Part 1: Targeting Cancer Pathways
Tumor Resistance

October 22, 2014

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Part 1: Targeting Cancer Pathways
Tumor Resistance

October 22, 2014

Participating Experts

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Dynamic Re-Wiring of Signaling Networks as Mechanisms for Improving Combination Therapy for Cancer

Michael B. Yaffe
Koch Institute for Integrative Cancer Biology
Depts of Biology & Biological Engineering
Broad Institute & MIT
Dept. of Surgery, Beth Israel Deaconess Med Ctr,
Harvard Medical School
Dynamic Re-Wiring of Signaling Networks as Mechanisms for Improving Combination Therapy for Cancer

Protein kinases
Growth Factor receptors
DNA Damage
RNA-Binding Proteins
Cytokines
Signaling and Systems Biology are the ‘Missing Data’ that links Genotype to Phenotype… Mutational spectra to tumor responses.....
Why Use Systems Biology of Signaling to Treat Cancer?

1. Targeted monotherapies for cancer, including EGFR inhibitors, B-Raf inhibitors, and ALK inhibitors do not cure the disease. They target signaling molecules and result in impressive remission of the disease, but ultimately the disease recurs in nearly all patients as the tumors develop resistance.

2. Most forms of combination chemotherapy for cancer are not synergistic. Instead, most common drug combinations function by targeting heterogeneity with the tumor cell population – they represent ‘de-personalized’ medicine. However, these combinations have the advantage of non-overlapping toxicities.

3. **Systems Biology** is the key to (1) identifying new nodes in clinically relevant pathways; (2) designing and optimizing effective synergistic combination therapies; (3) Predicting patients who will respond to a drug, at least initially; (4) developing approaches to minimize development of chemo-resistance.

**TWO KEY CONCEPTS**: **SYNTHETIC LETHALITY** and **DYNAMIC NETWORK RE-WIRING**.
Dynamic network re-wiring is bad for molecularly targeted therapies alone.

Before Rx  PLX4032—15 wks  PLX4032—23 wks

Wagle et al., J. Clin. Oncol. 2011
Dynamic network re-wiring is bad for molecularly targeted therapies alone.

But it can be beneficial for combination chemotherapy using molecularly targeted drugs **PLUS** DNA damaging cytotoxic agents…

Conventional DNA-damaging chemotherapy

Targeted therapies

Mike Lee
Combination Drug Screen in Breast Cancer

“TRIPLE-NEGATIVE” =
- No ER expression
- No PR expression
- No HER2 amplification

Luminal (A and B)
48 – 78 %

HER2
10-30%

TNBC
15-20%

EGFR over-expression
(30% overall; 45-75% TNBC)
Combination Drug Screen for Triple Negative Breast Cancer

**Signaling inhibitors**
- Erlotinib (EGFR)
- Gefitinib (EGFR)
- Lapatinib (EGFR/HER2)
- MM-121 (ErbB3)
- PD98059 (MEK)
- BMS-345541 (NF-κB)
- Rapamycin (mTOR)
- NVP-BEZ235 (PI3K/mTOR)
- Wortmannin (PIKKs)

**DNA Damage**
- Camptothecin
- CDDP
- Etoposide
- Doxorubicin
- Temozolomide
- Taxol

**Time course**
Start - End
Efficacy of EGFR Inhibition in BT-20 TNBC Cells Depends on Timing of Drug Delivery

Apoptotic Response at 8 hours after doxorubicin treatment
Subtype Dependent Responses to Treatment

Luminal (A and B) 48 – 78 %

HER2 7-12%

TNBC 15-20%

BT-20 (TNBC)

MDA-MB-453 (HER2 OE)

MCF7 (Luminal)

Hs578Bst (Normal)
Understanding “Dynamic Re-wiring”

Gene Expression Profiling

Putative Response Network

Collect Large Dataset of Treatment Responses

Data-driven Modeling

Identify EGFR-driven Subset

Confirm Utility of Treatment Strategy In vivo

Lee, MJ et al. (2012) *Cell*
Understanding “Dynamic Re-wiring”

