon</td>
<td>35</td>
<td>1</td>
<td>6</td>
<td>42</td>
</tr>
<tr>
<td>Small intestine</td>
<td>35</td>
<td>0</td>
<td>&lt;1</td>
<td>35</td>
</tr>
<tr>
<td>Biliary tract</td>
<td>26</td>
<td>0</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>Endometrium</td>
<td>17</td>
<td>&lt;1</td>
<td>5</td>
<td>22</td>
</tr>
<tr>
<td>Lung</td>
<td>19</td>
<td>&lt;1</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Skin (melanoma)</td>
<td>1</td>
<td>1</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Cervix</td>
<td>8</td>
<td>9</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>5</td>
<td>10</td>
<td>1</td>
<td>16</td>
</tr>
</tbody>
</table>

Data were compiled from the Catalogue of Somatic Mutations in Cancer (COSMIC) version 67. All human cancers that had total Ras mutation frequencies above 15% are listed.

Stephen et al., Canc Cell 2015
Major types of RAS point mutations


Mutational spectrum disease specific

Binding pocket inaccessible for GTPase

Mutation specific association with effectors
Mutation specific effector preference

Different K-Ras mutations –
Different Effectors

G12C/V
Lung

G12D
1) What is RAS and what are the RAS pathways?

2) How can RAS be targeted?
Challenges of directly targeting mutant RAS

- **Difficulty of competitive inhibitors of GTP**
  - GTP binds RAS with picomolar affinity
  - GTP physiologically present in high concentrations
  - Potential toxicity of wild type inhibitors

→ **Proof of principle of mutation specific covalent inhibitors of GDP “off” form**
  - At present relatively unspecific
  - Inhibitors for each mutation needed

→ **Inhibitors of effectors only available approach at present**
Potentially targetable effector proteins
Targeting BRAF

- **Mutant BRAF V600E targetable with mutant-specific inhibitors**
- **Clinical activity in Melanoma**
  - Verumafenib
  - Dabrafenib
  - Combination of Dabrafenib with MEK inhibitor

- Targeting mutant BRAF does not target wild-type BRAF

- Paradoxical Ras pathway activation in wildtype cells

Holderfield et al, Nat Rev Canc 2014
Toxicity of targeting RAS pathway

- **KRAS inhibitors**: unknown, potentially low (mutant-specific)

- **BRAF inhibitors**: paradoxical Ras pathway activation in normal tissue
  Fatigue, diarrhoea
  Skin toxicity, secondary (non-invasive) SCC
  (Reduced by combination with MEK inhibitor!)

- **MEK inhibitors**: Rash, fatigue, diarrhoea
  Visual disturbances, central serous retinopathy
  Retinal vein occlusion

- **ERK inhibitors**: Intolerable side effects of first generation drugs
It ain’t gonna be easy

But why would it be?
Outline

1) What is RAS and what are the RAS pathways?

2) How can RAS be targeted?

3) Anatomy of RAS in myeloma
Lauren Aronson
RAS mutations in myeloma pathogenesis

Exome sequencing 463 newly diagnosed cases (Myeloma XI)

Walker et al, Nature reviews Oncology
Mutational spectrum using unbiased, whole exome sequencing
Ras mutations contribute to a “myeloma” mutational signature
Alterations of the RAS pathway and prognosis

- 3 of the 5 most commonly mutated genes in myeloma belong to the RAS pathway
- Although they are negatively correlated they are not mutually exclusive
- They have no impact on survival

Eileen Boyle *Blood* 2014;124(21): abstract 637
Alterations of the RAS pathway and prognosis

- 3 of the 5 most commonly mutated genes in myeloma belong to the RAS pathway.

- Although they are negatively correlated they are not mutually exclusive.

- They have no impact on survival.

Impact of NRAS, KRAS and BRAF mutations on PFS and OS.

Eileen Boyle *Blood* 2014;124(21): abstract 637
Results: interaction between mutations and CNSA

- Positive correlations:
  - NRAS + HRD
  - KRAS + t(11;14)

- Negative correlations
  - NRAS and KRAS
Meta-analysis of Ras pathway mutations

Myeloma XI: n = 463, presentation  
Lohr: n = 203, presentation & relapse

72 RAS or RAS pathway genes
Frequency of mutations

<table>
<thead>
<tr>
<th></th>
<th>My XI</th>
<th>Lohr</th>
</tr>
</thead>
<tbody>
<tr>
<td>% RAS genes mutated</td>
<td>51%</td>
<td>38%</td>
</tr>
<tr>
<td>% patients with RAS mutation</td>
<td>50%</td>
<td>53%</td>
</tr>
<tr>
<td>% patients with &gt;1 RAS mutation</td>
<td>26%</td>
<td>30%</td>
</tr>
<tr>
<td>KRAS</td>
<td>22%</td>
<td>26%</td>
</tr>
<tr>
<td>NRAS</td>
<td>20%</td>
<td>20%</td>
</tr>
<tr>
<td>BRAF</td>
<td>8%</td>
<td>6%</td>
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</table>
Mutational spectrum

