# Developing quantitative slide-based assays to assess target inhibition in oncology drug discovery and development

TIGA Workshop
June 25-26, 2010

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Millennium Pharmaceuticals

# Outline

- Imaging @ Millennium
- Technology development & Integration
- Applications in Oncology
  - Assess in vivo potency
  - Biomarker development
  - Assess clinical activity
- Challenges / Unmet needs

# Millennium

- Cambridge, Massachusetts U.S.A.
- Oncology-focused

Global Oncology Pipeline

| | | | Preclin | Phase I | Phase II | Phase III |
|---|---|---|---|---|---|---|
| Protein Homeostasis | MLN4924 | NAE Inhibitor | | | | |
| | MLN9708 | Proteasome Inhibitor | | | | |
| Anti-Angiogenesis | motesanib* | VEGFR/PDGFR Inhibitor | | | | |
| | tandutinib | PDGFR Inhibitor | | | | |
| | AMG386*† | Anti-Angiopoietin Peptibody | | | | |
| Growth Signaling Inhibition | TAK-285 | HER2/EGFR Inhibitor | | | | |
| | TAK-701* | HGF Antibody | | | | |
| | TAK-733 | MEK Inhibitor | | | | |
| | AMG479*† | IGF-1R Antibody | | | | |
| Cell Cycle Inhibition | SGN-35* | Anti-CD30 | | | | |
| | MLN8237 | Aurora A Kinase Inhibitor | | | | |
| | CBP501* | Cell Cycle Dysregulator | | | | |
| | TAK-901 | Pan-Aurora Kinase Inhibitor§ | | | | |
| Hormone Regulation | TAK-700 | Androgen Synthesis Inhibitor | | | | |
| | TAK-448 | Metastin Analog | | | | |
| Apoptosis Inducer | conatumumab*† | DR5 Antibody | | | | |
| Immunomodulator | mifamurtide‡ | Macrophage Activator | | | | |
| ADDITIONAL INDICATIONS / NEW FORMULATIONS | | | | | | |
| Protein Homeostasis | VELCADE® | Proteasome Inhibitor (Follicular NHL, First Line MCL, Subcutaneous Formulation) | | | | |

* In-Licensed
† Japan Only Developed by TBDC
§ TAK-901 also inhibits multiple other kinases
‡ Approved in EU only
- Motesanib diphosphate is being developed by Millennium in collaboration with Amgen, Incorporated
- CBP501 is being developed by Millennium in collaboration with CanBas Limited
- SGN-35 is being developed by Millennium in collaboration with Seattle Genetics, Inc.
- VELCADE® is co-developed by Millennium and Johnson & Johnson Pharmaceutical Research & Development

**These compounds are either investigational or studied in new indications. Efficacy and safety have not been established.**

# Cellular Imaging @ Millennium

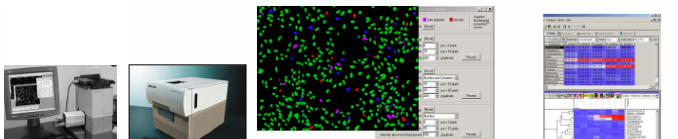
- Develop image-based cellular assays
  - Better understanding of the Mechanism of Action of target inhibition
  - Better biological readout to drive medicinal chemistry
  - Better understanding of the pharmacodynamics for preclinical models and clinical biopsies
- Develop technologies that allow us to move rapidly and efficiently from MoA → *in vitro* → *in vivo* PD
- Provide imaging technology across project teams and departments
  - Cell Biology, Lead Discovery, Cancer Pharmacology, Biochemistry, Molecular Technologies, Clinical

# Tissue-based imaging enables direct and indirect biomarkers of target inhibition

- Mechanism of Action: understand target inhibition
  - Direct and indirect pathway markers
  - Cell morphology assay
- Terminal outcome: understand cell fate
  - Apoptosis
  - Senescence
- These assays can be utilized for in vivo preclinical PD assays and clinical biomarker assays
  - Adopt to variety of tissue and biopsy types

# Cellular Imaging @ Millennium

## High Content Screening



Imaging Systems



Application Modules



AcuityXpress™

MetaXpress™ Image Analysis

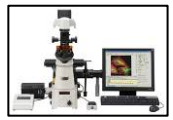
Cellular Informatics

MDCStore Database

Acapella
Image analysis system with distributed data processing.

## Live-cell Timelapse / Confocal microscopy / Basic

x 2



x 3

DEFINIENS Understanding Images

File Share

MetaMorph

## Automated slide-scanning (If & colorimetric)

x 4

Nuance™

DEFINIENS Understanding Images

File Share

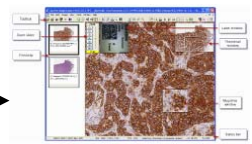
MetaMorph

## Automated slide-scanning (Aperio Technologies)

ScanScopeXT

Spectrum

Aperio-prd



ImageScope

ScanscopeFL

LIMS integration

Image analysis & visualization stations

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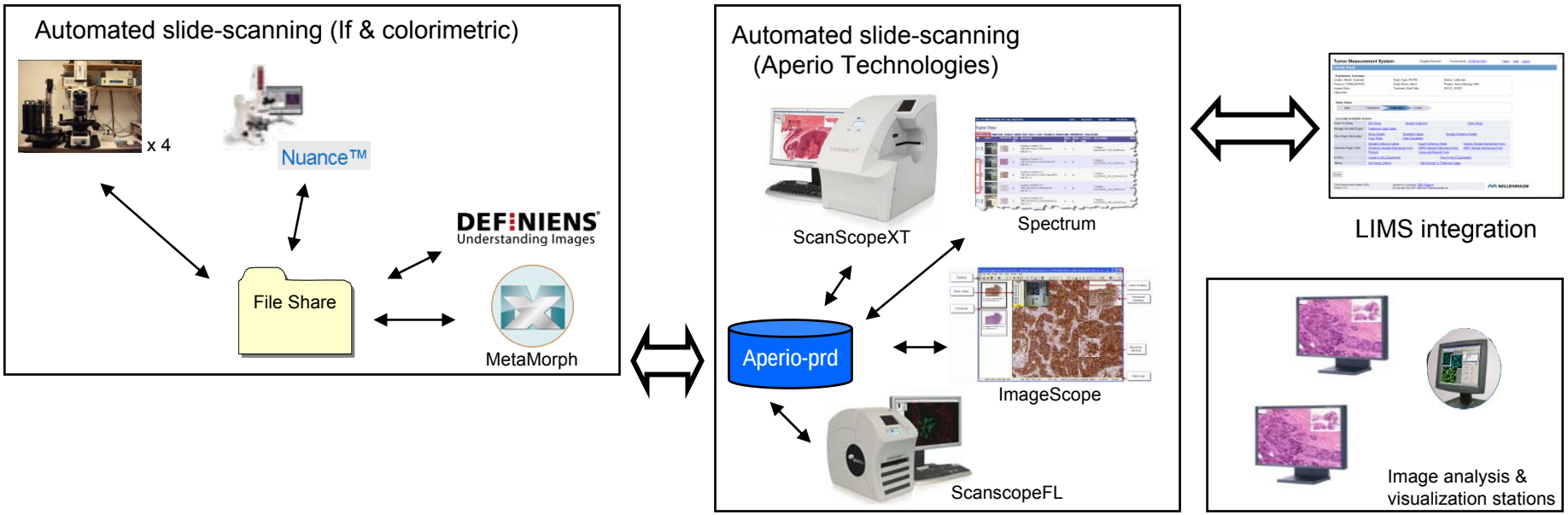
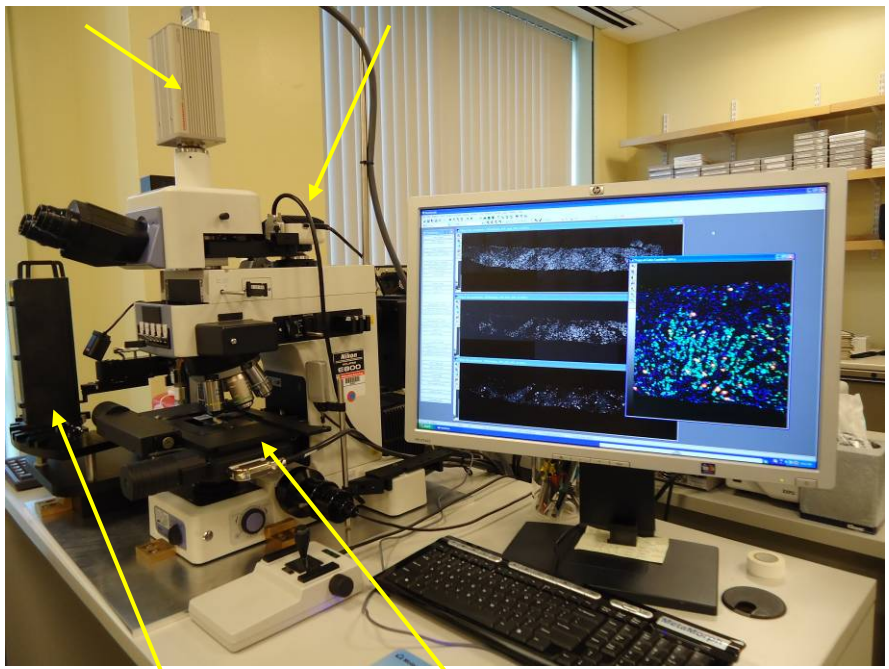
# Tissue-Based Imaging @ Millennium

