(The Before and Beyond) Phase I trials
Pharmacology of drugs targeting the MAPK pathway

Jordi Rodon
(The Before and Beyond) Phase I trials
Pharmacology of drugs targeting the MAPK pathway
Outline

1. BRAF inhibitors: are they all the same?
   - RAF inhibitors and pharmacological properties
   - 2nd Gen RAFi, “Paradox-breakers”
2. MEK inhibitors
3. Dual... and triple combos
4. New kids on the block:
   - ERKi
   - Drugs targeting KRAS ...
The pharmacological landscape of the MAPK pathway

Non-specific Kinase Inh
Sorafenib
Regorafenib

BRAFV600E specific
Vemurafenib
Dabrafenib
Encorafenib (LGX818)
RAF265/CHIR265
BMS-908662/XL281
(GSK2118436)

2nd generation BRAFi
PLX8394/ PLX7904

BRAFi

PanRAFi

RO5126766/ CH5126766
(BRAF/MEKi)

CCT196969, CCT241161
LY3009120
ARQ-736
MLN2480

Inhibitors of membrane association
Minerval
FTIs
GGTs
Deltarasin

Mutation Specific
Direct KRAS inhibitor G12C

Targeting RAS at RNA level
Anti-sense oligonucleotids
siRNA

MK8353
GDC–0994
BVD523
SCH772984
VTX11e

E6201 (MEK/MEKK1 inh)

Allosteric MEK1/2i
Trametinib (GSK1120212)
Pimasertib (AS703026)
Selumetinib (AZD6244)
PD0325901
Refametinib (BAY86-9766)
TAK733
MEK162 (ARRY438162)
WX554
RO4987655
ARRY-300
AS703988
AZD8830
E6201

Allosteric MEK1i
Cobimetinib (GDC0973)
Non-specific
Sorafenib
Regorafenib

BRAFV600E specific
Vemurafenib
Dabrafenib
Encorafenib (LGX818)
RAF265/CHIR265
BMS-908662/XL281
(GSK2118436)

2nd generation
PLX8394

BRAFi

PanRAFi

KRAS

PAMi

MEKi

The “Paradox”
PK/PD correlations and dose selection: dabrafenib as a paradigmatic case

Dabrafenib was administered orally once, twice (BID), or three times daily (TID). No MTD was defined. A recommended phase II dose (RP2D) was chosen based on safety, pharmacokinetic, pharmacodynamic, and response data.

Pharmacokinetic assessment of dabrafenib demonstrated a less than-dose-proportional increase in exposure after repeat dosing above 150 mg BID.

A favorable activity and tolerability profile was demonstrated at 150 mg BID. There was no improvement with TID.

Predicted target inhibition of pERK (>80%) was achieved at 150 mg BID, with a similar magnitude of inhibition at higher doses.
The Devil is in the Details:

RAF inhibitors and Pharmacological properties

Drugs with “unique” properties
In vitro Comparison of the targeting efficacy of marketed inhibitors.

Indirect comparison of vemurafenib and dabrafenib in patients with BRAFV600 mutant metastatic melanoma

Vemurafenib
(960 mg po bid)

BRIM3
Chapman et al.
NEJM 2011

Dabrafenib
(150 mg po bid)

BREAK3
Hauschild et al.
Lancet 2012
Dabrafenib exposure decrease over time, as a result of induction of its own metabolism (CYP3A).

The major route of elimination is via oxidative metabolism (via CYP2C8 and CYP3A) and biliary excretion.

