Developing therapies for Ras-driven tumors

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Disclosures:

Genentech (Consultant)
The Ras pathway is one of the most commonly deregulated pathways in cancer.

Growth factor receptors
- breast, lung, GI, brain, melanoma, many more

Exchange factors
- Rac
  - melanoma
- Rho

Ras
- Lung, colon, pancreatic, melanoma, leukemia, bladder, ovarian

PI3 Kinase
- Breast, ovarian, lung, colon

PTEN
- Brain, prostate, Breast, colon

AKT
- Breast, ovarian

GAP proteins (NF1)
- PNS tumors, GBM, lung pheochromocytoma, leukemia, neuroblastoma, melanoma, colon

RAF
- Melanoma, lung, thyroid

MEK
- Lung

ERK

The Ras pathway is one of the most commonly deregulated pathways in cancer.
There are still no effective therapies for Ras-driven tumors.

So far, Ras itself has not been readily “targetable” (although drugs for a subset of specific KRAS mutations are in development).

No single agent will likely be curative.

How can we use our insight into Ras signaling and cancer biology to develop rational combination therapies for Ras-driven tumors?
Promising therapeutic strategies for Ras-driven cancers

1. Combine inhibitors that target multiple Ras effector pathways (but identify cancer specific signaling nodes within these pathways)

2. Co-target Ras effectors and epigenetic vulnerabilities

3. Co-target Ras effectors along with cancer cell-specific vulnerabilities
Considerations for developing **translatable** therapies

1. Agents that kill cells in vitro may not kill tumors in vivo (must test potential therapies in robust animal models: GEMMs, xenografts, PDX)

2. Cytostasis in most instances doesn’t translate to therapeutic efficacy in humans (need to see cell death/ regression)

3. If a therapy is ever going to be successfully translated we must attempt to recapitulate doses that are achievable in humans, when possible (and verify PK/PD)

4. Deconstructing how a specific drug combination works helps us select individuals that are the most likely to respond

Elucidating the MOA → biomarker discovery
**In vitro**

- **Cell number**
  - Time (days)
  - **veh**
  - **drug 1,2**
  - **50% loss of cells**

- **In vivo**
  - **Tumor size**
  - Time (days, weeks)
  - **veh**
  - **drug 1,2**
  - **50% shrinkage**

---

**This**

- Cell number vs. Time (days)
- Drug 1,2 vs. Veh
- 50% loss of cells

---

**Not this**

- Cell number vs. Time (days)
- Drug 1,2 vs. Veh
- No 50% loss of cells
1. Agents that kill cells in vitro may not kill tumors in vivo (must test potential therapies in robust animal models: GEMMs, xenografts, PDX)

2. Cytostasis in most instances doesn’t translate to therapeutic efficacy in humans (need to see cell death/ regression)

3. If a therapy is ever going to be successfully translated we must attempt to recapitulate doses that are achievable in humans, when possible (and verify PK/PD)

4. Deconstructing the mechanism by which a specific drug combination works, will ultimately help us select individuals that are the most likely to respond

Elucidating the MOA → biomarker discovery
The Ras pathway is one of the most commonly deregulated pathways in cancer.
**KRAS** mutant lung cancer

Engelman *et al.*

**NF1** mutant MPNSTs, melanoma

Maertens *et. al*, Malone *et al.*

\[
\text{Ras-GTP} \rightarrow \text{Ras-GDP}
\]

AF6, PI3K, PLCε, RaIGEF, Raf, Rin1, Tiam1, p190, RASSF

\[
\text{MEK} \rightarrow \text{MEKi} \quad \text{No response}
\]
KRAS mutant lung cancer

Engelman et al.

NF1 mutant MPNSTs, melanoma

Maertens et. al, Malone et al.

KRAS

mutant lung cancer

OR

No response
KRAS mutant lung cancer

Engelman et al.

NF1 mutant MPNSTs, melanoma

Maertens et. al, Malone et al.

Tumor regression

OR +

MEKi → Tumor regression

mTORi

mTOR

PI3Ki

AF6  PI3K  PLCε  RaIGEF  Raf  Rin1  Tiam1  p190  RASSF

Ras-GDP  Ras-GTP

NF1

Engelman et al.
Dual inhibition of mTORC1 and MEK causes tumor regression

Many clinical trials developed, and have failed
- wrong drugs (too toxic, not potent enough)
- wrong target (AKT)

Phase II trial of MEK inhibitor selumetinib in combination with the mTOR inhibitor AZD2014, + non-invasive biomarker study (Aerang Kim, Brigitte Widemann)
Clinical challenge: Targeting two major pathways at levels required for a therapeutic response may not be tolerable in humans.