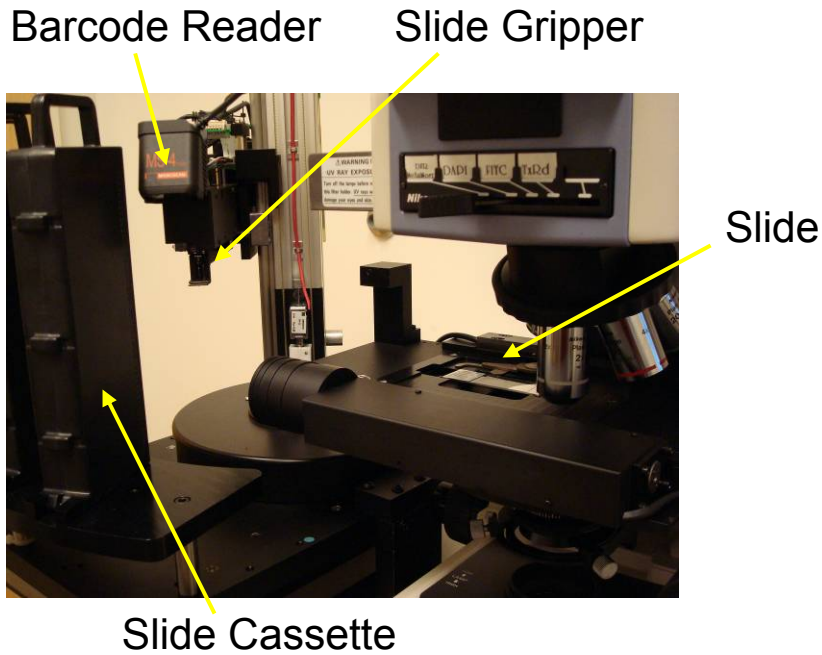



- 4 custom-developed systems
- 7000 slides (IF) per year
- Integration of image data with Aperio server (in development)

- ScanScopeXT = ~9000 slides (< 1 yr)
- Integration with in-house LIMS system to populate Spectrum with specimen data (drug, dose, staining, etc)
- Image analysis software: Aperio, Definiens, Metamorph

# Automated tissue-scanning system




- Currently – 4 systems
- Automated stage, focus
- Multi-channel fluorescence & brightfield
- Automated 200 slide loader

# Developed suite of tissue imaging tools

- Acquisition (2D and 3D)
  - Flexibility to deal with a variety of samples that include xenograft, clinical tumor/skin biopsies, clinical blood smears, etc.
  - Ease of use
  - Automation
    - Sample metadata (dose, timepoint, sample ID, etc)
    - Sample collection
- Analysis
  - Quick assessment of biological assay
  - Parallel development of automated analysis algorithms

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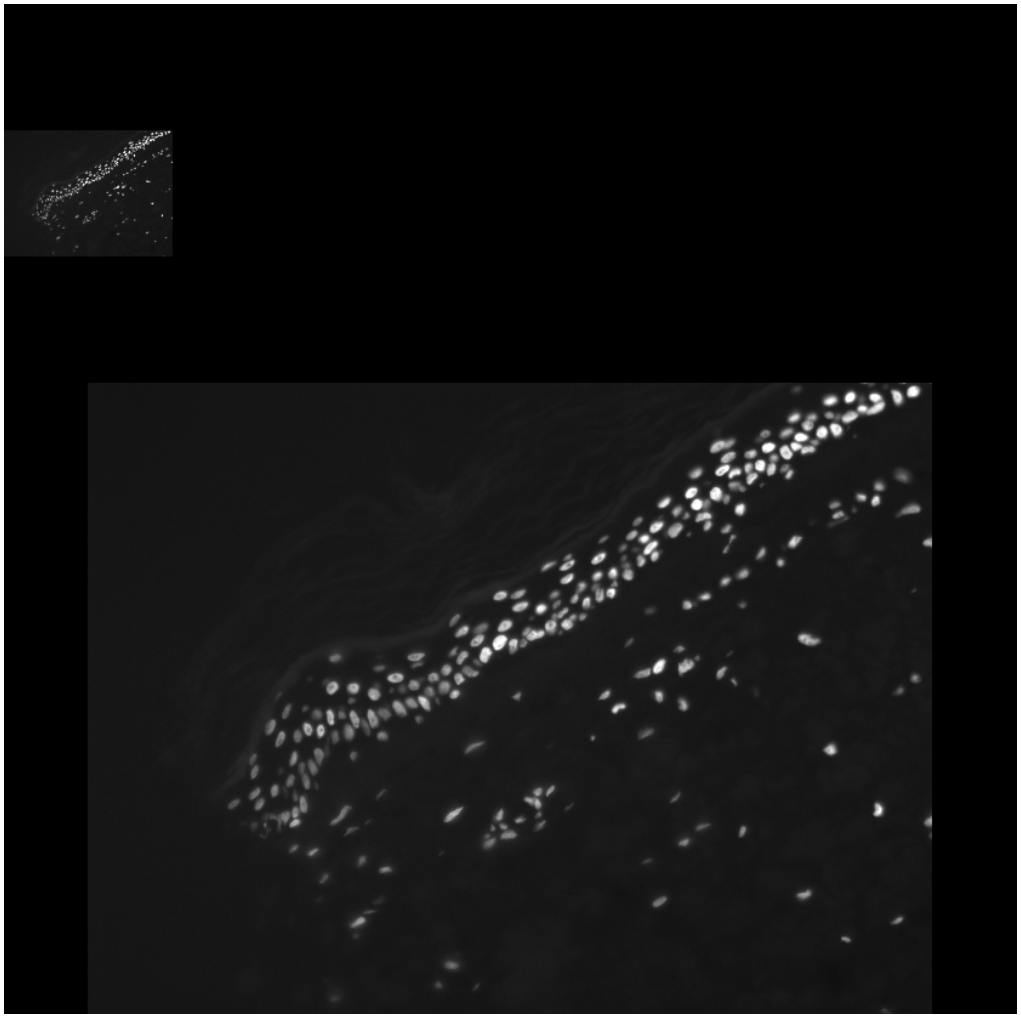
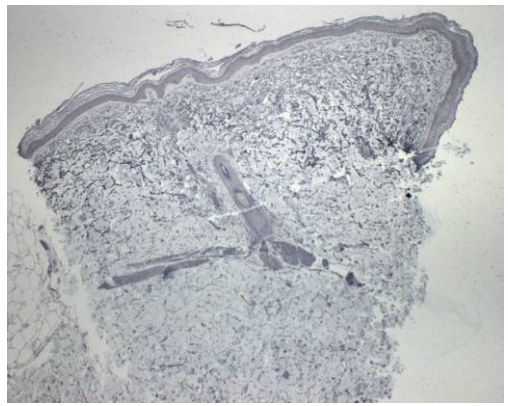
MetaMorph