Dabrafenib could be the victim and the perpetrator of drug-drug interactions with strong inhibitors of CYP2C8 and/or CYP3A4.
Encorafenib. A BRAFi with special pharmacological properties

Selectivity… Potency and a long dissociation t 1/2

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dissociation t1/2, h</th>
<th>B-Raf (V600E)</th>
<th>B-Raf</th>
<th>C-Raf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multikinase inhibitor</td>
<td>Sorafenib</td>
<td>22 nM</td>
<td>38 nM</td>
<td>6 nM</td>
</tr>
<tr>
<td>Multikinase inhibitor</td>
<td>Regorafenib</td>
<td>28 nM</td>
<td>19 nM</td>
<td>2.5 nM</td>
</tr>
<tr>
<td>BRAF (V600E selective)</td>
<td>Vemurafenib</td>
<td>100 nM</td>
<td>31 nM</td>
<td>48 nM</td>
</tr>
<tr>
<td>BRAF (V600E selective)</td>
<td>Dabrafenib</td>
<td>3.2 nM</td>
<td>0.8 nM</td>
<td>5.0 nM</td>
</tr>
<tr>
<td>BRAF (V600E selective)</td>
<td>Encorafenib (LGX818)</td>
<td>0.5 nM</td>
<td>0.4 nM</td>
<td>0.3 nM</td>
</tr>
</tbody>
</table>

The dissociation half-time represents the time needed for half the ligands to dissociate from the receptor to which they were initially bound.
Day 15 exposures were consistently lower (30%-60%) than those on day 1, probably due to induction of CYP enzymes.

A case for pulsatile therapy?
Pulsatile therapy with BRAFi?

Clinical observations

A case of Concurrent *RAF*- and *NRAS*-Mutant Malignancies

Fisher et al, SMR Annual Meeting, 2012
Stuart et al, AACR 2013
Abdel-Whab O. et al. Cancer discovery, 2014
Pulsatile therapy with BRAFi?

Preclinical observations

Das Thakur, M. Nature, 2013

Sun et al. Nature April 2014
Pulsatile therapy with BRAFi?

The hypothesis

[Diagram showing continuous and intermittent dosing with emergence of resistance.

Das Thakur MD, Cancer Res. 2013

Pulsatile therapy with BRAFi?

Experimental: Arm I (continuous dosing) Dabrafenib PO BID and trametinib PO QD

Experimental: Arm II (intermittent dosing) Dabrafenib PO BID and trametinib PO QD on days 1-7 and 29-56.
**ClinicalTrials.gov**

A service of the U.S. National Institutes of Health

ClinicalTrials.gov Identifier: NCT02012231

Phase I/IIa Study to Evaluate the Safety, PK, PD, and Preliminary Efficacy of PLX8394 in Patients With Advanced Cancers.

- Melanoma
- Thyroid Cancer
- Colorectal Cancer
- Non-small Cell Lung Cancer
- Cholangiocarcinoma
- Histiocytosis
- Hairy Cell Leukemia

- Inhibit RAF signaling in BRAF(V600E) without paradoxical effects in wild-type cells.
- Block the growth of vemurafenib-resistant BRAF(V600E) cells that express mutant BRAF(L505H), NRAS or BRAF splice variants

2nd Gen RAFl, “Paradox-breakers”: PLX8394 / PLX7904

Chen YH, Oncogene 2013
“Paradox-breaking” BRAFi: PanRAFi

CCT196969
CCT241161
PLX7904
LY3009120

- 2\textsuperscript{nd} generation pan-Raf inhibitors inhibit A-, B-, and C-Raf
- Minimal paradoxical pathway activation in B-Raf wild type background, avoiding skin side effects (such SCC)
- Being active against monomers, inhibit BRAF V600E cells and nonV600E/K mutant tumor cells
- May be active against vemurafenib- or dabrafenib-resistant cells:
  - Being active against Raf homodimers, inhibit p61BRaf and KIAA1549-BRAF splice variants
  - Being active against Raf heterodimers, inhibit Nras & Kras mutant cells.

Chen YH, Oncogene 2013
Peng SB, AACR 2014
MEK inhibitors

BRAF/MEKi:
RO5126766/CH5126766

Allosteric MEK1i
Trametinib (GSK1120212)
Pimasertib (AS703026)
Selumetinib (AZD6244)
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Refametinib (BAY86-9766)
TAK733
MEK162 (ARRY438162)
WX554
RO4987655
ARRY-300
AS703988
AZD8830
E6201