Gene Expression Profiling

Putative Response Network

Collect Large Dataset of Treatment Responses

Data-driven Modeling

Identify EGFR-driven Subset

Confirm Utility of Treatment Strategy In vivo

Lee, MJ et al. (2012) Cell
Working Model

TNBC before Erlotinib treatment

TNBC chronically treated with Erlotinib

RTK (EGFR)

DNA DAMAGE

CASP8

CASP3

CASP9

DEATH

RTK (EGFR)

DNA DAMAGE

CASP8

CASP9

DEATH

ERLOTINIB

ONCOGENIC SIGNATURE

ONCOGENIC SIGNATURE
Testing Time-Staggered Inhibition In Vivo

TUMOR VOLUME (mm$^3$)

DAYS

- □ DMSO
- - DOX
- - D/E
- ▲ E→D
Collaboration with Paula Hammond’s Lab: Nanoparticle Development for Time-Staggered Drug Delivery in vivo

Polylactic co-glycolic acid (PLGA)

Erlotinib

Stephen Morton, Mike Lee
Liposomal Delivery Vehicles

- Small Molecule Inhibitor
- Hydrophilic Cytotox

Erlotinib

Doxorubicin
Liposomal Delivery Vehicles

- Doxil®
  - Small Molecule Inhibitor
  - Hydrophilic Cytotox Liposomal Delivery Vehicles

- Doxorubicin
- Erlotinib

Graph showing the release of Dox - conjugate and Erlotinib over time.

Chemical structures of Erlotinib and Doxorubicin.
Folate/PEG Decorated Combo Liposomes

<table>
<thead>
<tr>
<th>Liposomal Formulation</th>
<th>Mean $d_h$ (nm)</th>
<th>PDI</th>
<th>$\zeta$-Potential (mV)</th>
<th>Cytotox:Inhibitor Mass Loading Ratio</th>
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<tr>
<td>DFP</td>
<td>156</td>
<td>0.1</td>
<td>-20</td>
<td>N/A</td>
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<tr>
<td>DEFP</td>
<td>151</td>
<td>0.16</td>
<td>-20</td>
<td>3:2</td>
</tr>
</tbody>
</table>
Combination Erl-Dox Nanoparticles in vivo

Erlotinib
Doxorubicin + Erlotinib
Doxorubicin
Dynamic Network Rewiring through Time-Staggered EGFR Inhibition also kills NSCLC tumors
Dynamic Network Rewiring through Time-Staggered EGFR Inhibition also kills NSCLC tumors
Generalizing Time-Staggered Inhibition of RTK Signaling for Tumor Sensitization

**ERLOTINIB** (EGFR)

**LAPATINIB** (HER2/EGFR)

**BT-20** (TNBC)

**MBA-MB-453** (HER2)

**BT-474** (HER2)
Conclusions

1. The EGFR pathway cross-talks with the DNA damage response pathway in a subset of TNBC cells and NSCLC cells to suppress an extrinsic apoptotic pathway, limiting the efficacy of cytotoxic chemotherapy.

2. Signaling pathways in cancer cells can be “dynamically re-wired” to enhance cell killing by DNA damage. The underlying mechanisms, along with biomarkers for patient selection and response can be obtained using systems biology approaches to combination therapy.

3. The concept of dynamic re-wiring can shift the focus from drug development to novel approaches to drug delivery…creating new IP for old drugs.

4. We need to test these systems-based insights about dynamic network modulation for optimizing combination therapies using kinase inhibitors and DNA damaging agents in the clinic.
Acknowledgements

Funding
NIH NCI (ICBP)
NIGMS, NIEHS, DOD

Collaborators
MIT
Paula Hammond
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Mike Lee
Stephen Morton
Albert Ye
Alexandra Gardino
Anne Margriet Heijink
Andrea Tentner
Gerry Ostheimer
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Salt Lake City, UT
Resistance to Tyrosine Kinase Inhibitors in Lung Cancer

Jeffrey Engelman, MD, PhD
Massachusetts General Hospital
Cancers with EGFR mutations are highly sensitive to EGFR kinase inhibitors

Lynch et al, NEJM 2004

Lux-Lung 2
Cancers with ALK translocations are highly sensitive to ALK kinase inhibitors.

