



# **Image Analysis with Phosphorylated Protein Biomarkers in Phase I Oncology Clinical Trials Dosing Studies**

**Jason Hill, Ph.D.**

**Targeted Molecular Diagnostics**



# What TMD Does



## *Pharmaceutical Services*

**Novel Approach to  
Drug Development  
Using Biomarkers**



## *Physician and Hospital Services*

**Targeted  
Diagnostics to  
Guide Cancer  
Treatment**

## Slide 2

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J5

We develop unique biomarker assays to support drug development

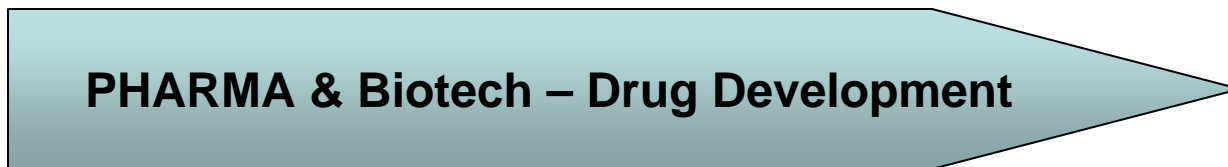
In pre-clinical studies, this can help elucidate drug mechanism of action, can also help differentiate one drug from a similar drug

These markers can be used to monitor drug pharmacodynamics in patient specimens which I'll be speaking about today or to be

predictive for either response or resistance of a particular patient to a drug

JHill, 3/3/2008

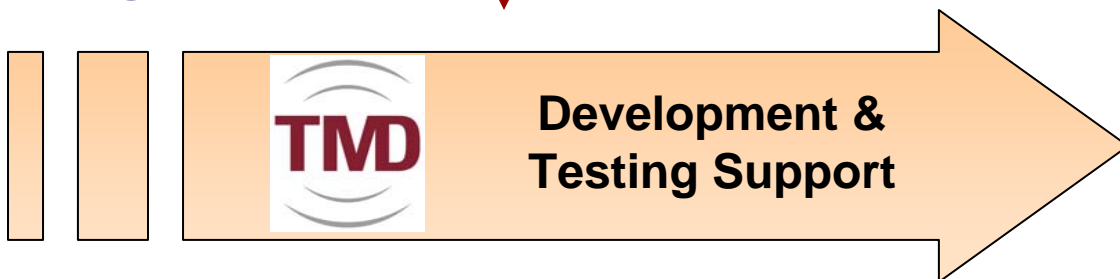
# PreClinical Development Support Through Phase III and Beyond



Master Research Services Agreement

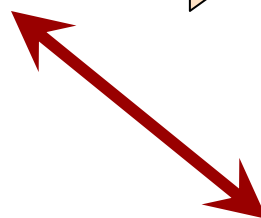


Master Laboratory Services Agreement



Technology Transfer, Regulatory & Promotion Support

- Diagnostic companies need to manufacture and sell the final test
- Frequently have a hard time understanding pharma/biotech companies. We can help.



### Slide 3

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J6

TMD provides biomarker testing and development support for pre-clinical through Phase III trials

Our senior team also has experience developing and launching diagnostic tests, so we can prepare a test for FDA approval.

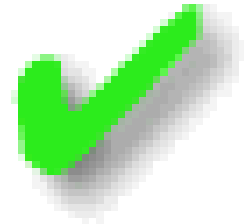
Because we speak the language of pharma and the language of diagnostic companies, we can help find the right partner for manufacture, etc.

