Current Cancer Drug Development

<table>
<thead>
<tr>
<th>Stage</th>
<th>Oncology compounds</th>
<th>All compounds</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Number entering</td>
<td>Number entering</td>
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<tr>
<td>Preclinical testing</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Phase I</td>
<td>61</td>
<td>63</td>
</tr>
<tr>
<td>Phase II</td>
<td>17</td>
<td>25</td>
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<tr>
<td>Phase III</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Registration</td>
<td>5</td>
<td>11</td>
</tr>
</tbody>
</table>

...at an average cost of $1B per drug

adapted from: Sharpless and DePinho; Nature Reviews Drug Discovery ‘06
Work Horse Mouse in Cancer Drug Development

(xenograft: cells from one species transplanted into another)

in cancer drug testing: subcutaneous injection of established human cancer cell lines into immunocompromized mice

adapted from: Sharpless and DePinho; Nature Reviews Drug Discovery ‘06
“We’ve cured plenty of cancers in mice, but only very few in humans…. The mouse is not a good model”

many reasonable people
Cancer is a Dynamic and Evolutionary Process

adapted from Hanahan and Weinberg, Cell 2000
Mutant Mice in Integrated Disease Analyses: Two Decades of Development

cell biology & cell imaging

histopathology, physiology

cell-based tests

animal imaging

genomics, proteomics

simple and complex genetics

0% 50% 100%
Rb Pathway Commonly Aberrant in Human Cancers

INK4s → cdk4,6 → cyclin Ds → pRb → E2Fs → G1 → S

most human cancers
A Tool for Cell-Specific Inactivation of pRb Function

SV40 T Ag

N

C

Rb
107
130

p53
p300/
CBP

T121

Rb
107
130

pRb, p107, p130
Inactivation
Cancer Evolution as Deciphered in GEM

Normal cell → proliferation + cell death → loss of many biological controls → progression

Aberrant signals to proliferate

Evolutionary selection for impaired cell death

Evolution/selection

Cancer models
Choroid plexus
Mammary/breast
Prostate
Ovarian
Astrocytoma

Common among most cell types

Cell type/tissue specific

pRb

p53 or Pten

High-grade Astrocytoma

Grade III: anaplastic astrocytoma
Grade IV: glioblastoma

most common brain tumors
poor prognosis
no effective treatments

diffuse invasion
angiogenesis
pseudopalisading necrosis

diffusely differentiated

RB↓ or CDK4↑ or INK4a↓
EGFR or PDGFR↑
(K-Ras ↑)
Pten↓
Astrocytoma Model Engineering

**mouse genotype**

**NORMAL**

\[ \text{TgGZT}_{121} \] GFAP \[ \text{Lac Z} \] \[ \text{T}_{121} \]

\[ \text{K-Ras}^{+/\text{IsIG12D}} \]

\[ \text{Ptent}^{+/\text{fl}} \] 4 5 6

\[ \text{X GFAP-CreERT}_{\text{TAM}} \]

(K. McCarthy UNC) + 4-OHTam

\[ \text{TgGdZT}_{121} \]

\[ \text{K-Ras}^{+/\text{G12D}} \]

\[ \text{Ptent}^{+/\text{-}} \]

*adult astrocyte genotype*

Cancer-associated

Qian Zhang
Diffuse grade II-III astrocytoma

Inducible Astrocytoma Model Assessment

- K-Ras → Normal
- Pten → Normal
- Rbf → Diffuse grade II-III astrocytoma
- Rbf Pten → Diffuse grade II-III astrocytoma accelerated
- Ras Pten → no BT
- Rbf Ras → AA
- Rbf Ras Pten+/- → GBM
- Rbf Ras Pten → GBM + sk. tu.

Time post 4OHT:
- 2 mo
- 4 mo
- 1 yr

Angiogenesis
Invasion
Necrosis

sk. tu.
Inducible Astrocytoma Models

Diffuse grade II-III astrocytoma

- Rbf
- Ras

AA

angiogenesis

GBM

angiogenesis necrosis invasion

Pten +/−

4OHT

2 mo

4 mo

1 yr
GEM Astrocytoma: Human Disease Properties

T121;K-Ras\textsuperscript{G12D}  T121;K-Ras\textsuperscript{G12D},Pten\textsuperscript{+/-}  T121;K-Ras\textsuperscript{G12D},Pten\textsuperscript{-/-}

Qian Zhang, Chao Yin
R. Miller; D. Louis
Malignant Vessels in GEM-GBM

Bullitt, Lin, Van Dyke UNC
Scheme for Integrated Disease Analysis

inducible cell-type-targeted events

widespread induction

focal induction
GEM in Clinical Translation

Cancer genome
Cancer transcriptome
Cancer proteome
Serum proteome
frequent aberrations

Cancer initiation and progression
Engineer programmed aberrations

GEM model
genome transcriptome proteome biomarker discovery target discovery target validation preclinical trials
In Vivo Pathway Analyses:

What are the **Critical** Cause-Effect Relationships *in the Context of Natural Microenvironment*?
Inducible Astrocytoma Models

- Diffuse grade II-III astrocytoma
- Inducible Astrocytoma Models
  - 4OHT
  - 2 mo
  - 4 mo
  - 1 yr

- $\text{Rbf, Ras}$
- $\text{Rbf, Ras, Pten}^+/-$
- $\text{Rbf, Ras, Pten}$

GBM: glioblastoma multiforme
AA: anaplastic astrocytoma

Angiogenesis
Necrosis
Invasion
Ras activates multiple effector signaling pathways
Pathways to Astrocytoma

