Cancer Drug Discovery: Targets, Phenotypes, or Both?

John Moffat

New Zealand Society for Oncology  July 3 2013
Outline

Oncology discovery at Genentech.

How have cancer drugs been discovered?
  • The emergence and current dominance of targeted therapies

Why do targeted drugs fail?
  • Strengths and weaknesses of Target-first and Phenotype-first discovery.

Maximizing the chance of success with targeted drugs
  • Targeted combinations.
  • ‘Targeted Phenotypic’ drug discovery.
Genentech
1 DNA Way, South San Francisco, CA

Founded in 1976 by Bob Swanson and Herb Boyer to commercialize recombinant DNA technology.

- Produced first recombinant human proteins.

Wholly owned by Roche
~11,000 employees

Genentech Research and Early Development (gRED) runs as independent unit.

~1300 Researchers.

Largest single-site biotech research facility in the world.
How are new drugs being discovered?

Targeted Discovery drives the Genentech Oncology Pipeline

- Balance between biologics and small molecules
- Focus on Pathways for Small Molecule Targets
- Companion Diagnostics are key.

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<thead>
<tr>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
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<tbody>
<tr>
<td>AKT inh (GDC-0068)</td>
<td>RG7414 Anti-EGFL7</td>
<td>B-RAF inh + MEK inh</td>
<td>Bevacizumab (VEGF)</td>
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<td>Anti-PD-L1</td>
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<td>Obinutuzumab</td>
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Source: Genentech Public website www.gene.com
How have cancer drugs been discovered?

Milestones in Cancer Drug Discovery

- Cell Lines
- NCI screening program
- v-Src proto-oncogenes
- Tyrosine kinases
- HTS Human Genome
- Kinome Cancer Genomes
- 5-FU Vincristine Taxol (disc.) Etoposide Taxol (appr.) Imatinib Herceptin Vemurafinib

Phenotypic Discovery

Targeted Discovery
There is a continuum of approaches to drug discovery

Phenotypic

- Phenotypic screening identifies lead and optimized drug candidate without knowledge of molecular target.
- Phenotypic screening identifies lead, molecular target is identified and used to optimize drug candidate.
- Specific pathway or molecular response screened – target hypothesized but not directly assayed.
- Target with hypothesized function screened – inhibitors optimized with cell assays for phenotype or molecular response.
- Target with hypothesized function screened – inhibitors optimized biochemically.

Targeted
Antiproliferative assays yielded many of the antimitotics and cytotoxics in use;
- They’re unlikely to yield anything novel from further random screens.

Most other hallmarks do not correspond directly to in vitro-screenable phenotypes, but have molecular proxy biomarkers:
- Gene expression
- Kinase activity
- Protein localization

Hanahan & Weinberg, Cell 2011 144, 646-674
How have new drugs been discovered?

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NCEs approved for cancer in United States 1999-2012

- Kinase inhibitors have dominated recent approvals.
- De novo phenotypic screening has not delivered any first-in-class NCEs.
- Only one drug with unknown target has been approved

**Kinase Inhibitors**

* First-in-class

§ Clinical activity primarily driven by target other than nominal discovery target
How have new first-in-class drugs been discovered?

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Kinase Inhibitors

* First-in-class

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How are new drugs being discovered?

Current Clinical Trials

- Targeted drugs make up the largest proportion of NCEs in Phase II and III.
- Kinase inhibitors constitute >50% of TDD NCEs.
- A significant number of phenotypically-discovered phase II/III drug candidates are cytotoxics.
Why do cancer drugs fail in clinical development?

There is a ~3-5% success rate from phase 1 to approval.

Reasons:

- **Pharmacokinetics**
  - Unable to attain therapeutic exposures
  - Mostly Phase I

- **Toxicity/Tolerability**
  - Inadequate therapeutic window
  - Phase I/II

- **Efficacy**
  - **Inhibiting the target doesn’t have intended effect.**
  - Preclinical phenotype is not predictive
  - The drug doesn’t inhibit the intended target.
  - Phase II/III

- **Strategic**
  - *E.g.* Insufficient differentiation from existing drugs.
  - Portfolio decision
  - Any phase

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**Figure 1 | Phase II failures: 2008–2010.** The 108 failures are divided according to reason for failure when reported (87 drugs) (a) and therapeutic area (b).

**Figure 1 | Phase III and submission failures: 2007–2010.** The 83 failures are divided according to therapeutic area (a) and reason for failure (b).

*Arrowsmith, Nat Rev Drug Discov 2011, 10 87*
If we’re so smart, why do targeted drugs still fail due to efficacy?

Resistance is rapidly acquired.

Signaling networks are redundant and robust.

Therapeutic hypothesis may be flawed.
  – The target does not do what it was predicted to do.

Molecular mechanism of action not optimal.
  – Target is not engaged in the way that results in the expected response.