JHill, 3/3/2008

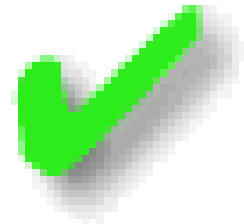
# TMD Certifications



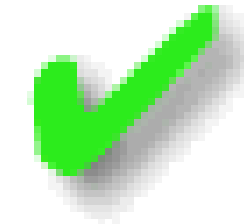
**College of American Pathologists**



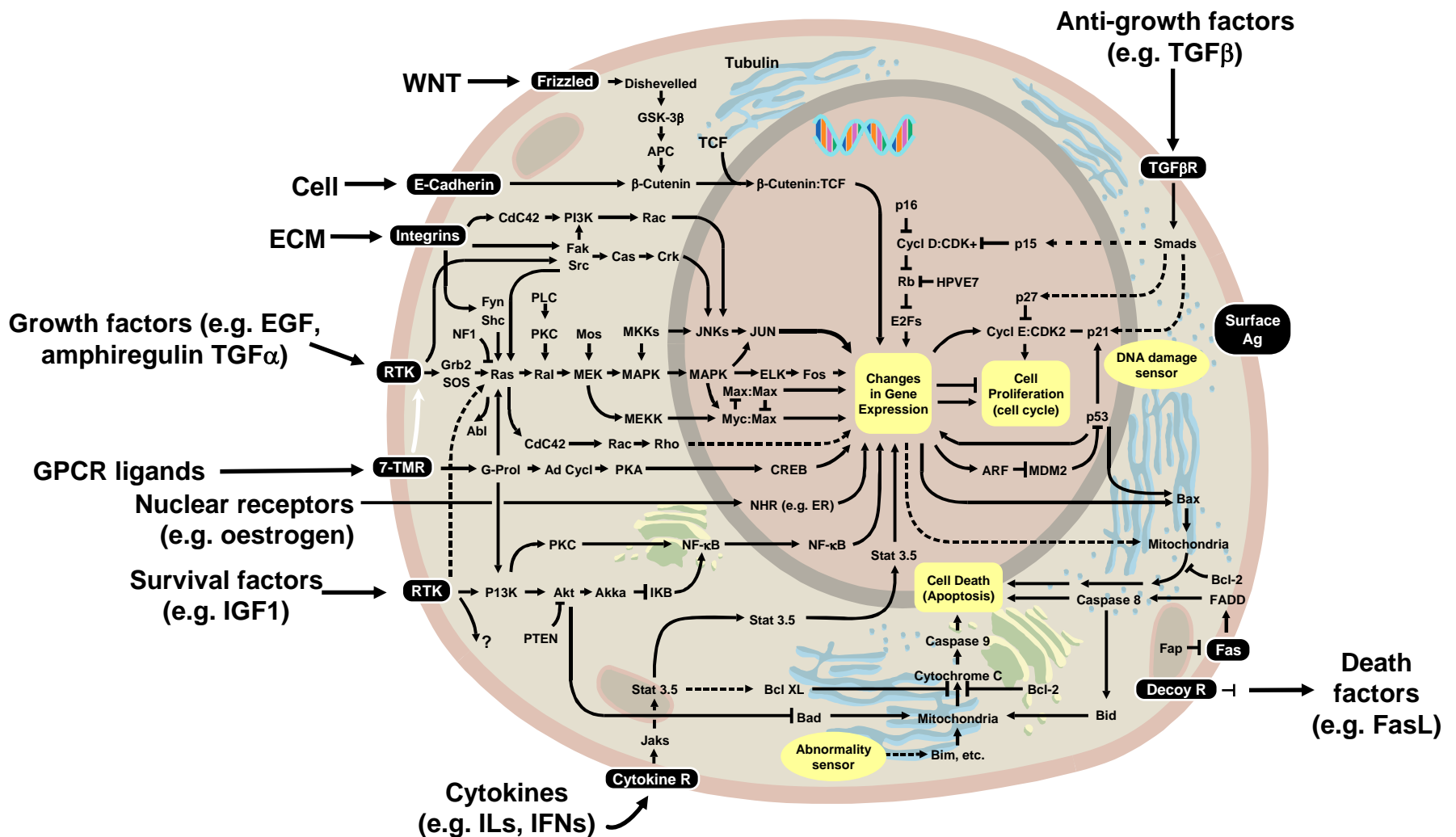
**Clinical Laboratory Improvement  
Amendments (CLIA)**



**Good Clinical & Laboratory  
Practice**



# Complexity of Signaling Pathways in Cancer



Hanahan D, Weinberg RA. Cell 2000;100:57-70

# Uses of Morphological Biomarkers in Drug Development



- Analyze effect on target/downstream pathways in pre-clinical studies e.g.
  - Phosphorylation (TKIs: Iressa, Tykerb, Gleevec)
  - Acetylation (HDACi: SAHA, MS-275)
  - Methylation (Vidaza)
- Transition assays to clinical specimens, can be used for e.g.
  - Selecting/Guiding dose in Phase I/II
  - Identifying potential biomarkers of response and/or resistance
- Refine Response/Resistance Biomarkers in Phase II
- Select Patients and Standardize in Phase III