Shaw et al JCO 2009, Kwak et al NEJM 2010
# Acquired resistance to targeted therapies

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Drug</th>
<th>Median Duration of Response (months)</th>
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</thead>
<tbody>
<tr>
<td>EGFR mutant lung cancers</td>
<td>gefitinib/erlotinib</td>
<td>11</td>
</tr>
<tr>
<td>EML4-ALK lung cancers</td>
<td>ALK inhibitors (crizotinib)</td>
<td>8-10</td>
</tr>
<tr>
<td>BRAF mutant melanomas</td>
<td>BRAF + MEK inhibitors (dabrafenib and trametinib)</td>
<td>9</td>
</tr>
<tr>
<td>HER2 amplified breast cancer</td>
<td>lapatinib</td>
<td>9</td>
</tr>
<tr>
<td>FLT3 AML</td>
<td>quizartinib</td>
<td>3</td>
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<tr>
<td>CKIT GIST</td>
<td>imatinib</td>
<td>20</td>
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</tbody>
</table>

**EML4-ALK lung cancer**
Sensitivity to tyrosine kinase inhibitors

Sensitive
(mutant EGFR or EML4-ALK)

Downstream signaling
(PI3K and MEK)

Cell growth/
Survival

Downstream signaling
(PI3K and MEK)

Cell growth/
Survival

Engelman et al, Science, 2007
Resistance to tyrosine kinase inhibitors

**Sensitive**
- EGFR
- EML4-ALK
- Downstream signaling (PI3K and MEK)
- Cell growth/Survival

**Resistant**

**Mutation/Amplification**
- EGFR T790M
- ALK L1196M
- ALK G1269A
- ALK G1206Y
- ALK G1202R
- ALK 1151 ins
- MET amplification
- HGF/MET activation
- HER2/HER3 activation
- IGF-IR activation
- PIK3CA mutation
- BRAF mutation
- EGFR activation
- C-KIT amplification

**Bypass Tracks**
- Defect growth arrest/apoptosis
- EMT
- SCLC
- Loss of BIM
Resistance to tyrosine kinase inhibitors

**Sensitive**
- Downstream signaling (PI3K and MEK)
- Cell growth/Survival

**Resistant**
- Mutation/Amplification
- Downstream signaling (PI3K and MEK)
- Cell growth/Survival
- Bypass Tracks
- Downstream signaling (PI3K and MEK)
- Cell growth/Survival
- Defect growth arrest/apoptosis

**MORE POTENT INHIBITORS:**
- CLO-1686, AZD9291
- LDK378, CH5424802

- EGFR T790M
- ALK L1196M
- ALK G1269A
- ALK G1206Y
- ALK G1202R
- ALK 1151 ins

- MET amplification
- HGF/MET activation
- HER2/HER3 activation
- IGF-IR activation
- PIK3CA mutation
- BRAF mutation
- EGFR activation
- C-KIT amplification

- EMT
- SCLC
- Loss of BIM
Resistance to tyrosine kinase inhibitors

Sensitivity:
- Downstream signaling (PI3K and MEK)
- Cell growth/Survival

Resistance:
- Mutation/Amplification
- Downstream signaling (PI3K and MEK)
- Cell growth/Survival

Bypass Tracks:
- EMT
- SCLC
- Loss of BIM

Defect growth arrest/apoptosis

COMBINATIONS: e.g., MET and EGFR inhibitors
- MET amplification
- HGF/MET activation
- HER2/HER3 activation
- IGF-IR activation
- PIK3CA mutation
- BRAF mutation
- EGFR activation
- C-KIT amplification
About One-Third of Crizotinib-Resistant Tumors Harbor ALK Resistance Mutations

About one-third of Crizotinib-resistant tumors harbor ALK resistance mutations. This includes ALK amplification (amp), ALK mutation (mut), and other mechanisms such as L1196M, G1269A, S1206Y, G1202R, 1151Tins, L1152R, and C1156Y. Bypass tracks involving EGFR and CKIT are also observed in about one-third of Crizotinib-resistant tumors. Unknown mechanisms account for the remaining tumors.