Allosteric MEK1/2i
Cobimetinib (GDC0973)
Family tree of MEK inhibitors

1st Generation

CI 1040
Sept. 18, 2000

2nd Generation

PD 0325901
AZD-6244

better biological/pharmaceutical properties
more potent against MEK1 and MEK2

3rd Generation

E-6201
ARRAY-300
RDEA-436
AZD-8330

AS-703026
RDEA-119
RO 4987655
XL-518
GSK1120212
BAY 86-9766
GDC-0973
MSC1936396

From Pat Lorusso
Pharmacology meets combination strategies

...Dual combinations
...triple combinations
Mechanisms of resistance to BRAFi and potential combos

Dual combinations BRAFi+ MEKi: Comparison of G3 toxicity

<table>
<thead>
<tr>
<th></th>
<th>Vemurafenib</th>
<th>Dabrafenib</th>
<th>Dabrafenib (150 mg BID)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any G3 NA (around 49%)</td>
<td>147 (48%)</td>
<td>117 (49%)</td>
<td>198 (57%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>5 (8%)</td>
<td>4 (1%)</td>
<td>0</td>
</tr>
<tr>
<td>Rash</td>
<td>1 (2%)</td>
<td>4 (1%)</td>
<td>0</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>2 (3%)</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>AST / ALT elevated</td>
<td>5 (8%) / 6 (10%)</td>
<td>4 (1%) / 9 (3%)</td>
<td>14 (6%) / 4 (2%)</td>
</tr>
<tr>
<td>CreatinKinase elevated</td>
<td>2 (3%)</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>1 (2%)</td>
<td>15 (4%)</td>
<td>0</td>
</tr>
<tr>
<td>Decreased EF</td>
<td>3 (1%)</td>
<td>13 (4%)</td>
<td>3 (1%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>NA</td>
<td>48 (14%)</td>
<td>NA</td>
</tr>
<tr>
<td>Cutaneous squamous cell carcinoma</td>
<td>7 (11%)</td>
<td>1 - 3 (2 - 5%)</td>
<td>27 (11%) / 9 (17%)</td>
</tr>
</tbody>
</table>

Most common toxicity any grade: pyrexia, nausea, diarrhea, chills, vomiting.

Dose interruption: 55%; Dose reduction: 33%; Permanent discontinuation: 13%
**RAF/MEK inhibitor, RO5126766**

- **V600E BRAF**
- **WT BRAF**
- **CRAF**
- **MEK1**

| IC50 (nM) | 8.2 | 19 | 56 | 160 |

**FIH n=52 Cohorts**

- Once daily (QD) 0.1-2.7 mg
- 7 days on/7 days off 2.7-5.0 mg
- 4 days on/3 days off 2.7-4.0 mg

**Main toxicities**

<table>
<thead>
<tr>
<th>All grade</th>
<th>G1 (46%)</th>
<th>G2/3 (15%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>(94)</td>
<td>(36)</td>
</tr>
<tr>
<td>Gastrointestinal (Diarrhea, constipation, dry mouth, stomatitis)</td>
<td>(71)</td>
<td>0</td>
</tr>
<tr>
<td>Metabolic/nutrition (CPK elevation, hypoalbuminemia)</td>
<td>(67)</td>
<td>6</td>
</tr>
<tr>
<td>General (Edema, fatigue)</td>
<td>(67)</td>
<td>1</td>
</tr>
<tr>
<td>Ocular</td>
<td>(58)</td>
<td>3</td>
</tr>
<tr>
<td>Musculoskeletal (Myopathy, myalgia, joint swelling)</td>
<td>(19)</td>
<td>10</td>
</tr>
</tbody>
</table>

**No SCC or keratoacanthoma**

- **Main toxicities**
  - Blurred vision (36%)
  - Macular edema (11%)
  - Photopsia (4%)

- **Main grade**
  - G1 (46%)
  - G2/3 (15%)