DEFINIENS
Understanding Images

# High resolution and efficient scanning of clinical samples



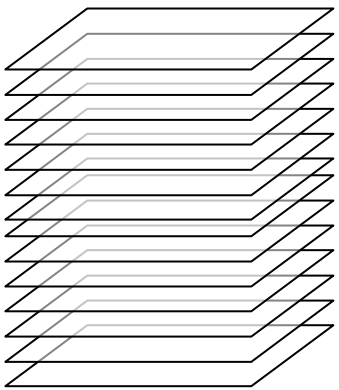
20x objective



100um

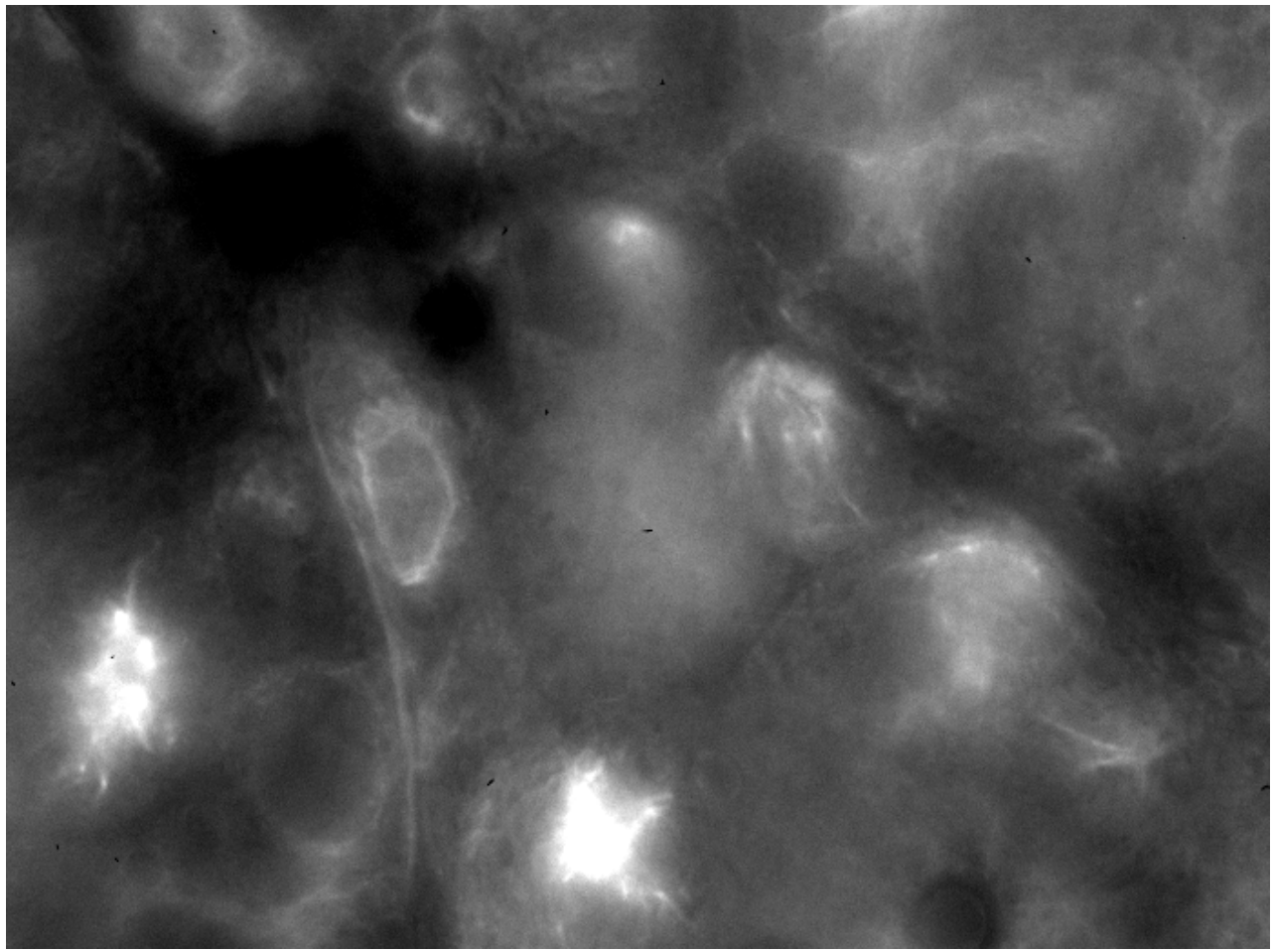
- Multi-mode
- Multi-channel IF

# Capture entire volume of cells for 3D morphology assays



Z-axis

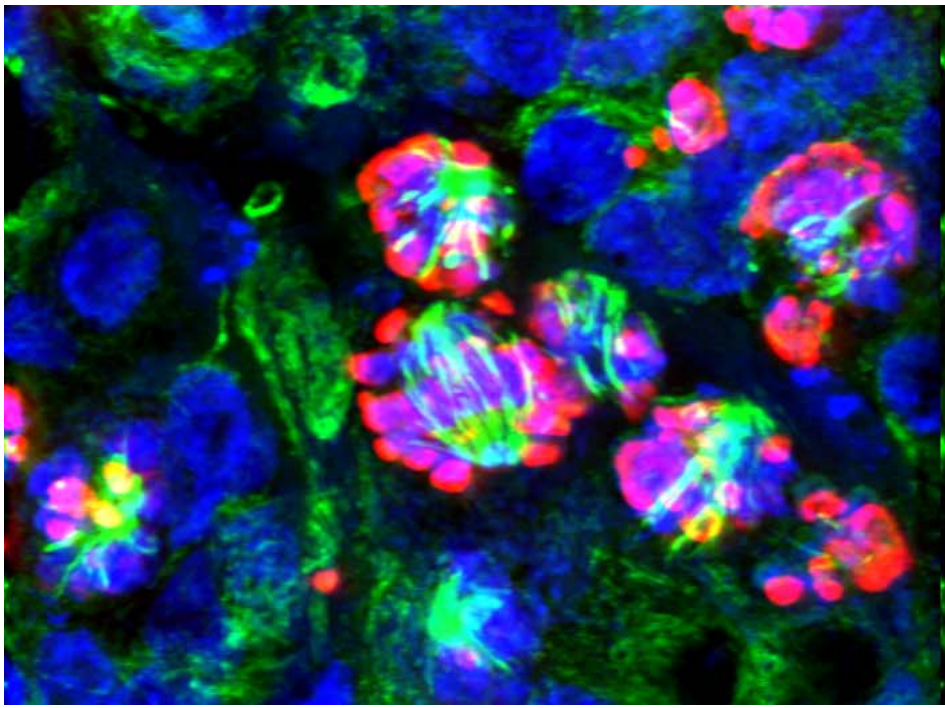
15 optical sections
@ 0.5 um intervals



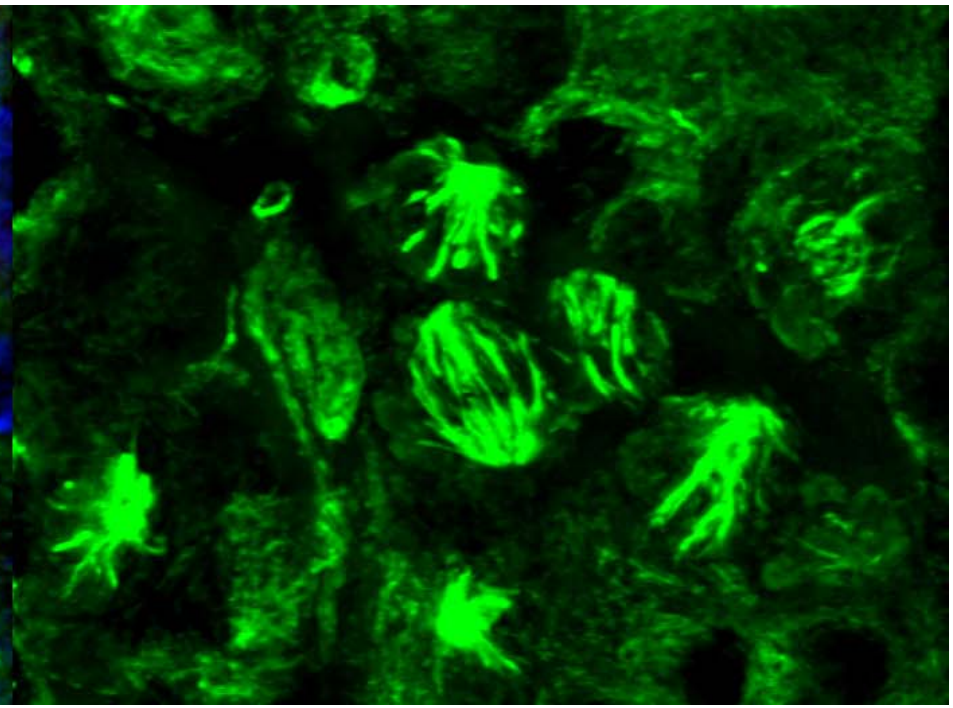
aTubulin

# Visualization of 3D cellular morphology

3 dimensional rotation, +- 30 degrees



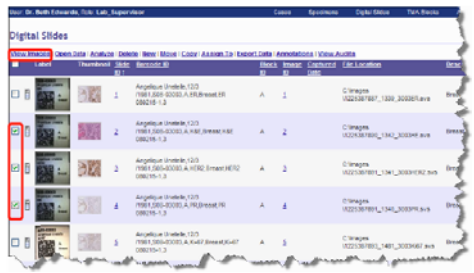
aTubulin / pHisH3 / Dapi

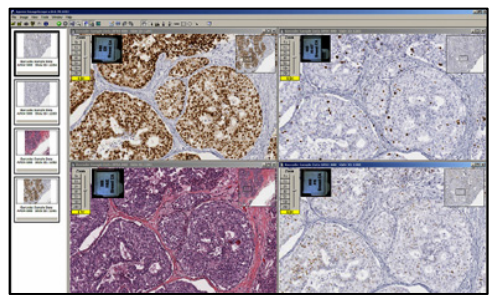


aTubulin

# Investment in Aperio Technologies

- Automated whole slide scanner
  - Fluorescence
