Co-targeting of the PI3K/AKT/mTOR and RAS/RAF/MEK/ERK Pathways as a Strategy for Cancer Treatment

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Molecular Targeted Cancer Therapy

• Limitations of traditional chemotherapy
• Increasing awareness of specifically mutated pathways in cancer
• Efforts to develop drugs that specifically target abnormal pathways
PI3K/Akt/mTOR & RAS/RAF/MEK/ERK Signaling Pathways

RTK

IRS → PI3-K → PIP3 → AKT → mTOR

PI3-K

PTEN

PIP2

PI3-K

PTEN

PDK1

PHLPP

mTORC2

P

AKT

mTORC1

4E-BP1

p70S6K
Co-targeting of the PI3K and RAS Pathways for the Treatment of Carcinoid Tumors
Increasing Incidence of Carcinoid

Carcinoid Syndrome and Fibrosis

- Flushing
- Bronchoconstriction
- Liver Metastases
- Primary carcinoid
- Pulmonary and Tricuspid Valvular Heart Disease
- Hyperperistalsis/Diarrhea
- Blood ↑ Chromogranin A
- Blood ↑ Serotonin
- Urine ↑ 5-HIAA
- Patchy Hyperpigmentation
Experimental Design

• Purpose: Understanding the role of PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways in carcinoid disease.
  1) Assess the frequency of PI3K and RAS mutations
  2) Determine the effects of inhibition of these pathways on cell proliferation, apoptosis, and secretion
  3) Evaluate the effects of combined inhibition of both of these pathways
The BON cell line – a model of carcinoid tumors
# Mutational Analysis

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Source</th>
<th>PIK3CA</th>
<th>KRAS</th>
<th>NRAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BON</td>
<td>Human pancreatic carcinoid</td>
<td>E545A</td>
<td>WT</td>
<td>Q61R</td>
</tr>
<tr>
<td>QGP-1</td>
<td>Human pancreatic somatostatinoma</td>
<td>E545A</td>
<td>G12V</td>
<td>WT</td>
</tr>
<tr>
<td>NCI-H727</td>
<td>Human lung carcinoid</td>
<td>E545A</td>
<td>G12V</td>
<td>WT</td>
</tr>
<tr>
<td>UMC-11</td>
<td>Human lung carcinoid</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
</tr>
</tbody>
</table>
PI3K Inhibition Results in Anti-neoplastic Effects

Apoptosis (BON)

Cell Viability

BKM120

BEZ235
Final Tumor Volume and Weight of Mice Treated with Orally Administered BEZ235 x 25 days

**BEZ235 - Average Tumor Volume**

- Vehicle Control: ~250 mm³
- BEZ235: ~50 mm³

**BEZ235 - Average Tumor Weight**

- Vehicle Control: ~0.25 grams
- BEZ235: ~0.15 grams

Vehicle Control Treatment
PI3K Inhibition Increases Signaling through the RAS/RAF/MEK/ERK Pathway

<table>
<thead>
<tr>
<th></th>
<th>BKM120</th>
<th>BEZ235</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C 1.0 2.5 5.0uM</td>
<td>C 10 100 1000nM</td>
</tr>
<tr>
<td>pERK</td>
<td>![pERK Image]</td>
<td>![pERK Image]</td>
</tr>
<tr>
<td>ERK</td>
<td>![ERK Image]</td>
<td>![ERK Image]</td>
</tr>
<tr>
<td>B-actin</td>
<td>![B-actin Image]</td>
<td>![B-actin Image]</td>
</tr>
</tbody>
</table>
Combined PI3K and MEK Inhibition Decreases Cell Proliferation

BON cells treated with BKM120, PD901, or combination x 72h

\[
\begin{array}{c|cccc}
\text{Treatment} & \text{DMSO Control} & \text{BKM120} & \text{PD901} & \text{BKM120/PD901} \\
\hline
0 \times 10^6 & 1 & 0.2 & 0.4 & 0.6 & 0.8 & 1 & 1.2 \\
\end{array}
\]