**PK concentration vs PBMC pERK (all doses)**

**Tumor biopsies pERK expression (IHC)**

- CRC-BRAF V600E mutant 1.8mg QD

**Martinez M, Clin Cancer Res 2012**
Other combos

- PI3K/AKT/MTORi + MEKi
- PI3K/AKT/MTORi + BRAFi
- EGFRi (and other surface receptors)+ MEKi
- EGFRi (and other surface receptors)+ BRAFi
- Immunotherapy (anti CTLA4, antiPD1/PDL1) + BRAFi
- Immunotherapy (anti CTLA4, antiPD1/PDL1) + MEKi
- HSP90i + BRAFi
- METi + BRAFi
- CDKi + BRAFi
- VEGFRi + BRAFi
- FGFRi + BRAFi
Logic 2: A phase II, multi-center, open-label study of sequential LGX818/MEK162 combination followed by a rational combination with targeted agents after progression, to overcome resistance in adult patients with locally advanced or metastatic BRAF V600 melanoma.

Part I

**BRAF**\(^{V600}\) melanoma

- LGX818
- MEK162

Relapse/PD

Biopsy

Part II

- +
  - PI3Ki: BKM120
  - FGFRi: BGJ398
  - cMETi: INC280
  - CDKi: LEE001

Identification of putative resistance mechanisms to BRAF/MEK combination and assign pts to rational triple combination.

Clinicaltrials.gov: NCT02159066
Encorafenib (BRAFi) + cetuximab (Anti-EGFR Antibody) +/- BYL719 (α-specific PI3K inhibitor) in Patients with BRAF V600E Mutated Colorectal Cancer

Dabrafenib (BRAFi) + Trametinib (MEKi) + Panitumumab (Anti-EGFR Antibody) in Patients with BRAF V600E Mutated Colorectal Cancer

Encorafenib (BRAFi) + MEK162 (MEKi) + LEE011 (CDK 4/6 inh)

Dabrafenib (BRAFi) + Trametinib (MEKi) + Ipilimumab (CTLA4 inh)

Dabrafenib (BRAFi) + Trametinib (MEKi) + MED14736 (antiPDL1)

Dabrafenib (BRAFi) + Trametinib (MEKi) + Pembrolizumab (antiPD1)

Vemurafenib (BRAFi) + Interleukin 2 + INFa2b

Dabrafenib (BRAFi) + Trametinib (MEKi) + AT13387 (HSP90 inh)

Dabrafenib (BRAFi) + Trametinib (MEKi) + AMG232 (HDM2-P53 inh)

MTD was not reached

Acneiform Rash:

- D + P: 47% grade 1, 6% grade 2
- D + P + T: 25% grade 1; 25% grade 2, 6% grade 3
- P: 57% grade 1-2 rash, 7% grade 3 rash (label)

Pyrexia:

- D + P: 0% grade 1, 24% grade 2
- D + P + T: 13% grade 1, 6% grade 2

van Geel Robin M, 2014: Bendell J, ASCO 2014
Cross-Study Comparison of Phospho-ERK Modulation Between DUAL (D+P, D+T) vs TRIPLE (D+P+T) Therapy in CRC and Monotherapy (D) Therapy in Melanoma

Comparison of p-ERK modulation using dabrafenib based combination therapies in BRAFm CRC and BRAFm melanoma. Treatment in BRAFm CRC was dabrafenib (150 mg BID), trametinib (1.5-2 mg QD) and/or panitumumab (4.5-6 mg every two weeks). Treatment in BRAFm melanoma was dabrafenib (70-200 mg BID). Average +/- SD for median pERK decrease in CRC was D+P (n= 7): -11 % (± 35.7 %), -28; D+T (n=9): 47 % (± 24 %), -36.7%; D+P+T (n=2): -84 (± 14 %), -84%. Average +/- SD for pERK decrease in melanoma was D (n=8): 76 % (± 20 %), -84%.