Slide 6

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J4

tell Wayne analogy

JHill, 3/2/2008



# Why Semi-quantitative IHC for understanding the local environment efficacy / toxicity?

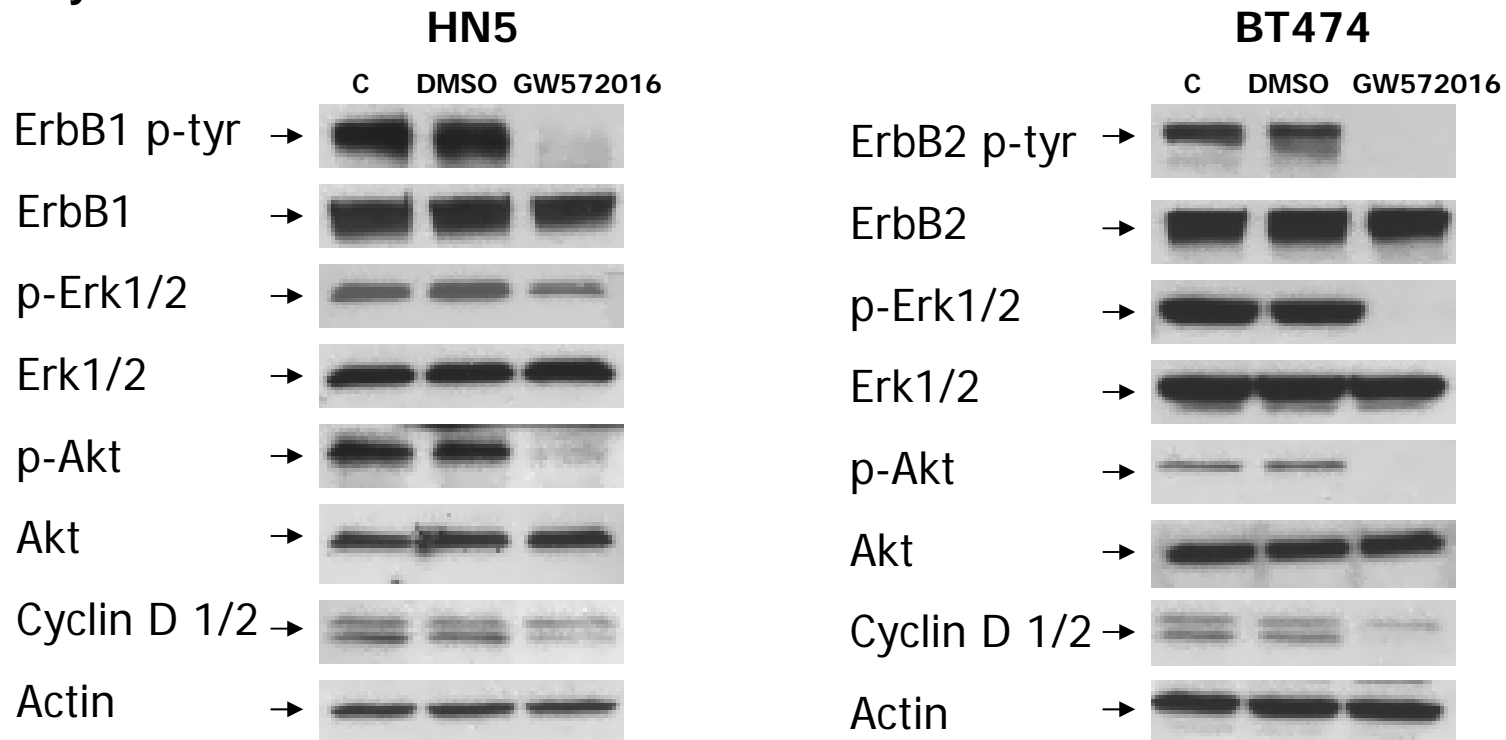
- We all hope for magic bullet serum markers, but...
  - Until then we must understand what is happening in the organ and tissue of interest
  - Much higher, more specific signals in tissues than in serum
    - the location in the tissue matters
- Preclinical and clinical trials are run by pathologists (DVM / MD), they are trained to look at tissues with IHC
- Infrastructure for IHC already in place at preclinical and clinical trials CROs (*ever tried running protein-based mass spec at a large CRO?*)
- HER2/ER/PR with semiquantitative IHC is an accepted standard now for patient stratification with Herceptin
- IHC is inexpensive and easy to run, with hundreds of assays available  
Some limitations:
  - Perceived as non-quantitative
  - Requires good histology and attention to standardization
  - Computer-assisted semiquantitative IHC
  - Requires certified pathologists