  - Brightfield
- Spectrum: Image management system
- ImageScope: Image visualization and analysis
- Integration with existing image analysis tools (MetaMorph, Definiens) as well as Aperio tools



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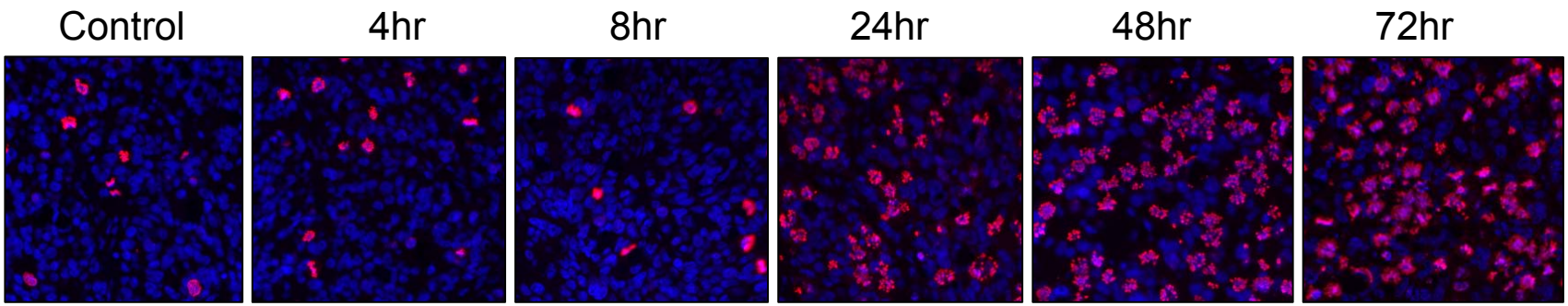
# Application examples

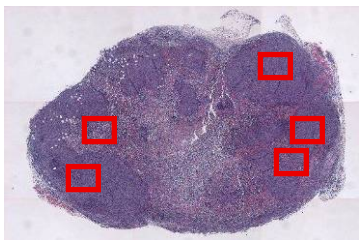
- Direct and indirect pathway biomarkers
- Preclinical biomarkers
- Clinical biomarkers


- Preclinical biomarker
  - Lead optimization efforts to measure potency of compounds
  - Understand temporal response of biomarker for optimal sampling point and to help define clinical sampling

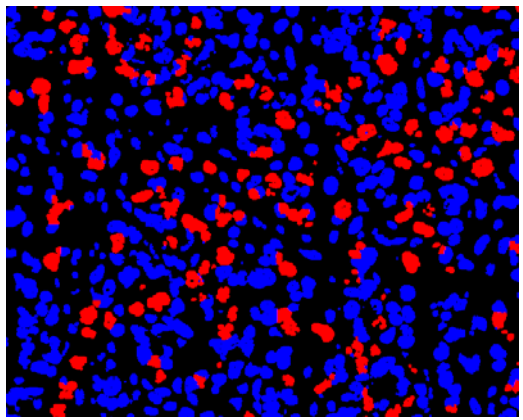
# Pathway inhibition in pre-clinical models




