Decoding rafenibs

<table>
<thead>
<tr>
<th>PLX4032, RG7204</th>
<th>vemurafenib</th>
<th>RO51...</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V 600 E mutated raf inhibitor</td>
<td></td>
</tr>
<tr>
<td>GSK2118436</td>
<td>dabrafenib</td>
<td></td>
</tr>
<tr>
<td></td>
<td>“Da” B-raf inhibitor</td>
<td></td>
</tr>
<tr>
<td>BAY 43-90...</td>
<td>sorafenib</td>
<td></td>
</tr>
<tr>
<td></td>
<td>So, so raf inhibitor</td>
<td></td>
</tr>
</tbody>
</table>

Nanoparticle delivery of siRNA to treat melanoma

Antoni Ribas, M.D.
University of California Los Angeles

Mark E. Davis, Ph.D.
Jonathan Zuckerman, B.S.
California Institute of Technology
Disclosures

• Antoni Ribas: No relevant conflicts
• Mark E. Davis is a founder of Calando

Inhibiting Undruggable Targets: RNAi and siRNA

• siRNA: Widely used in the laboratory in vitro for specific, rapid and efficient gene knockdown in almost any type of cell
• The problem: How can this process be used therapeutically?
RNA Interference (RNAi)

2006 Nobel Prize in Medicine

RNA interference (RNAi) is a method to silence genes by double-stranded RNA

C. Mello

“ If a person has a tumor, why not take a gene that’s essential for that tumor and administer double-stranded RNA corresponding to that gene to shut down the growth of that tumor?” – A. Fire, Nobel Prize Lecture 2006

“If you could get RNA to the target you could have some really cool therapeutics. Delivery is the major issue in all of it.” – A. Fire

siRNA used in vitro

siRNA used in vivo
Luciferase Knockdown in Sarcoma by Tf-targeted, siRNA-Containing Nanoparticles

Intravenous administration of anti-luciferase siRNA using nanoparticles leads to inhibition of luciferase expression in tumors \textit{in vivo}

Jeremy Heidel, Mark E. Davis, Caltech

\textbf{siRNA-mediated Gene Silencing}

Protein

mRNA

DNA
siRNA-mediated Gene Silencing

Ribonucleotide Reductase subunit 2 (RRM2) Silencing with siRNA in Human Melanoma Cell Lines

Effects of RRM2 silencing in a panel of melanoma cell lines

HT-144 melanoma

siR2B+5 efficiently silences RRM2 mRNA and protein expression

John Zuckerman, MSTP Student, UCLA-Caltech
In vitro anti-proliferative effects of RRM2 knockdown by siR2B+5 siRNA in a panel of melanoma cell lines

% Viability Compared to Untreated Control

siR2B+5 anti-proliferative effects are independent of the driver oncogenic mutations in melanoma

RRM2 Silencing by siR2B+5 is Mediated by RNAi

5'-RLM-RACE detection of siRNA-induced mRNA cleavage fragment

Targeted CDP/siRNA nanoparticles

predicted 209bp amplicon resultant by the siRNA-induced cleavage site (10 base pairs from the 5' end of the antisense strand)
Targeted Nanoparticle Delivery of siRNA in Humans: First-in-class Phase 1 Clinical Trial

Mark E. Davis, Ph.D.
Chemical Engineering
California Institute of Technology

Cyclodextrin containing polymer (CDP)
AD-PEG-hTf
RRM2 siRNA:

5' gauuuagccaagaaguucaga 3'
3' cgcuaaaucgguucuucaagu 5'

Schematic of Delivery and Function
Patient Characteristics (n=15)

| Age            | Median 62  
|----------------|----------
| (range 53-85)  |  

| Gender | Male: 12, Female: 3  

| Performance status | ECOG 0: 6, ECOG 1: 9  

| # prior lines of therapy | 0: 1, 1: 4, 2: 5, >2: 5  

| Sites of metastasis | Skin-SC-LN: 2, Visceral: 12, Bone: 1  

| Cancer Histologies | Melanoma: 3, Colon: 2, Esophageal: 1, Cholangiocarcinoma: 1, Anal squamous cell: 1, Mesothelioma: 1, Neuroendocrine: 1, RCC: 1, Prostate: 1, Ovarian: 1, Adenocarcinoma of unknown primary: 1, Leiomyosarcoma: 1  