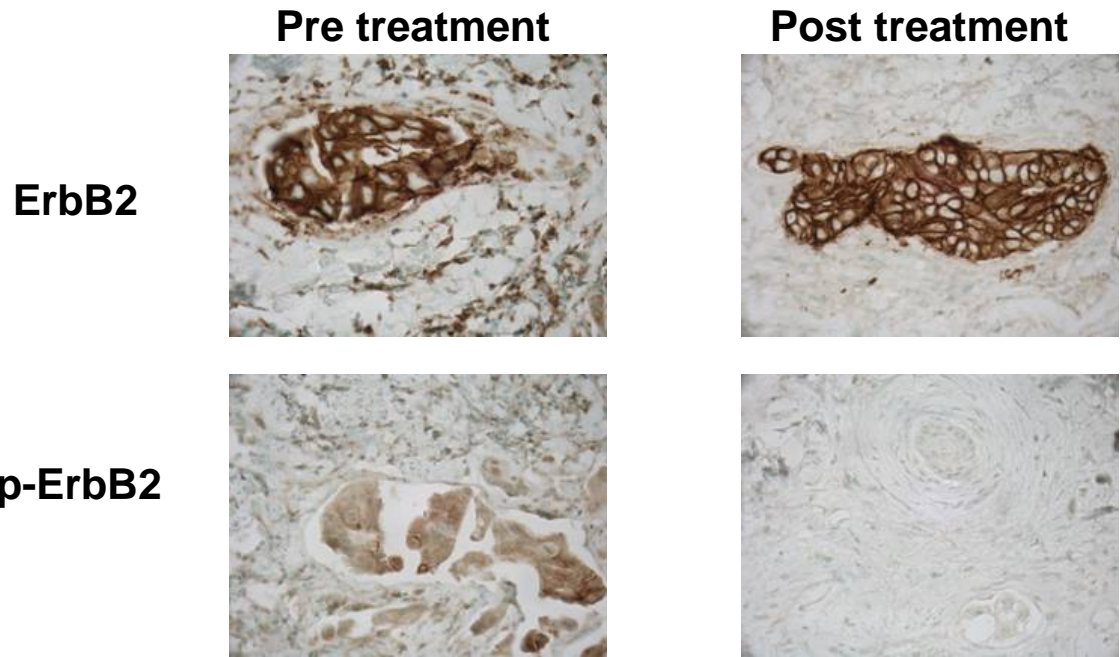
# Phospho-biomarkers in Pre-clinical Development



- Small molecule reversible inhibitor of the tyrosine kinase activity of ErbB1 and ErbB2
- Inhibits p-ErbB1 and p-ErbB2 and downstream signaling molecules p-Erk1/2, p-Akt and cyclin D