Mitotic Index (dapi / pHH3)



HT29 Xenograft

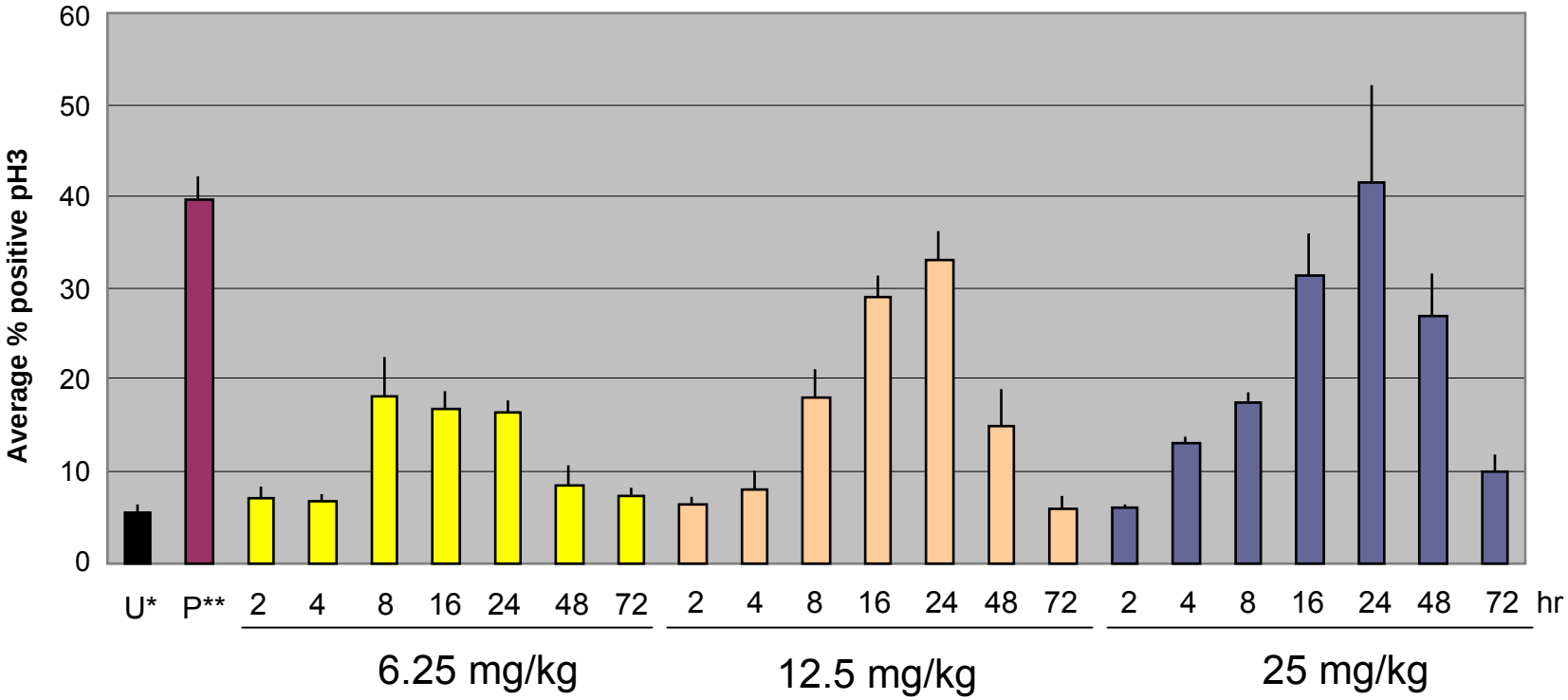


~ 9000 slides over 2 year period

Automated analysis

- Count total cells
- Count mitotic cells

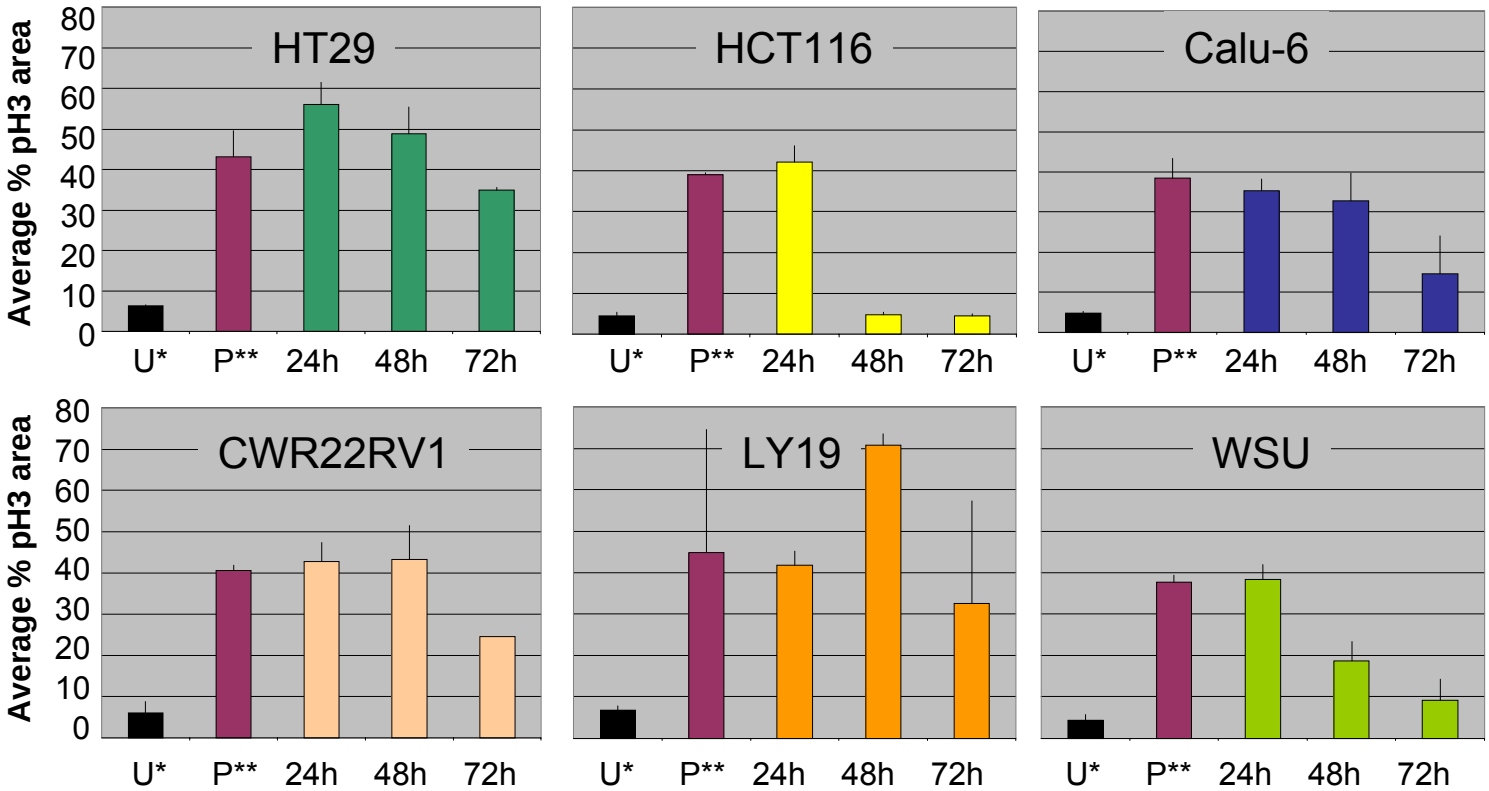
# Preclinical PD: dose and temporal response