Can we preemptively identify more cancer-specific targets within these pathways?
NF1

PI3K (p110α,β,δ)

→

AKT (1,2,3)

→

mTOR (mTORC1)

→

mTOR (mTORC1)

→

S6K1 S6K2 4EBP1

→

EIF4E

↓

Cell death

← critical component of the eIF4F translational machinery
Mnk phosphorylates and activates eIF4e (increases protein translation)

eIF4E phosphorylation is only important in cancer cells
Its dispensable in normal cells (high translational demand of CA)
PI3K
(p110α,β,δ)
→ Akt ← mTORC2
(1,2,3)
→ mTORC1
→ S6K1 S6K2

Ras
→ C-Raf
→ MEKi MEK1/2
→ ERK 1/2

NF1

Cancer specific target = greater therapeutic window?
Genetic ablation of MNKs cooperates with MEKi to kill NF1 mutant cancer cells

siMNK1/2 → Mnk1/2 → EIF4E

PD901 → MEK

shMnk2  siMnk1

shMNK2  siMnk1
MNK inhibitors cooperate with MEKi to kill NF1 mutant cancer cells

CGP57380 (CGP) --- Mnk1/2 → ELF4E

PD901 --- MEK

CGP57380: - + - +
PD901: - - + +

*p-cercosporamide (a natural product) works as well
<table>
<thead>
<tr>
<th>Drug</th>
<th>Targets</th>
<th>Stage</th>
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<tbody>
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<td>MNK1/2</td>
<td>Preclinical tool</td>
</tr>
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</tr>
<tr>
<td>Merestinib (c-Met, multi-TK)</td>
<td>MNK1/2&lt;br&gt;MET, FLT3, AXL, ROS1&lt;br&gt;VEGFR2</td>
<td>Phase I &lt;br&gt;(not publically available)</td>
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<tr>
<td>Cabozantinib (c-Met, multi-TK)</td>
<td>MNK1/2&lt;br&gt;MET, FLT3, AXL, ROS1, VEGFR2</td>
<td>Approved</td>
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Performed binding/kinase studies: MNK is a direct cabozantinib target
Cabozantinib and MEKi kill MPNSTs and KRAS mutant lung NSCLC

**NF1** mutant MPNSTs

**KRAS** mutant lung cancer
Cabo cooperates with MEKi to promote tumor regression in vivo.

Cabo dose: equiv to utilized dose (60 mg)
MEKi dose: equiv to human dose (but only 1x/day)
Ruled out other Cabozantinib targets, both genetically and chemically: (MET, AXL, VEGFR2, c-Kit)

Death can be rescued by a phosphomimetic eIF4E mutant (dephosphorylation at MNK site is required for response)
### MNK kinase inhibitors available in 2017

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<td>MNK1/2</td>
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MNK is an important therapeutic target in these Ras-driven cancers (biomarker p-eIF4E)

MEK and MNK suppression causes tumor regression

MNK is an unrecognized direct target of cabozantinib: may be re-purposed (Cabo/MEKi trials, Merestinib/MEKi?)

Specific MNK inhibitors still may ultimately provide a greater therapeutic window

Lock et al, 2016
1. Combine inhibitors that target multiple Ras effector pathways (but target cancer specific signaling nodes within these pathways)

2. Co-target Ras effectors and epigenetic vulnerabilities

3. Co-target Ras effectors along with a cancer cell-specific vulnerability (adaptive pathways)
Can we develop more effective therapies by co-targeting specific oncogenic and epigenetic defects?
The Ras pathway is one of the most commonly deregulated pathways in cancer.