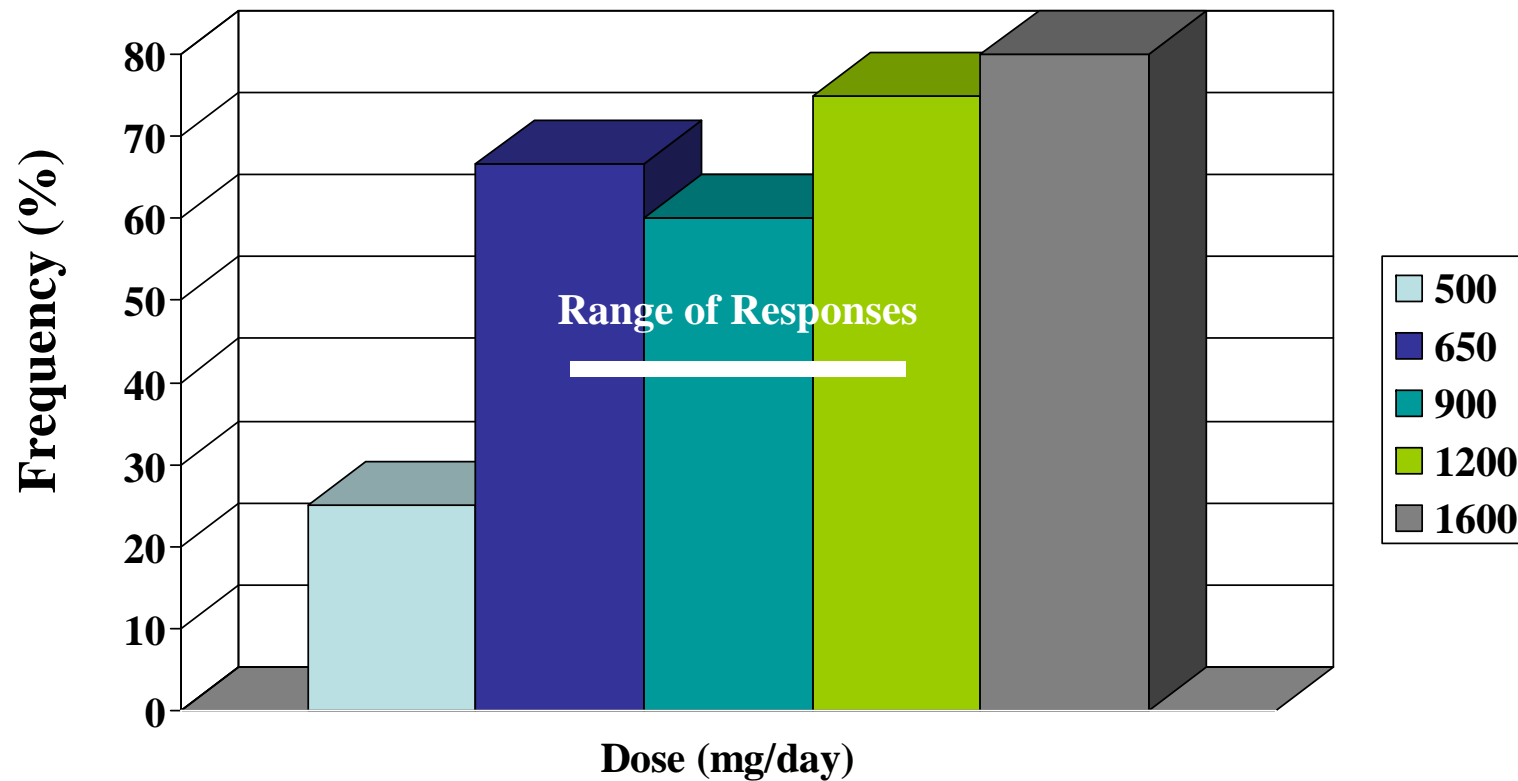
# Phospho-biomarkers in Clinical Development



# More Is Not Necessarily Better



Frequency of Achieving >75% Inhibition of p-ErbB1, p-ErbB2, p-Erk1/2, or p-Akt Expression in Tumors at Day 21



J2

# J7 Using PD Phospho-biomarkers to Guide Dose Selection



- Phospho-biomarkers that are drug targets or downstream signaling molecules can be used to monitor target inhibition
- When combined with an analytical method such as quantitative IHC, degree of target inhibition can be measured and used to guide patient dosing



## Slide 11

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J2

draw comparison of retrospective (lapatinib) vs prospective (src)

generate excitement - 1st clinical PD biomarker trial of its kind that we're excited to be involved with