*Untreated control
**Positive control

- Increase in pH3 begins at 8hrs
- pH3 continues to rise with increasing dose and peaks at 24 hrs

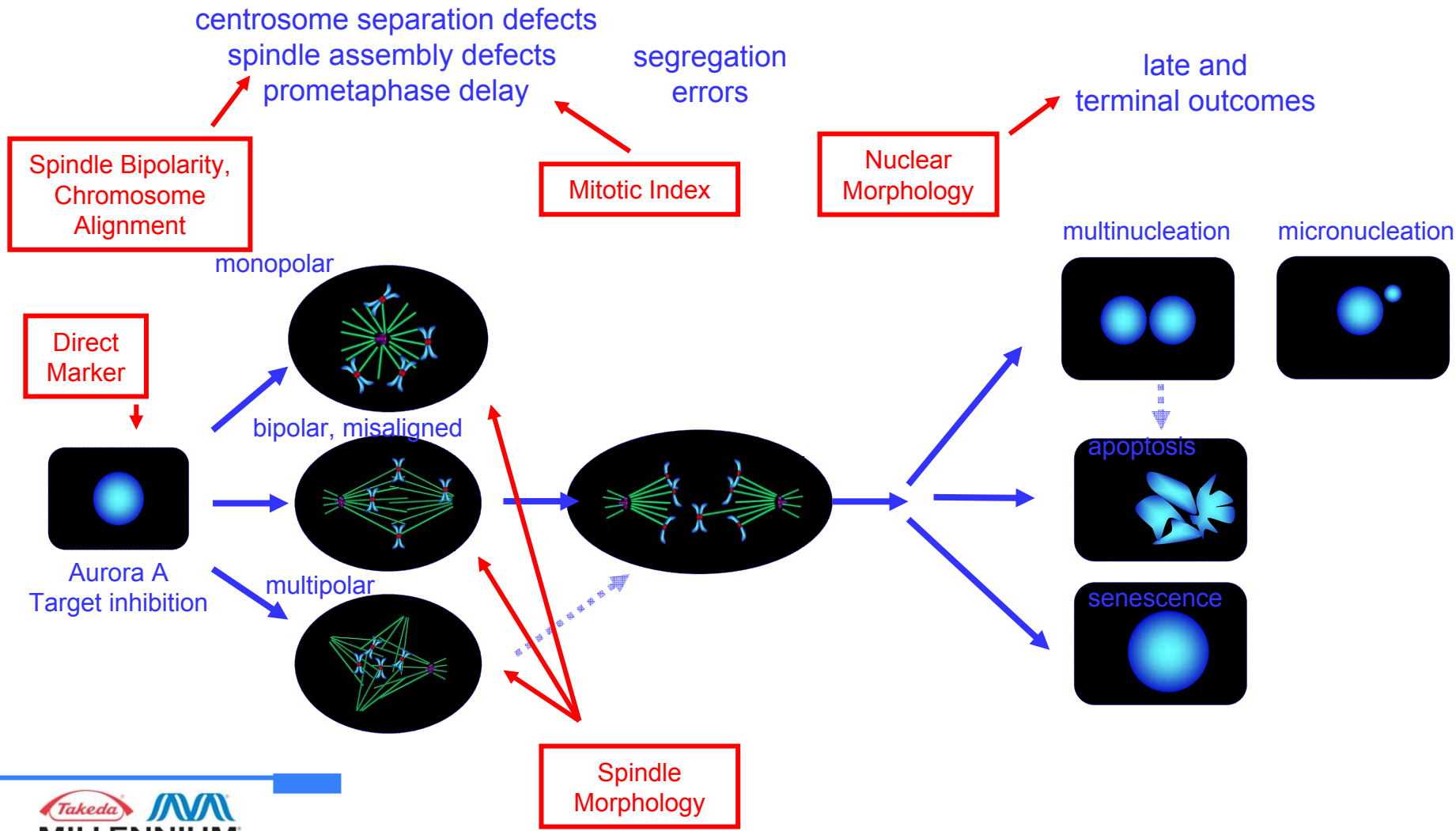
# Evaluation of PD Response in different models




- PD response in colon, lung, prostate and lymphoma xenografts after a single 50 mg/kg po dose

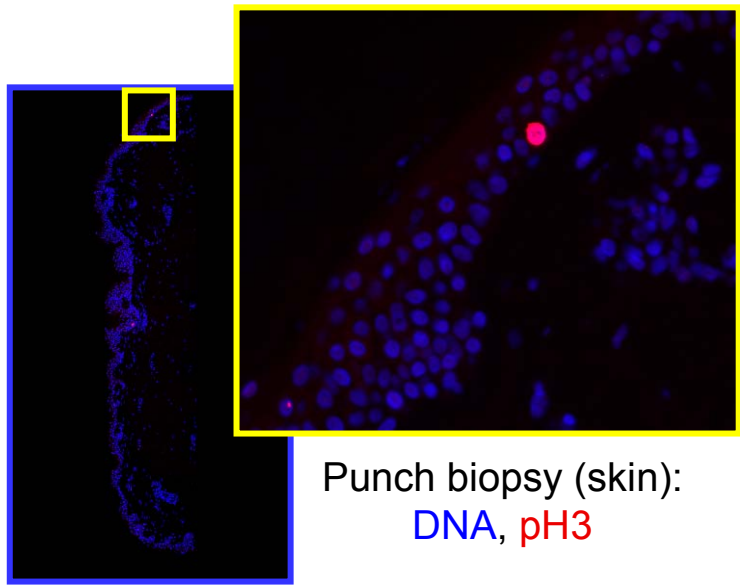

- MLN8237: Aurora A Kinase inhibitor
  - Pharmacodynamic evaluation in Phase 1 clinical studies in advanced solid tumors
  - Includes image-based PD biomarker strategy to assess activity