Strategies to address risks:

Multi-targeting
  Either in a single molecule or with a targeted combination

Use Target-specific Phenotypes for candidate selection
Strategies for targeted phenotypic discovery

Our goal is to design screening, lead optimization and candidate selection assays that:

1. Increase confidence in target-phenotype causality.

2. Understand the molecular mechanism of action of the drug on the target and the network.

3. Improve probability that the functional response is predictive of clinical response.
A broadly applicable approach to *in vitro* phenotypic profiling: High Content screening

What is being measured?

- Multiple parameters from multi-channel fluorescent staining.
  - Object number and area (cells, nuclei, organelles, spots)
  - Average or integrated intensity
  - Subcellular localization and distance relationships
  - Object shape parameters
  - Pixel intensity distributions (texture)

HCS is an ideal platform for compound selectivity and MoA profiling;

- In many cases a specific phospho-epitope can be detected and quantitated.
- Possible to multiplex multiple readouts into a single assay to distinguish on- and off-target effects.
- Inherently multiparametric: Detect and differentiate cytostatic and cytotoxic effects without even trying.
Simple high-content assays can differentiate multiple MoAs

Standard metabolism-based assays as proxies for cell proliferation or viability can fail to differentiate compound mechanisms of action.

- Cell Titer-Glo: Total amount of ATP in the well
- MTS: Amount of tetrazolium-reducing activity in the well

vs.

High-Content imaging (DNA stain only)

- Number of cells
- Sub-populations
- Cell cycle distribution

Chan, Kleinheinz, Peterson & Moffat, PLoS One 2013
Acquired Resistance
Molecular Mechanisms of Action
Target identification
Robustness of Oncogenic Signaling Networks

Acquired insensitivity to chronic inhibition:
- Upregulation/activation of compensating pathways, e.g. via loss of feedback repression or epigenetic alterations.
- Selection of resistance mutations in the target, pathway or in a compensating pathway.

Intrinsic insensitivity to acute inhibition:
- Redundancy within or between pathways
- Adaptation via loss of feedback repression

The textbook models of linear one-way signaling cascades does not reflect the reality of robust dynamic networks.

Phenotypic profiling of compound activities reveal unexpected complexities of response.
Molecular Mechanisms of Action

Same Target, Different Phenotypes – RAF inhibitors

RAF kinase inhibitors inhibit growth of B-RAF<sup>V600E</sup> cells

but

Some RAF inhibitors enhance growth of B-RAF<sup>WT</sup> cells, and some don’t.

This paradoxical effect depends on compound-induced dimerization of B-RAF and C-RAF

Hatzivassiliou G et al. Nature 2010
ERK is generally depicted as an essential signaling node in the Ras/RAF pathway.

However, complete inhibition of ERK1 & 2 in K-Ras mutant cell lines does not completely inhibit cell cycle progression.

The magnitude of phenotypic response varies with different ERK inhibitors that are equivalent in biochemical potency.

Differential effects on pathway feedback correlate with functional differences.
Acquired resistance to mono-targeted drugs

Responses to Vemurafenib treatment of B-RAF$^{V600E}$-mutant melanoma illustrate the extraordinary efficacy that can be achieved by targeting a dominant driver mutation.

- And the rapidity with which resistance can be acquired when targeting a single node in a complex network.

Wagle et al J Clin Oncol 2011, 29 3085
Acquired Resistance

Possession of drugs targeting multiple nodes enables rapid response to acquired mutations

*E.g.* MEK inhibitors were already in development before Vemurafenib resistance was observed. MEK+RAF Combination trials have moved rapidly into and through Phase III.

Genentech/Roche

**A Study of Vemurafenib And GDC-0973 in Patients With BRAF-Mutation Positive Metastatic Melanoma**

- **Condition:** Malignant Melanoma
- **Interventions:** Drug: vemurafenib; Drug: GDC-0973

**A Phase 3 Study Comparing GDC-0973, a MEK Inhibitor, in Combination With Vemurafenib vs Vemurafenib Alone in Patients With Metastatic Melanoma**

- **Condition:** Malignant Melanoma
- **Interventions:** Drug: vemurafenib; Drug: Placebo; Drug: GDC-0973

GSK

**A Study Comparing Trametinib and Dabrafenib Combination Therapy to Dabrafenib Monotherapy in Subjects With BRAF-mutant Melanoma**

- **Condition:** Melanoma
- **Interventions:** Drug: dabrafenib plus trametinib; Drug: dabrafenib plus trametinib placebo

**LCCC 1128: Open Label Phase II Trial of the BRAF Inhibitor (Dabrafenib) and the MEK Inhibitor (Trametinib) in Unresectable Stage III and Stage IV BRAF Mutant Melanoma; Correlation of Resistance With the Kinome and Functional Mutations**

- **Conditions:** Stage III Melanoma; Stage IV Melanoma; Unresectable Melanoma; BRAF Mutant Melanoma
- **Intervention:** Drug: BRAF inhibitor dabrafenib and MEK inhibitor trametinib

www.clinicaltrials.gov
Part of Genentech’s strategy has been to focus on pathways, not just targets.