- RTKs -> Ras
- Ras -> ERK, JNK
- ERK -> P21, cell survival, proliferation
- JNK -> proliferation
- Pten -> PI3K
- PI3K -> PIP3, PIP2
- PIP2 -> PKC, AKT
- AKT -> cell survival
- PI3K -> metabolism, growth
- motility, invasion

Genes and Proteins:
- T121
- pRb
- E2Fs
Pathways to Astrocytoma

- **Ras**
- **RTKs**
- **P**
- **E2Fs**
- **pRb**
- **T\textsubscript{121}**
- **PI3K**
- **PIP\_3**
- **PKC\_\zeta**
- **AKT**
- **PI3K**
- **PIP\_2**
- **Pten**

**Processes:**
- proliferation
- metabolism, growth
- motility, invasion
- cell survival
In Vitro Pathway Analyses:

What are the likely *Mechanistic* Cause-Effect Relationships of *Pathways Perturbed In Vivo*?

What are the *Critical Therapeutic Targets*?
inducible cell-type-targeted events

widespread induction

focal induction

Inducible primary cultures

Induced primary cultures

primary tumor cultures

Scheme for Integrated Disease Analysis
Scheme for Integrated Disease Analysis

Inducible cell-type-targeted events

Widespread induction

Focal induction

Inducible primary cultures

Orthotopic syngeneic transplants

Induced primary cultures

Primary tumor cultures
Orthotopic Syngeneic Transplant Model for “Rapid” Pathway/Microenvironment Assessment

Ryan Bash, Natalie Karpinich, Ryan Miller
inducible cell-type-targeted events

widespread induction

focal induction

Inducible primary cultures

Induced primary cultures

orthotopic syngeneic transplants

primary tumor cultures

Scheme for Integrated Disease Analysis
High-grade Astrocytoma

- Poorly differentiated
- High mitotic index
- Diffuse invasion
- Angiogenesis
- Pseudopalisading necrosis

Genes involved:
- RB
- CDK4 or INK4a
- EGFR or PDGFR
- Pten
- K-Ras
EGFR Signal Activation via Ras Activation
Targeting EGFR in Cancer

adapted from Ciardiello & Tortora, 2002; courtesy of David Threadgill (UNC)
Inducible Astrocytoma Models

- **Rb**, **Ras**
- **Diffuse grade II-III astrocytoma**
- **Angiogenesis**

- **Rb**, **Ras**, **Pten**
- **AA**
- **Angiogenesis**

- **Rb**, **Ras**, **Pten**
- **GBM**
- **Angiogenesis**
- **Necrosis**
- **Invasion**

- **Rb**, **Ras**, **Pten**
- **GBM**
- **Angiogenesis**
- **Necrosis**
- **Invasion**

* 4OHT
* 2 mo
* 4 mo
* 1 yr
EGFR Inactivation INCREASES Severity

Tumor Volume

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EGFR Status</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>TR (n=11)</td>
<td>EGFR+/+</td>
<td></td>
</tr>
<tr>
<td>TRE+/− (n=5)</td>
<td>EGFR+/−</td>
<td></td>
</tr>
<tr>
<td>TRE−/− (n=5)</td>
<td>EGFR−/−</td>
<td></td>
</tr>
</tbody>
</table>

185340
TRE+/−
Cortex Tu

185571
TRE+/−
Cortex Tu

EGFR
PDGFRα
b-actin

CD31

Q. Zhang, D. Threadgill
Pathways to Astrocytoma

**RTKs** → **Ras**

- **T121** → **pRb** → **E2Fs** → proliferation

**Ras** → **ERK** → **JNK**

- **PI3K** → **PIP3** → **PIF2** → cell survival, metabolism, growth

- **PKCζ** → **AKT** → motility, invasion
Mutations in the Epidermal Growth Factor Receptor and in KRAS Are Predictive and Prognostic Indicators in Patients With Non–Small-Cell Lung Cancer Treated With Chemotherapy Alone and in Combination With Erlotinib

Disease Models at the Frontiers of Basic and Clinical Discovery

Therapeutic development

- cell biology & imaging
- animal imaging

Diagnosis
- histopathology, physiology

Risk assessment
- Therapy response
- Pharmacogenomics

complex genetics

Target discovery
- Diagnosis
- Early detection
- Monitor treatment
- Target validation
- Therapeutic testing

biochemistry genomics

genomics, proteomics

HT cell-based screens
Why Have Spontaneous Cancer Models *not* been Incorporated into Drug Discovery Preclinical Assessment?

DuPont

FDA

expensive compared to xenografts

old dogs and new tricks

academic-private technology transfer

requires major expertise in cancer mechanisms, pathways, GEMM, genetics, drug development *and* clinical care

requires uncommon research culture
NCI-CAPR
Center for Advanced Preclinical Studies

...to facilitate the improvement of preclinical assessment and clinical trial design for effective cancer diagnosis and treatment

Current Interactome

Projected Interactome

a new paradigm for translational science
NCI-CAPR
Center for Advanced Preclinical Studies

• Predict possible outcomes/patient stratification to inform clinical trial design
  • Therapeutic target discovery and validation
  • Biomarker/molecular signature identification via comparative (human, canine, murine) analyses
• Cancer model and “tool” mouse development for UNMET needs.
  • Annotated tissue/fluids/nucleic acids banks
  • Consultation.
• Integrated preclinical/clinical LIMS development
• Develop effective preclinical testing strategies in murine cancer models (GEM, humanized orthotopic xenografts)
• Comparative assessment of predictive power among murine cancer models
• Develop molecular/cellular imaging strategies for therapeutic/diagnostic assessment
• Develop technologies to overcome barriers to scale up and throughput while limiting sacrifice in predictive power.