Standard patient population for a first-in-human phase 1 clinical trial
Ribas, Kalinoski, Heidel, Peterkin, Seligson, Zuckerman, Choi, Yen, Davis, Tolcher. ASCO 2010

Pharmacokinetics (PK) of CALAA-01

Short plasma half life of minutes

No change in PK with repeated treatment cycles

Preliminary result >ULOQ, retest in progress
### Study Agent-Related Toxicities

<table>
<thead>
<tr>
<th>Dose Level</th>
<th># Pts</th>
<th>Constitutional and Allergic Toxicities</th>
<th>GI Toxicities</th>
<th>Laboratory Abnormalities</th>
<th>Other Toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mg/m²</td>
<td>3</td>
<td>G1 Fatigue</td>
<td>G2 Constipation</td>
<td>G1 Rash, G1 Edema, G1 Desquamation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1 Flushing</td>
<td>G1 Dysgeusia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 mg/m²</td>
<td>4</td>
<td>G2 Allergic reaction (2), G2 Fatigue</td>
<td>G1 Constipation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1 Fevers</td>
<td>G1 Abdominal pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 mg/m²</td>
<td>3</td>
<td>G2 Rigors</td>
<td>G1 Rash</td>
<td>G1 Thrombocytopenia</td>
<td>G1 Anorexia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1 Fatigue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1 Fever</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 mg/m²</td>
<td>4</td>
<td>G2 Chills (4), G2 Fatigue, G2 Rigors</td>
<td>G2 Nausea</td>
<td>G3 Anemia, G2 Vomiting, G2 Thrombocytopenia</td>
<td>G2 Sinus bradycardia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1 Flushing</td>
<td>G1 Constipation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1 Fever</td>
<td>G1 Diarrhea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 mg/m²</td>
<td>1</td>
<td>G2 Fatigue, G2 Headaches</td>
<td>G1 Vomiting</td>
<td>G2 Thrombocytopenia</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1 Diarrhea</td>
<td>G1 Dysgeusia</td>
<td></td>
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</tr>
</tbody>
</table>

Most side effects were mild to moderate with no DLTs (ASCO 2010)

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### Patient 02-005, 30 mg/kg CALAA-01

May, 13, 09 Baseline  | July, 6, 09 | Aug, 24, 09 | Stable disease

**5/13 C1D1**  | **5/21 C1post biopsy**  | **6/03 C2D1**  | **7/17 C2pre biopsy**  | **7/20 C3D1**  | **7/30 C2post biopsy**  | **8/10 C4D1**  | **8/31 C5D1**  | **9/21 C6D1**  | **Stable disease**

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10/5/11
Cytokine Release after Dosing
Patient 02-005, 30 mg/m² cohort

Patient 02-005 Cohort 5

IL-10 (pg/mL) vs. TNF-α (pg/mL) vs. IL-6 (pg/mL)

- Dose-dependent accumulation of targeted nanoparticles in tumors
- Specific mRNA and protein knock-down
- Engagement of the RNAi mechanism in humans through systemic delivery of siRNA
Conclusions of Nanoparticle Delivery of siRNA in Humans

- Systemic delivery of siRNA via targeted nanoparticles is safe and can induce specific, siRNA-mediated gene silencing
- This approach can potentially be expanded to any currently undruggable cancer therapy target

NRAS Mutation in 20% of Melanomas

After 30 years of knowing that RAS is an oncogene, nobody has been able to "drug" it
siRNA against N-Ras (s55) has anti-proliferative effects on melanoma cell lines with activating N-Ras mutations

Potent Knockdown of N-Ras is achieved in wt and mtN-Ras cell lines

s55 induces G1 cell cycle arrest in mutated (mt)N-Ras cells but not in wild type (wt)N-Ras cells

Anti-proliferative effects of s55 treatment are observed in mtN-Ras cell lines and not wtN-Ras cell lines

% Viable Cells Compared to Untreated Control

Acknowledgements

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Ribas lab:

Tony Tolcher, M.D.
START

WAM Team