KRAS is mutated at more codons compared to NRAS

- KRAS Q61H: large intestine, lung, pancreas
- NRAS Q61K: skin
- NRAS Q61R: skin, thyroid

In solid cancers, KRAS mostly mutated at G12
NGS in myeloma

Long ‘tail’ of RAS mutations

![Graph showing mutation frequency of various RAS-related genes in myeloma.](image-url)
Pathway mutation analysis

30% patients have >1 mutation in a RAS pathway gene

Myl XI
59 patients >1 RAS mutation

- KRAS/NRAS (42%)
- 2x KRAS (31%)
- KRAS/BRAF (42%)

Lohr
32 patients >1 RAS mutation

- 2x KRAS (45%)
- NRAS/RASA2 (23%)
- KRAS/NRAS (33%)
### Co-occurring RAS/RAF mutations

**Mutations occur at sub-clonal level**

#### Hypothetical phylogenetic relationship

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Cancer Cell Fraction (%)</th>
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<tbody>
<tr>
<td>BRAF G466A</td>
<td>9</td>
</tr>
<tr>
<td>BRAF D594H</td>
<td>10</td>
</tr>
<tr>
<td>KRAS K117N</td>
<td>27</td>
</tr>
<tr>
<td>NRAS Q61L</td>
<td>38</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>84</strong></td>
</tr>
</tbody>
</table>

L Melchor, ASH 2014
Abstract 0640
1) What is RAS and what are the RAS pathways?

2) How can RAS be targeted?

3) Anatomy of RAS in myeloma

4) Targeting RAS pathways in myeloma
Targeting RAS in myeloma
Choosing cell lines with patient-relevant mutations

<table>
<thead>
<tr>
<th>AMO1</th>
<th>H929</th>
<th>JIM3</th>
<th>JNN3</th>
<th>KMM1</th>
<th>KMS11</th>
<th>KMS12-BM</th>
<th>L363</th>
<th>LP1</th>
<th>MM.1S</th>
<th>OPM2</th>
<th>RPMI-8226</th>
<th>SKMM2</th>
<th>U266</th>
</tr>
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<tbody>
<tr>
<td><strong>KRAS</strong></td>
<td>A146T</td>
<td>G12A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G12A</td>
<td>G12A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NRAS</strong></td>
<td>G13D</td>
<td>G13D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q61H</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BRAF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K601N</td>
</tr>
</tbody>
</table>

H929: NRAS G13D

Mutations don’t necessarily reflect spectrum seen in patients
No good model of K-Ras Q61 mutations available
Targeting RAS in myeloma

Drug screen

- Deltarasin
  - RAS inhibitory peptide
- BRAF/RAF1: GDC0879
  - RAFl: GW5074, ZM336372
- GDC0623
  - MEK
- GSK1120212
- FR180204
  - ERK

No effect

8 cell lines
8 inhibitors
24 and 72 hour
Wst-1 assay
Targeting RAS in myeloma
Role for MEK inhibitors in NRAS & BRAF mutant cells

- **RAS wildtype**
  - KMS11
  - LP1

- **KRAS mut**
  - MM.1S
  - RPMI8226
  - AMO1

- **NRAS mut**
  - H929
  - L363

- **BRAF mut**
  - U266

**Pathway:**
- **Deltarasin**
  - RAS inhibitory peptide
- **BRAF/RAF1**: GDC0879
  - RAF1: GW5074, ZM336372
- **GDC0623**
  - GSK1120212
- **FR180204**

**Inhibitors:**
- GDC0623
- GSK1120212
- Trametinib

**Concentration (μM):**

Graph showing concentration vs. response for different cell lines with varying RAS mutation status.
Targeting RAS in myeloma

Role for MEK inhibitors in NRAS & BRAF mutant cells

RAS wildtype
- KMS11
- LP1

KRAS mut
- MM.1S
- RPMI8226
- AMO1

NRAS mut
- H929
- L363
- L363

BRAF mut
- U266

GDC0623

GSK1120212

Trametinib

C Heuck, Abstract 4775
Targeting RAS in myeloma

Targeting of KRAS via PDEδ

Deltarasin

BRAF/RAF1: GDC0879
RAF1: GW5074, ZM336372

GDC0623
GSK1120212

FR180204

Deltarasin

Targeting RAS in myeloma

Targeting of KRAS via PDEδ

- Deltarasin
- BRAF/RAF1: GDC0879
  - RAF1: GW5074, ZM336372
- GDC0623
- GSK1120212
- FR180204

Graphs showing concentration (μM) over time (24hr and 72hr) for different RAS types:

- RAS wildtype
- KRAS mut
- NRAS mut
- BRAF mut

Species included:
- KMS11
- LP1
- MM.1S
- RPMI8226
- AMO1
- H929
- L363
- U266
Targeting RAS in myeloma

Evidence of a therapeutic window?