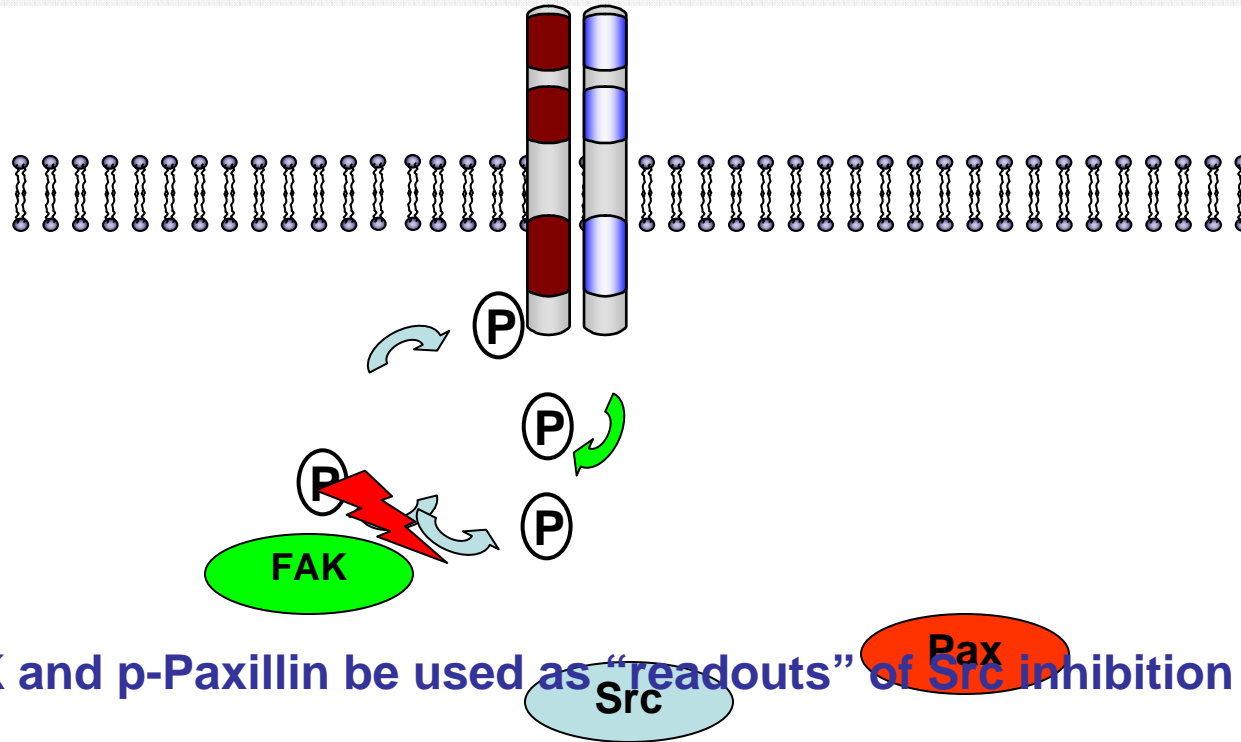
JHill, 3/2/2008

J7

if you can monitor phospho-biomarkers in real time, they can be used to study drug PD in patients and guide patient dosing selection

JHill, 3/3/2008

# Biomarker Imaging to Guide Phase I/II Dosing



Can p-FAK and p-Paxillin be used as “readouts” of Src inhibition in patients?

FAK becomes active upon recruitment to the plasma membrane and autophosphorylates itself

Src is recruited to the plasma membrane, autophosphorylates itself and further phosphorylates and activates FAK

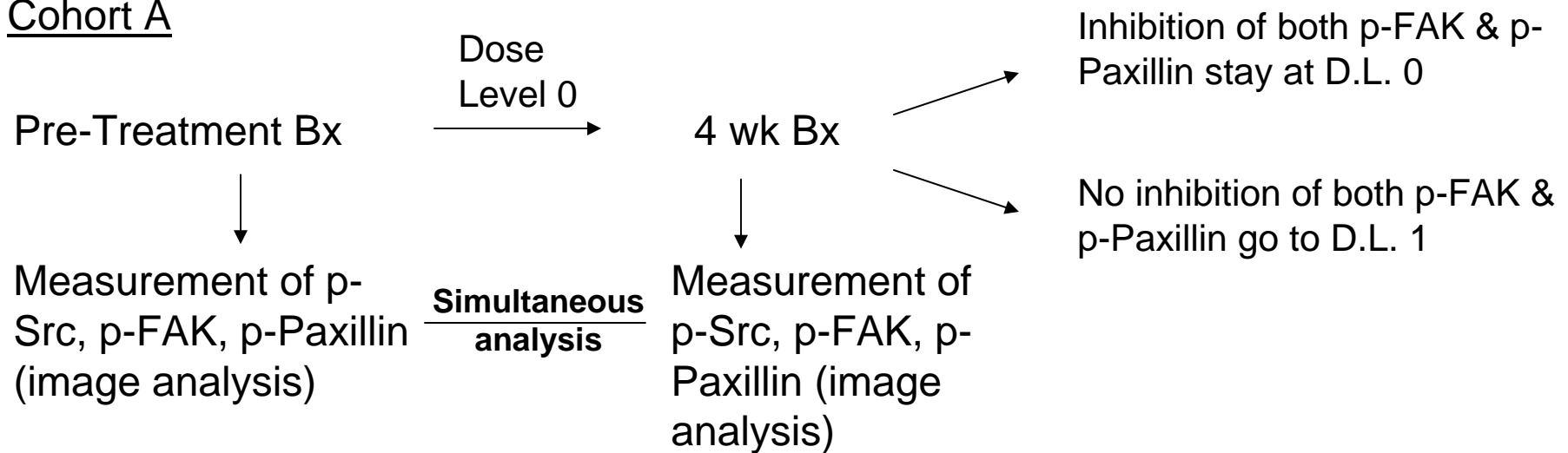
Paxillin is recruited to FAK and is phosphorylated by Src