# Biomarker strategy based on MoA of Aurora A inhibition

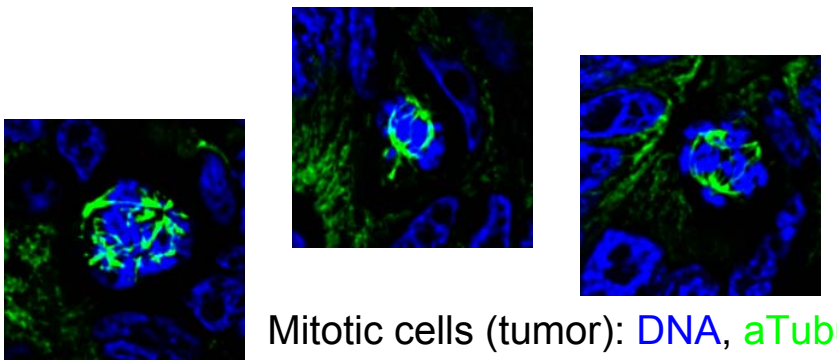


# Assess MLN8237 pathway inhibition in clinical patient biopsies

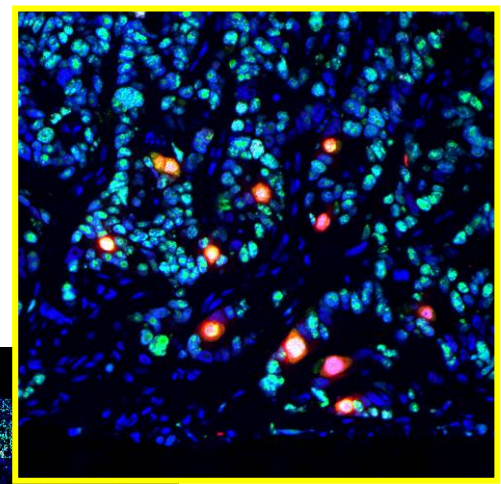
- Mitotic Index in surrogate tissue (skin)
- Mitotic Index (tumor)
- Spindle bipolarity (tumor)
- Chromosome alignment (tumor)



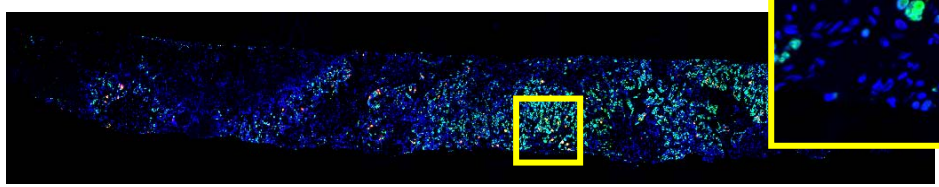
Punch biopsy (skin): DNA, pH3



Mitotic cells (tumor): DNA, aTubulin



Needle biopsy (tumor): DNA, Ki67, pH3



# MLN8237 clinical trials 14001/14002

## Biopsy schedules

- Two P1 trials in patients with advanced solid tumors
  - C14001 in US; C14002 in Spain
- Secondary Objectives
  - Evaluate MLN8237 PD effect on Aurora A inhibition in skin / tumor biopsies

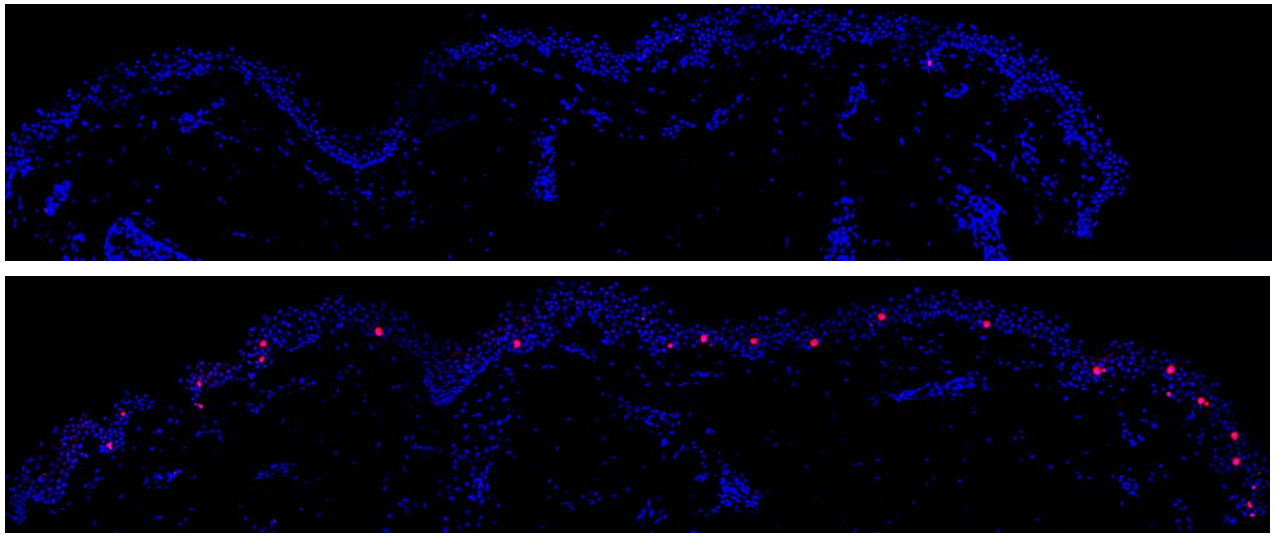
| | Day 1 | | | Day 7 | |
|---|---|---|---|---|---|
| | pre-treatment | ~6h post-dose | ~24h post-dose | ~6 post-dose | ~24h post-dose |
| 14001 skin biopsy | ✓ | ✓ | ✓ | | |
| 14002 skin biopsy | ✓ | ✓ | | ✓ | ✓ |
| 14002 tumor biopsy | ✓ | ✓ | | ✓ | |

# PT 703, a case study to highlight pharmacodynamic assays used

- 33 year old woman with neural sheath sarcoma
- 150 mg QD dose group (Spain)
- Completed 4 cycles of treatment
- Usable tissue and high dose make this a good case study