Courtesy of Alice Shaw
Results from Repeat Biopsy Program

- **No identified AR mechanism** 26%
- **T790M** 52%
  - alone 42%
  - with EGFR amp 10%
- **MET amp** 5%
- **BRAF** 2%
- **EGFR Amp** 15%
  - with T790M 10%
  - alone 4%
  - with SCLC 1%
- **PI3K** 5%
  - with SCLC 3%
  - alone 2%
- **SCLC** 8%
  - with EGFR amp 1%
  - alone 4%
  - with PI3K 3%

- **N=106**

Unpublished data, Lecia Sequist
Sequist et al, Science Translational Medicine, 2011
Katyama, Shaw, et al, Science Translational Medicine, 2012
Results from Repeat Biopsy Program

Small Cell Lung Cancer Transformation

SCLC 8%
with EGFR amp 1%
alone 4%
with PI3K 3%

EGFR Amp 15%
with T790M 10%
alone 4%
with SCLC 1%

T790M 52%
alone 42%
with EGFR amp 10%

N=106

unpublished data, Lecia Sequist
Sequist et al, Science Translational Medicine, 2011
Katyama, Shaw, et al, Science Translational Medicine, 2012
Heterogeneity of resistant clones within individual patients explain paradoxical clinical findings
Serial Biopsies Reveal:
Dynamic Populations of Different Clones

<table>
<thead>
<tr>
<th>Histology</th>
<th>Adeno</th>
<th>Adeno</th>
<th>Adeno</th>
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<td>Genotype</td>
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<tr>
<td>T790M</td>
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<tr>
<td>EGFR TKI status</td>
<td>Sensitive</td>
<td>Resistant</td>
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<tr>
<td>Treatment</td>
<td>Chemo</td>
<td>Erlotinib</td>
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<tr>
<td>Timeline</td>
<td>2007</td>
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Sequist et al, Sci Transl Med 2011
### Serial Biopsies Reveal Fluctuating Dynamics of Cell Populations

<table>
<thead>
<tr>
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<th>Adeno</th>
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<td></td>
<td></td>
<td>PIK3CA</td>
<td></td>
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<td>EGFR TKI status</td>
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<td>Resistant</td>
<td>Sensitive</td>
<td>Resistant</td>
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<td>Tumor Burden</td>
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<td>Treatment</td>
<td>Erlotinib</td>
<td>C+RT</td>
<td>Erlotinib</td>
<td>C+ RT</td>
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<tr>
<td>Timeline</td>
<td>2008</td>
<td>2009</td>
<td>2010</td>
<td></td>
</tr>
</tbody>
</table>

Sequist et al, Sci Transl Med 2011
Multiple Populations in a Single Tumor Nodule: “Microscopic heterogeneity”

Pre-treatment “Sensitive”

Rx erlotinib

“persistor cells”

Post-treatment “Response”

Rx erlotinib

Resistant

Rx erlotinib

Sensitive again

D/C erlotinib

“FLARE”

+ T790M

Resistant

NO T790M

Sensitive
Each patient may have his/her own “pie chart” of resistance mechanisms.

Pre-treatment “Sensitive”

Rx erlotinib

Post-treatment “Response”

Rx erlotinib

Resistant

+ T790M

Sensitive

Resistant

T790M 52%

alone 42%

with EGFR amp 10%

EGFR Amp 15%

with T790M 10%

alone 4%

with SCLC 1%

PI3K 5%

with SCLC3%

alone 2%

BRAF 2%

MET amp 5%

No identified AR mechanism 26%

SCLC 8%

with EGFR amp 1% alone 4%

with PI3K 3%
ARE WE FAILING TO FULLY SUPPRESS THE TARGET?