**Growth factor receptors**

- **Rac**
  - melanoma

- **Rho**

- **PTEN**
  - Brain, prostate, breast, colon

- **PI3 Kinase**
  - Breast, ovarian, lung, colon

- **AKT**
  - Breast, ovarian

- **GAP proteins (NF1)**
  - Lung, colon, pancreatic, melanoma, leukemia, bladder, ovarian

- **MPNSTs: as deadly as pancreatic cancer**
  - Melanoma, lung, thyroid
  - PNS tumors, melanoma, leukemia, neuroblastoma, lung, glioma, pheochromocytoma, colon

- **ERK**
  - Lung

**KRAS mutant NSCLC**

- Breast, lung, GI, brain, melanoma, many more
Performed array CGH on 51 human MPNSTs:
- Identified FREQUENT homozygous deletions in SUZ12 and EED

Sequencing:
- Identified many additional SUZ12 inactivating mutations
- Identified many additional EED inactivating mutations
Performed array CGH on 51 human MPNSTs:
- Identified FREQUENT homozygous deletions in **SUZ12** and **EED**

**Sequencing:**
- Identified many additional **SUZ12** inactivating mutations
- Identified many additional **EED** inactivating mutations

**PRC2**

- PRC2 traditionally thought of as an “oncogenic complex”
  (GOF$^\text{mut}$ in lymphoma, overexpressed in solid tumors)

**X- Transcriptional repression**
Mutations identified in patient tumors

Develop genetically engineered mouse models

Mutations identified in patient tumors

Functional biochemical/cellular studies

• Prove causality (MPNST, GBM)
• Elucidate function
• Conceptualize therapies
Can we develop a therapy by co-targeting the effects of \textit{NF1} and \textit{SUZ12} loss?

First: identify a drug that reverses the epigenetic effects of \textit{SUZ12} loss
**NF1\text{mut} tumors frequently have co-occurring SUZ12/EED (lof)mut**
NF1\textsuperscript{mut} tumors frequently have co-occurring SUZ12/EED (lof)mut

Transcriptional repression

Transcriptional re-activation

PRC2

EZH2 SUZ12 RbAp46/48 EED

Me3 K27 Me3 K27

AC K27 AC

BRD4 TF

X

Histone marks and epigenetic machinery
**NF1**mut tumors frequently have co-occurring SUZ12/EED (lof)mut

- **Histone marks and epigenetic machinery**
  - EZH2
  - SUZ12
  - RbAp46/48
  - EED
  - PRC2

- **X- Transcriptional repression**

- **BRD4 inhibitors** (JQ1, GSK525762, OTX015)

- **Transcriptional Re-activation**
Combined BRD4i plus MEKi promote tumor regression in vivo
Cooperative suppression of Ras-driven transcription

NF1 mutation

- RAS
  - PI3K
  - RAF
  - MEKi
  - AKT
  - MEK
  - mTOR
  - ERK

Suppression of Ras TXN output

Suppression of Ras TXN output

SUZ12 or EED mutation

BRD4i + MEKi

Ras-responsive genes

DeRaedt et al., Nature 2014
Is this strategy more broadly applicable to other Ras–driven tumors (e.g. KRAS mutant)?

If so can identify precise biomarkers that might predict response?
Leading cause of cancer death in men and women

- More than one million deaths annually
- Average 5-year survival rate: 15%

Oncogenic Drivers of Lung Adenocarcinoma

TCGA, 2014
Effects of MEK and BRD4 inhibitors in Ras-driven lung NSCLC

- **Log2-fold change in cell no. (72 hours)**
  - **KRAS-mutant**
    - Veh
    - MEKi
    - BRD4i
    - MEKi/BRD4i
  - **NF1-mutant**
    - Veh
    - MEKi
    - BRD4i
    - MEKi/BRD4i

- **% change in cell number**
  - proliferation
  - death

- **Veh**
- **MEKi**
- **BRD4i**
- **MEKi/BRD4i**
Combined MEK/BRD4 inhibition triggers cell death in 50% of KRAS mutant lung cancer lines.
MEK/BRD4 inhibitors are effective in KRAS cancers in vivo

Model 1

Model 2

Log2 Fold Change in Tumor Volume

Percent Change in Tumor Volume

No treatment
MEKi
BRD4
combo
1. What is the mechanism of action?