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\end{array}
\]

DNA fragmentation Abs (405-490) nm

\[
\begin{array}{c|cccc}
\text{Treatment} & \text{DMSO Control} & \text{BKM120} & \text{PD901} & \text{BKM120/PD901} \\
\hline
0 & 0.5 & 1 & 1.5 & 2 \\
1 & 2.5 & 2 & 1.5 & 1 \\
2 & 2.5 & 2 & 1.5 & 1 \\
3 & 3 & 3 & 3 & 3 \\
\end{array}
\]
Co-treatment with a MEK Inhibitor Blocks the Increase in Secretion that Occurs with PI3K Inhibition

**Table:**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BON</th>
<th>QGP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapa (20 nM)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PIK-75 (0.5 μM)</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>PD98059 (10 μM)</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>±</td>
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<td>±</td>
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<tr>
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<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>±</td>
</tr>
</tbody>
</table>

**Graphs:**

- BON: NT (pg/ml) values for different treatments.
- QGP-1: NT (pg/ml) values for different treatments.

Key:
- Rapa (20 nM): -
- PIK-75 (0.5 μM): -
- PD98059 (10 μM): -
- **(0.5 μM)**
- **(10 μM)**
- PIK-75 (0.5 μM): +
- PD98059 (10 μM): +
Conclusions

• Mutations of the PI3K and RAS pathway are common in neuroendocrine cell lines

• PI3K inhibition
  – Effective as individual therapy
  – Upregulation of RAS/RAF/MEK/ERK
  – Increased Secretion

• PI3K inhibition combined with MEK inhibition
  – Enhanced effects
  – Blocks increase in secretion
Novel siRNA Co-targeting Strategy as Treatment for Colorectal Cancer
Discovery of siRNA

- Discovered in 1998 in nematodes
- 21-23 nucleotide segments of RNA
- Very specific, wide variety of targets
Experimental Design

• Purpose: Comparative analysis of a panel of siRNA directed toward specific components of either the PI3K or RAS pathways
• Cell Lines - Likely effected by mutational profile – HCT 116 and DLD-1
• Outcomes – proliferation, apoptosis
siRNA Knockdown of Selected Targets

PI3K/Akt/mTOR Pathway

RAS/RAF/MEK/ERK Pathway

HCT116

DLD-1
Combined PI3K and RAS siRNA Targeting

**Cell Viability**

**HCT116**

![Cell Count (x10^6)](chart)

**DLD-1**

![Cell Count (x10^6)](chart)

**Apoptosis**

**HCT116**

![DNA fragmentation Abs (405-490) nm](chart)

<table>
<thead>
<tr>
<th>siRNA</th>
<th>NTC</th>
<th>PIK3CA</th>
<th>Akt2</th>
<th>KRAS</th>
<th>PIK3CA / KRAS</th>
<th>Akt2 / KRAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>p110a siRNA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Akt2 siRNA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>KRAS siRNA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Western Blot**

- p110α
- Akt2
- KRAS
- p4eBP1
- Total 4eBP1
Conclusions

- P110α and KRAS are the most effective siRNA treatments in their individual pathways
- Combination treatments are more effective than single agents
- 4E-BP1, a downstream effector common to both pathways, may serve as a marker of effective treatment
Liver Directed siRNA Therapy in the Treatment of Colon Cancer
Barriers to siRNA delivery

• siRNA instability
• Enzymatic degradation
• Rapid renal clearance
Organ Directed Therapy

- Concentrated delivery to target organ
- Feasible in humans
- No animal models
Experimental Design

• Purpose: Develop a mouse model of portal vein catheterization for the chronic delivery of therapeutic agents to liver metastases
Portal Vein Catheter Video
Catheter Placement and Portal Vein Distribution
Delivery of DY-547 Labeled siRNA to Liver Metastases
Conclusions

• Portal vein catheterization is technically feasible
• Liver directed therapy results in enhanced uptake of siRNA as confirmed by IVIS imaging
• siRNA is able to be delivered to metastatic disease using this technique