Green cells staying alive because ALK is not fully suppressed
Antitumor Efficacy of Ceritinib

Change from baseline in sum of longest diameters (%)

- Patients with measurable disease at baseline and at least 1 post baseline assessment without unknown response for target lesion or overall response

N=228*

* Patients with measurable disease at baseline and at least 1 post baseline assessment without unknown response for target lesion or overall response
Ceritinib is Active Against Resistant Tumors With and Without ALK Resistance Mutations

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<tr>
<th>Best % response</th>
<th>22</th>
<th>26</th>
<th>32</th>
<th>34</th>
<th>43</th>
<th>44</th>
<th>45</th>
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<th>63</th>
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<tr>
<td>PFS on LDK378 (wks)</td>
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<td>71</td>
<td>12</td>
<td>8</td>
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<td>77</td>
<td>21</td>
<td>42</td>
<td>61</td>
<td>39</td>
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<tr>
<td>ALK FISH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>ALK amplification</td>
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</tr>
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</table>

Decrease from baseline (%)

Ceritinib is an active agent against resistant tumors with and without ALK resistance mutations.
Incomplete ALK inhibition may allow minimal bypass track activation to cause resistance.
Selection of new resistant clones on LDK378 (certinib)

**EML4-ALK** sequence:
- **WT**
- **S1206Y**
- **G1202R**

**Ba/F3 EML4-ALK V1**

- S1206Y v1 crizotinib
- S1206Y v1 LDK378
- G1202R crizotinib
- G1202R LDK378

*Friboulet et al, Cancer Discovery, 2014*
Selection of new resistant clones on LDK378

**EML4-ALK sequence:**
- Baseline: WT
- After 8 weeks of crizotinib: S1206Y
- After 34 months of crizotinib: WT
- After 12 weeks of LDK378: WT
- After 15 months of LDK378: G1202R

<table>
<thead>
<tr>
<th>Patient Id</th>
<th>EML4-ALK sequence at Crizotinib Resistance</th>
<th>EML4-ALK sequence at LDK378 Resistance</th>
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</thead>
<tbody>
<tr>
<td>MGH011</td>
<td>S1206Y</td>
<td>G1202R</td>
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<tr>
<td>MGH015</td>
<td>WT</td>
<td>WT</td>
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<tr>
<td>MGH023</td>
<td>WT</td>
<td>F1174C</td>
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<tr>
<td>MGH034</td>
<td>WT</td>
<td>WT</td>
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<td>MGH049</td>
<td>N/A</td>
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<td>MGH051</td>
<td>WT</td>
<td>G1202R</td>
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<tr>
<td>MGH057</td>
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<td>JCR013</td>
<td>N/A</td>
<td>WT</td>
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<tr>
<td>JCR021</td>
<td>G1269A (right lung)</td>
<td>F1174V (left lung) and G1202R (right lung)</td>
</tr>
</tbody>
</table>

_Friboulet et al, Cancer Discovery, 2014_
Autopsies reveal heterogeneity of resistance mechanisms

Autopsy #1
- T790M
- MET amplification

Autopsy #2
- EMT
- Adeno T790M

Develop Regimens: Alternating and Intermittent Therapeutic Combinations

Current Treatment

Future Regimen

EGFR TKI

EGFR TKI + Drug A (Combo #1)

EGFR TKI + Drug B (Combo #2)

Combo #3

Immunotherapy

EGFR TKI + Drug C (Combo #4)

EGFR TKI + Drug C (Combo #5)
Conclusions

• Resistance to tyrosine kinase inhibitors limits clinical impact
• Resistance can be mediated by mutation of the gene target or activation of bypass track
• Multiple resistant clones can co-exist in a single patient
• Future treatment regimens may require complex combinations to overcome resistance.
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Harvard Medical School
Boston, MA

Michael Deininger, M.D., Ph.D.
University of Utah
Salt Lake City, UT
Teaching Old Dogs New Tricks: Tyrosine Kinase Inhibitor Resistance due to Mutations in the Target Kinase

Michael Deininger, MD, PhD
Protein Kinases Regulate Key Cell Functions

518 PTKs (1.7% of HG)

90 TKs (+ 5 pseudogenes)

58 Receptor TKs

32 Non-receptor TKs

Several catalytically inactive or predicted to be inactive

Manning et al. Science 2002
Chronic Myeloid Leukemia

Chronic phase

Blastic phase
The Philadelphia Chromosome is the Cytogenetic Hallmark of CML
BCR-ABL Kinase Activity is Central to CML Pathogenesis

Deininger et al Blood 1997
Imatinib Greatly Improved Survival in Chronic Phase CML