2. How can we predict sensitivity or resistance?
BRD4 and MEK inhibitors cooperatively suppress Ras transcriptional output in NSCLC

Guerra et al. unpublished
Is sensitivity related to PRC2 status?
Sensitive lung cancers exhibit defects in PRC2 genes

- Different than MPNSTs
- Mostly heterozygous copy loss
- Mutations are rare
Sensitive lung cancers exhibit defects in PRC2 function

<table>
<thead>
<tr>
<th>Enriched in SENSITIVE Cells</th>
<th>NES</th>
<th>pvalue</th>
<th>FDR</th>
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<tbody>
<tr>
<td>BENPORATH_PRC2_TARGETS</td>
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<td>0.059</td>
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<tr>
<td>BENPORATH_EED_TARGETS</td>
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<td>0.076</td>
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<td>BENPORATH_SUZ12_TARGETS</td>
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<td>PASINI_SUZ12_TARGETS_UP</td>
<td>1.41</td>
<td>0.018</td>
<td>0.096</td>
</tr>
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</table>
PRC2 suppression confers sensitivity to BRD4/MEK inhibitors

Resistant → Sensitive

Note: The NF1 lung CA lines examined had intact SUZ12 and EED
Combined BRD4/MEK inhibitors are effective in a large percentage of KRAS mutant NSCLC

- They cooperatively suppress Ras transcriptional output
- PRC2 defects confer sensitivity
Do BRD4 inhibitors have additional targets in lung cancer?

- MEKi
- BRD4i

- RAS transcriptional output
- Other PRC2 targets???
HOXC10 is exclusively expressed in sensitive cell lines

- HOX genes are well established PRC2 targets
- HOX genes are known to play an important role in cancer
HOXC10 is potently suppressed by BRD4 inhibitors

MEKi → BRD4i → RAS transcriptional output → Other PRC2 targets???

V M B M/B vinculin

DMSO MEKI BRD4i COMBO
HOXC10 reconstitution prevents cell death
**HOX genes**

- Master developmental transcription factors, expressed largely during development (not adult tissue)

- Reciprocally regulated by PRC2 and TRX complexes

- HOX genes are known to be overexpressed and play an oncogenic role in cancer (e.g. HOXA9 in AML)

**HOXC10**

- Little known

- Overexpressed in breast cancer, oral squamous cell carcinoma, cervical cancer, and thyroid cancer

- In some settings expression correlates with poor outcome
HOXC10 is overexpressed in 55% of KRAS\textsuperscript{mut} mutant lung cancers (>3 SD, compared to mean)
MEK and BRD4 inhibitors trigger regression of HOXC10 expressing PDX tumors
A distinct subset of human lung cancers uniquely express HOXC10

HOXC10 expression is largely triggered by (heterozygous) defects in PRC2 components

These tumors are sensitive to combined BRD4/MEK inhibitors

BRD4 and MEK inhibitors function by 1) cooperatively suppressing Ras transcriptional output and 2) inhibiting HOXC10 expression

HOXC10 can be used as a predictive biomarker for patient selection
1. Combine inhibitors that target multiple Ras effector pathways (but target cancer specific signaling nodes within these pathways)

2. Co-target Ras effectors and epigenetic vulnerabilities

3. Co-target Ras effectors along with a cancer cell-specific vulnerability
Cancer cells must engage adaptive pathways to protect cells from damaging processes associated with transformation
e.g. Excessive DNA damage, oxidative stress, metabolic stress, proteotoxic stress, replicative stress
Co-targeting Ras effectors and cancer cell vulnerabilities

**Target 1:**
A driving oncogenic pathway

- **BRAF**
- **MEK/ERK**

**Target 2**
A protective/adaptive pathway that helps stressed cancer cells survive

- Suppress enzymes that regulate DNA repair genes
- Prevent lethal DNA damage in defective tumor cells *(Under review)*
- *Melanoma: Trial in discussion*

**Target 2** 
+ 

- Suppress anti-oxidant pathways
- Protect cancer cells from catastrophic oxidative stress *(Cancer Discovery, 2017)*
- *MPNST and Lung CA: Trial being developed*

- Suppress proteins that control proteostasis
- Protect cancer cells from ER stress associated with aneuploidy *(Cancer Cell, 2008)*
- *MPNST and Lung CA: 2 clinical trials conducted, ongoing*
1. Combine inhibitors that target multiple Ras effector pathways (cancer specific signaling nodes within these pathways, eg. MNK)

2. Co-target Ras effectors and epigenetic vulnerabilities (e.g. BRD4)

3. Co-target Ras effectors along with a cancer cell-specific vulnerability

- At least one Ras effector pathway must be targeted
- Different effectors (e.g. MEK, mTOR) are effective in different combinations
- A therapeutic index is more readily achieved if at least one drug capitalizes on a cancer-specific target or vulnerability
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