# Real Time Biomarker Measurement



## Cohort A



**If at least 10 pts in cohort A have inhibition of both p-FAK and p-Paxillin, cohort B will receive D.L. 0**

**If < 10 pts in cohort A have inhibition of both p-FAK and p-Paxillin, cohort B will receive D.L. 1**

Slide 13

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J1

PhosphoGuard

TAT

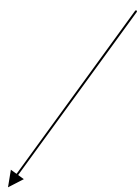
Simultaneous pre- & post- analysis

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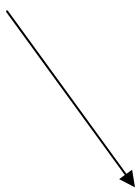
# Real Time Biomarker Measurement



- 3rd and final Bx in patients who progressed at 16 wks but had documented response
- By measuring 3 sequential Bx with image analysis, can ask the question:
  - Did patients who were progression free but then relapsed still have inhibition of the target and/or pathway?



**Pathway still inhibited:  
resistance due to alternative  
oncogenic pathway**



**Pathway no longer inhibited:  
resistance due mutation or  
compensatory dysregulation in  
target or downstream signaling  
molecules**

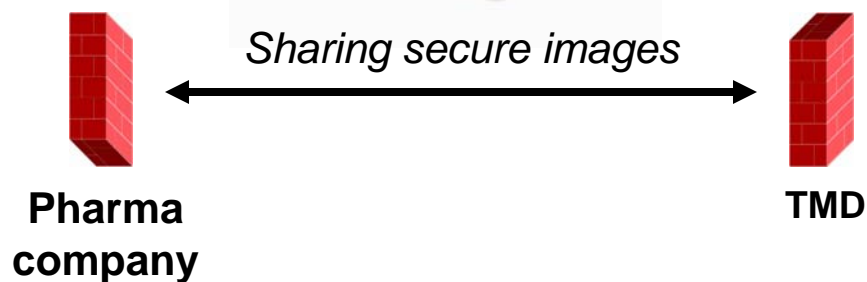
# Make IHC More Accessible

## The old way



- Only path and image analysis data can be shared between TMD and pharma
- Cumbersome and incomplete information from snap shots of slides

## The new way



- Weekly digital slide conferences between pharma and CROs
- Entire slide captured

← Before digital slides

After digital slides →



# The New Way Enables Us To...



- Share real-time results between TMD & pharma/biotech
- Perform and review complex, highly informative PD biomarkers in real-time by multiple pathologists/clinical scientists
- Provides our clients more confidence in the transfer and validation of developed assays



# Conclusions



- Phospho-biomarkers are integral to monitoring drug efficacy in the patient
- PD biomarkers can be used to guide patient dosing (Phase I/II) and eventually patient selection (Phase II/III)
- Quantitative IHC methodology is a practical way to measure biomarker changes in solid tumor specimens

J3

Slide 17

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J3

Acknowledge ScanScope Aperio make the last point possible

JHill, 3/2/2008

# **Aperio Science Webinar series**

**Repeated two times (7:00 a.m & 5:00 p.m. California Time)**



- **March: *Digital Pathology in Clinical Trials: Phosphorylation Assays in Phase I dosing studies***  
**Jason Hill, Ph.D. Targeted Molecular Diagnostics**

To register for these free events, please visit  
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