Quintas-Cardama et al, 2006
# BCR-ABL1 Tyrosine Kinase Inhibitors (TKIs)

<table>
<thead>
<tr>
<th><strong>A)</strong> Src/Abl inhibitors</th>
<th>PD180970</th>
<th>bosutinib</th>
<th>dasatinib</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B)</strong> Imatinib-type inhibitors</td>
<td>imatinib</td>
<td>nilotinib</td>
<td>INNO-406</td>
</tr>
<tr>
<td><strong>C)</strong> Allosteric, non-ATP competitive inhibitors</td>
<td>DCC-2036</td>
<td>GNF-2</td>
<td>ponatinib</td>
</tr>
<tr>
<td><strong>D)</strong> Abl/Aurora kinase inhibitors</td>
<td>MK-0457</td>
<td>PHA-739358</td>
<td>HG-7-85-01</td>
</tr>
<tr>
<td><strong>E)</strong> T315i inhibitors</td>
<td>XL228</td>
<td>PPY-A (SGX393-like)</td>
<td></td>
</tr>
</tbody>
</table>
Inhibitor Binding Conformations

Type II Inhibitor: INACTIVE CONFORMATION ("DFG-out")

Type I Inhibitor: ACTIVE CONFORMATION ("DFG-in")
Mechanisms of TKI Resistance

BCR-ABL Reactivation?

Yes

CML L-BC (Bcr-Abl\textsuperscript{T315I})

- pCrkL
- non-pCrkL

NT 1000 nM imatinib 50 nM dasatinib

No

Dasatinib resistant CML CP (Native Bcr-Abl)

- pCrkL
- non-pCrkL

NT 1000 nM imatinib 50 nM dasatinib
Resistance Mutations in BCR-ABL1

Preclinical Characterization of Ponatinib

Accelerated Mutagenesis Screen: Predicting Mutational Resistance to TKIs

1. Ba/F3 BCR-ABL cells
2. ENU mutagenesis (overnight) + ENU
3. Culture with inhibitor(s)
4. Monitor for outgrowth (~28 days)
5. Expand positive wells
6. Sequence BCR-ABL kinase domain for mutations
Mutation Screening in a T315I Background: Anticipating Combinations of Mutations

E255V has highest IC$_{50}$ shift ratio for ponatinib

Native BCR-ABL background

Outgrowth (% of wells) 11.7%

Frequency among recovered clones (%) E255V

BCR-ABL T315I background

Outgrowth (% of wells) 40.0%

Frequency among recovered clones (%) E255V/T315I

Ponatinib Phase 2 Study: Duration of Response

- 89% estimated to maintain MCyR for at least 2 yrs (95% CI: 82%, 93%)
- 21% estimated to maintain MaHR for at least 2 yrs (95% CI: 8%, 37%)
Ponatinib Phase 2 Study: OS in BP-CML and Ph+ ALL

- OS at 2 years in BP-CML: 18% (median 7 months)
- OS at 2 years in Ph+ ALL: 21% (median 8 months)

Ponatinib Resistance

Chronic Phase CML (17% of failures)

- 33%

Accelerated and Blastic CML/Ph+ ALL (83% of failures)

- 66%
- 55%
- 45%

Overall Mutational Status

- Compound mutant: 51%
- Native BCR-ABL1 or ponatinib-sensitive point mutant: 49%

Pt. 11-032: E255V/T315I
- Resistance due to BCR-ABL1 reactivation

Pt. 11-077: T315I
- Resistance despite sustained BCR-ABL1 inhibition
T315I-Inclusive Compound Mutations Confer Universal TKI Resistance

Cellular BCR-ABL TKI Sensitivity

Rationalizing Resistance due to E255V/T315I

Forcing BCR-ABL1 to Commit Mutational Suicide

Auto-Inhibition of ABL Kinase Activity

Nagar et al., Cell 2003
Allosteric Inhibitors Targeting the Myristoyl Binding Pocket

Inhibition of T315I in vitro with GNF-5 Plus Nilotinib

E505K = mutation in the myristoyl binding pocket

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Part 1: Targeting Cancer Pathways
Tumor Resistance

October 22, 2014

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