ERK1/2 are the central effectors of signaling downstream of Ras, RAF, and RTK oncogenes.

We have been developing inhibitors of ERK to complement our clinical programs in MEK and B-RAF.
Selection of drug-resistant cells

- Incubate 48 Hrs.
- Replace medium with no drug
- Grow Until ~ 80% Confluent
- Note time required

- Repeat process, selecting flask resistant to highest [drug].
- Bank pooled cells for characterization.
- Document passage number required for resistance.
ERK inhibitors overcome acquired resistance to MEK inhibitors

- Multiple cell lines with acquired MEK inhibitor resistance are cross-resistant to other MEK inhibitors but retain sensitivity to ERK inhibitors.

Hatzivassiliou G et al. Mol Cancer Ther 2012;11:1143-1154
ERK inhibitors overcome acquired resistance to MEK inhibitors

- Different mechanisms of resistance arise within the same population are sensitive to ERK inhibitors.

K-Ras copy number

MEK1 mutations

Hatzivassiliou G et al. Mol Cancer Ther 2012;11:1143-1154
Acquired Resistance
Mechanism of Action

Target identification
Several phenotypically-discovered drugs have recently had unsuccessful trials due to functional activity not being causally related to the nominal target. Even if these drugs are efficacious, development and approval path is probably more compromised than if they were advanced as target-unknown:

- Financial costs
- Opportunity costs
- Clinical Trial Failures
- Patient mistreatment

**The “survivin suppressants” NSC 80467 and YM155 induce a DNA damage response**

Trevor G. Glaros · Luke H. Stockwin · Michael E. Mullendore · Brian Smith · Bethanie L. Morrison · Dianne L. Newton

**Iniparib Nonselectively Modifies Cysteine-Containing Proteins in Tumor Cells and Is Not a Bona Fide PARP Inhibitor**

Xuesong Liu¹, Yan Shi¹, David X. Maag¹, Joann P. Pelma¹, Melanie J. Patterson², Paul A. Ellis¹, Bruce W. Surber³, Damien B. Reacy⁴, Niru B. Soni¹, Uri S. Ladrón⁵, Allison J. Xu⁶, Ramesh Iyer⁷, John E. Harlan⁷, Larry R. Solomon⁷, Cherrie K. Donawho⁷, Thomas D. Penning⁷, Eric F. Johnson⁷, and Alexander R. Shoemaker⁷

**TARGETED THERAPIES**

**Tivantinib—a cytotoxic drug in MET inhibitor’s clothes?**

Paolo Michieli and Federica Di Nicolantonio
Cautionary Tales

Is Tivantinib really a MET Inhibitor?

Identified by screening a designed compound library for antiproliferative activity.

Proposed MOA: non-ATP-competitive inhibitor of MET
- Shown to bind the inactive confirmation of MET, thus inhibit activation

Preclinical data: Tivantinib activity is not consistent with that of a “true” MET inhibitor
- Tivantinib does not inhibit Met in the Invitrogen kinase panel.
- No effect detectable on HFG-induced MET phosphorylation in Met-positive cell lines.
- Tivantinib is active on Met-negative cell lines
- Met-driven cell lines are not differentially sensitive to tivantinib in vitro

Clinical results suggest that Met status does not predict response.
- Patient stratification by Met status was inconclusive
- Phase II PFS endpoints not met
- Phase III NSCLC combination with Erlotinib (EGFR) discontinued
Phenotypic signature of Tivantinib is most similar to microtubule-disrupting agents.

A375 (Met-negative) cells analyzed by high-content for DNA content and nuclear morphology.

Linear discriminant analysis;

- Features: cell number, percent subG1, G0/G1, G2/M, >4N, mean intensity, mean area, mean roundness.
- Training set: DMSO, PD901, nocodazole, paclitaxel.

![Graph showing Tivantinib and Nocodazole effects on cell number](image)
Summary
Summary

Phenotypic assays are Central to Targeted Drug Discovery

- Compound library → Natural products → Drugs & analogs
  - High-throughput biochemical screen
  - Med Chem
  - Crystallography
  - Enzyme assay
  - Antiproliferation Assay

Target-dependent phenotypic assays

- PK
- Xenograft efficacy
- Safety
- Drug Candidate

Target → Cellular substrate modification → Cellular pathway modulation → Hallmark or Phenotype → Clinical Response

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Summary

Targeted-based drug discovery is the dominant approach for cancer drug discovery in terms of approved drugs, development pipelines and novelty.

But

Target-first drug discovery is at greater risk of failure if rigorous phenotypic characterization is not applied for:

- Target Validation
- Target-Phenotype causality
- Molecular Mechanism of Action optimization.

Hybrid strategies with targeted hypothesis-driven lead discovery and target-driven and phenotypic lead optimization components are probably the optimal approach.
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David Bailey