**RPMI8226**

**KMS11**

**HS5**

Caspase 3

Actin

Time (h)

Time (h)

% apoptosis

Control 1 2.5 5 7 10

Control 1 2.5 5 7 10

Control 1 2.5 5 7 10

% apoptosis

0 20 40 60 80 100

0 20 40 60 80 100

0 20 40 60 80 100

Alone Co-culture

Alone Co-culture

Alone Co-culture

1 2.5 5 7 10

1 2.5 5 7 10

1 2.5 5 7 10

RPMI8226 (10μM) KMS11 (7μM)
Targeting RAS in myeloma
Is specificity achievable?

![Graph showing protein expression over time](image-url)

- KRAS
- RAF1
- p-RAF1 (S338)
- MEK
- p-MEK (S217/221)
- AKT
- p-AKT (S473)
- Actin

**Time (h)**

- RPMI8226 (10μM)
- KMS11 (7μM)
Conclusions

Inhibitors of RAF and ERK ineffective in tested cell lines

Potential for using MEK inhibitors in NRAS and BRAF mutated cells

RAS pathway inhibitors have limited activity in KRAS mutant cells

Potentially targeting RAS at the apex of the pathway is a better therapeutic option

Deltarasin has strong anti-proliferative effect and induces apoptosis in cell lines

Questions remain about specificity and therapeutic window for such inhibitors
Potential biomarkers for MEK inhibitor sensitivity

MAF overexpression in t(14;16) or t(4;14) myeloma

Annunziata et al, Blood 2011
Association of combined MEK/Akti with Wildtype Ras status

Steinbrunn et al, BJH 2012
Potential re-sensitisation to Imids by MEK inhibitors

Ocio et al, Leukemia 2015
Association of RAS mutations with clinical behaviour

APEX study

- Single agent Velcade
- Response and TTP worse in NRASmut
- No difference in OS
- Effect not replicated in retrospective analysis of pts treated with doublet or triplet combinations (Yong et al, BJH 2015)
Multiply relapsed patient with V600E

Screen for mutations
SSCP
300 cases 4% mutated

“n = 1” experiment
Age 60yrs
FISH t(4;14)
Treatment Velcade, Revlimid,
DTPACE, ASCT
Disease status pp 0-54g/l
sfl 1500
Ca ++
10% plasma cells BM
BRAF mutation status V600E+

Treatment Velcade, Revlimid,
DTPACE, ASCT
Disease status pp 0-54g/l
sfl 1500
Ca ++
10% plasma cells BM
BRAF mutation status V600E+
Verumafenib before and after

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraprotein (g/l)</td>
<td>54</td>
<td>21</td>
</tr>
<tr>
<td>Sflc</td>
<td>1500</td>
<td>400</td>
</tr>
</tbody>
</table>
BRAF V600E and verumafenib

Andrulis et al., Canc Disc 2015

Sharman et al, 2015
Trametinib in myeloma

- **UAMS experience reported at ASH 2014**
  - Patients screened for KRAS, NRAS, BRAF mutations by NGS
  - GEP signature profiling

- 63 relapsed patients treated with trametinib based on mutation status or GEP
  - 41 trametinib mono
  - 22 different combinations

- Treatment responses
  - 3 CR
  - 2 VGPR
  - 8 PR
Conclusions

• Ras still / as ever a very promising target

• Ras mutation ≠ Ras mutation

• Ras mutation specific inhibitors in development

• Activity of targeted therapy (BRAF V600E)
  • Despite genetic heterogeneity in myeloma

→ Explore effector pathway inhibitors in smart combinations

→ Continue search for reliable biomarkers
Myeloma XI

Investigators:
Professor Gareth Morgan, Professor Graham Jackson,
Dr Faith Davies, Professor Nigel Russell

Molecular/translational studies:
Dr Martin Kaiser
Dr Brian Walker

MRD studies:
Dr Roger Owen

Immune studies:
Professor Mark Drayson

Clinical Trials Research Unit:
Professor Walter Gregory

We would like to thank all the patients and staff at over 100 centres throughout the UK whose participation made this study possible.

We are grateful to all principle investigators for their dedication and commitment to recruiting patients to the study.
Myeloma XI

Investigators:
Professor Gareth Morgan, Professor Graham Jackson
Dr Faith Davies, Professor Nigel Russell

Molecular and translational research studies:

**Martin Kaiser**
David Johnson
Lorenzo Melchor
Fabio Mirabella
Charlotte Pawlyn
John Jones
Eileen Boyle

**Brian Walker**
Christopher Wardell
Alexander Murison
Mike Bright
Lauren Aronson
Arpita Ray Sinha
Jacqueline Fok

**Poppy Begum**
Paula Proszek
Nasrin Dahir
Charlotte Smith
Sidra Ellis

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