Bendell J, ASCO 2014
New Kids on the block…

…ERKi

…targeting KRAS
**ERKi**

- *In vitro* profiles of ERK inhibitors can differ
  - Effects on phospho-ERK levels vary between different ERK inhibitor series:
    - Most chemical series show rapid and sustained increase in phospho-ERK
    - GDC-0994 has little acute effect on phospho-ERK *in vitro*
    - In contrast SCH772984 is reported to decrease phospho-ERK *(Cancer Disc. 2013; 3:742)*
  - Inhibition of phospho-p90RSK correlates well with biochemical potency
  - Biochemical activities (kinetic differences in phosphorylation and dephosphorylation of ERK) of diverse ERK inhibitors suggest a range of conformational effects

**HCT116 (KRAS CRC) 1 hr treatment**

<table>
<thead>
<tr>
<th>GDC-0994</th>
<th>ERKi # 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>uM</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.01 0.04 0.1 0.3 1</td>
</tr>
<tr>
<td>0.01 0.04 0.1 0.3 1</td>
<td></td>
</tr>
</tbody>
</table>

**ERK**

- ERK
- MEK
- MKP

**pERK**

- p-p90RSK
Inhibitors of membrane association
Minerval
FTIs
GGTIs
Deltarasin

Mutation Specific
Direct KRAS inhibitor G12C

Targeting RAS at RNA level
Anti-sense oligonucleotids
siRNA
Direct KRAS targeting

Mutant KRAS is a druggable target for pancreatic cancer

Elina Zerova Khvalevsky1, Rachell Gaba1, Izhak-Haim Rahu1, et al.

Elini Zerova Khvalevsky1, Rachell Gaba1, Izhak-Haim Rahu1, et al.

Pancratic ductal adenocarcinoma (PDAC) represents an urgent therapeutic challenge. PDAC is associated with the activity of the mutated KRAS gene, which is considered so far an undruggable therapeutic target. We propose an approach to target KRAS effectors in patients using RNA interference. In our phase I trial, we demonstrated that siRNA delivered via LQ100 shows sufficient potency against the mutated KRAS (G12C) (LODER). The SIG12 C DODER was assessed for its structural release, and delivery properties in vivo and in vivo. The effect of the SIG12 LODER on tumor growth was assessed in vivo, and periods of time, reduction of side effects and cell reduction (13). A prominent method of controlling the release rate of a drug in a sustained dosage is to embed the active agent within a polymeric matrix (14, 15). The polymer must be biocompatible and in the case of pancreatic administration, preferably biodegradable, to avoid the need to remove implant materials.

In the present study, we exploited the slow-release characteristics of the biodegradable polymer matrix, which we tested local drug delivery (LODER) for the treatment of solid tumors.

A phase I trial of a local delivery of siRNA against k-ras in combination with chemotherapy for locally advanced pancreatic adenocarcinoma.

Khvalevsky EZ, PNAS 2013
Inhibitors of membrane association of KRAS

Ras localization

Human pancreatic cancer (BXPC3)

Human glioma (SF767)

Glioma cell

Glioma cell +2OHOA

Minerval

Gemcitab

Min+Gem

Control

TMZ

2OHOA

TMZ+OHOA

Tumor Volume (% of Control)

Tumor Volume (% of Control)
**Smart downstream inhibition: MEKi+ERKi combo**

### RAS-mutant

- RTK
- RAS\textsuperscript{mut}
- RAF
- MEK
- ERK
- SPRY
- DUSP
- Cytoplasmic substrates (p90RSK)
- Transcription

### BRAF-mutant

- RTK
- RAS
- RAF\textsubscript{BRAFV600mut}
- MEK
- ERK
- SPRY
- DUSP
- Cytoplasmic substrates (p90RSK)
- Transcription

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**Uptake of MEKi+ERKi combo**

- **H2122, KRAS NSCLC**
  - GDC-0994 + Cobimetinib
  - ERKi #3 + Cobimetinib
  - GDC-0994 + MEKi GDC-0623

  - QD x 7 days (PD study)
    - Vehicle
    - Cobimetinib, 5 mg/kg
    - GDC-0994, 60 mg/kg
    - ERKi #2
    - Cobimetinib, 5 mg/kg + GDC-0994, 60 mg/kg
    - Cobimetinib, 5 mg/kg + ERKi #2

**A375, BRAF melanoma**

- Cobimetinib (20 nM)
- GDC-0994 (320 nM)
- ERKi #3 (640 nM)

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Moffat, J et al. AACR 2014
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Sheng-Bin Peng (Eli Lilly)
Thank you for your attention