# PT 703, a case study

## Skin mitotic index



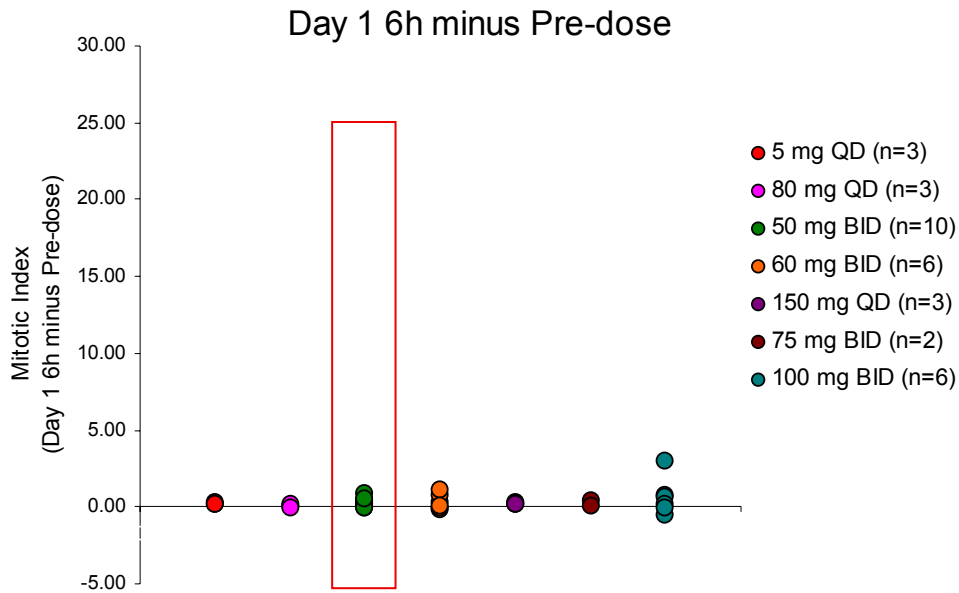
Day 1; Pre-dose = 1

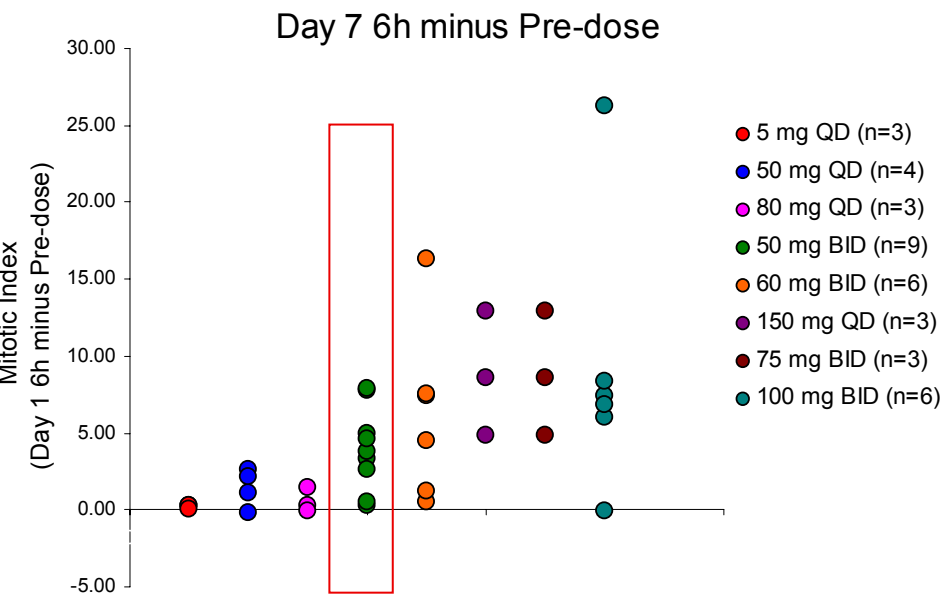
Day 7; 24 Hr Post-dose

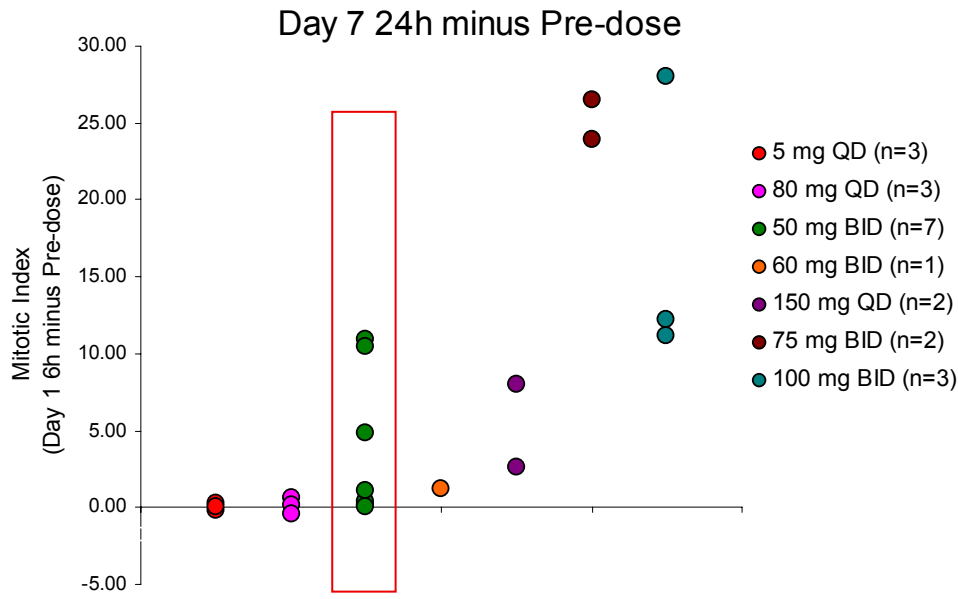
| Mitotic index (mitotic cells / mm BEL) | |
|---|---|
| Day 1; Pre-dose | = 0.10 |
| Day 1; 6 Hr Post-dose | = 0.39 |
| Day 7; 6 Hr Post-dose | = 3.62 |
| Day 7; 24 Hr Post-dose | = 8.08 |

# MLN8237 skin mitotic index (14002)

**Positive values are in a direction consistent with Aurora A inhibition*

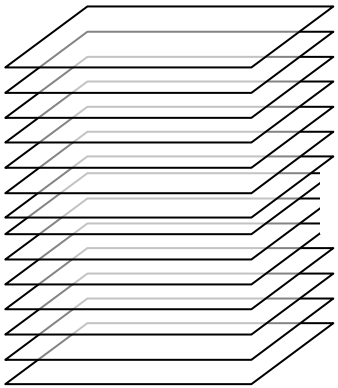


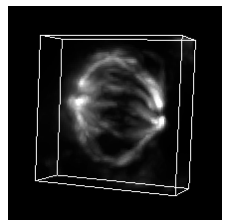
# Semi-automated method to measure mitotic spindle morphology changes in tissue

Image Acquisition → Image Processing (Deblur) + Visualization → Image Randomization → Scoring by blinded scorers



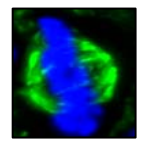

15 optical sections
@ 0.5 um intervals

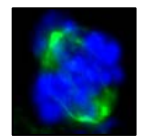
Deconvolution

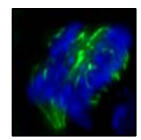


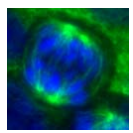
3D Rotation

**Score**



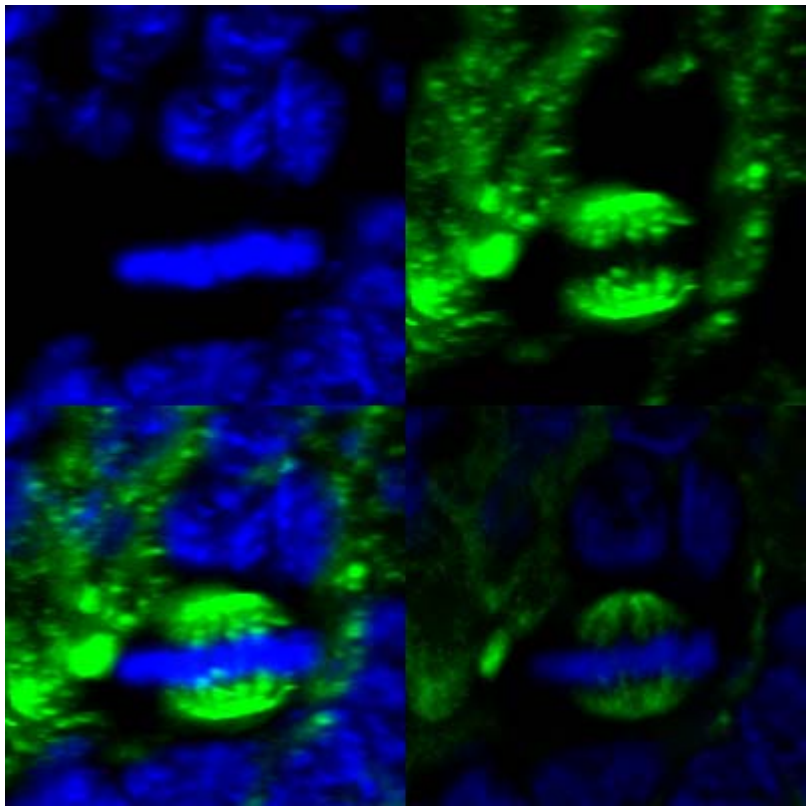



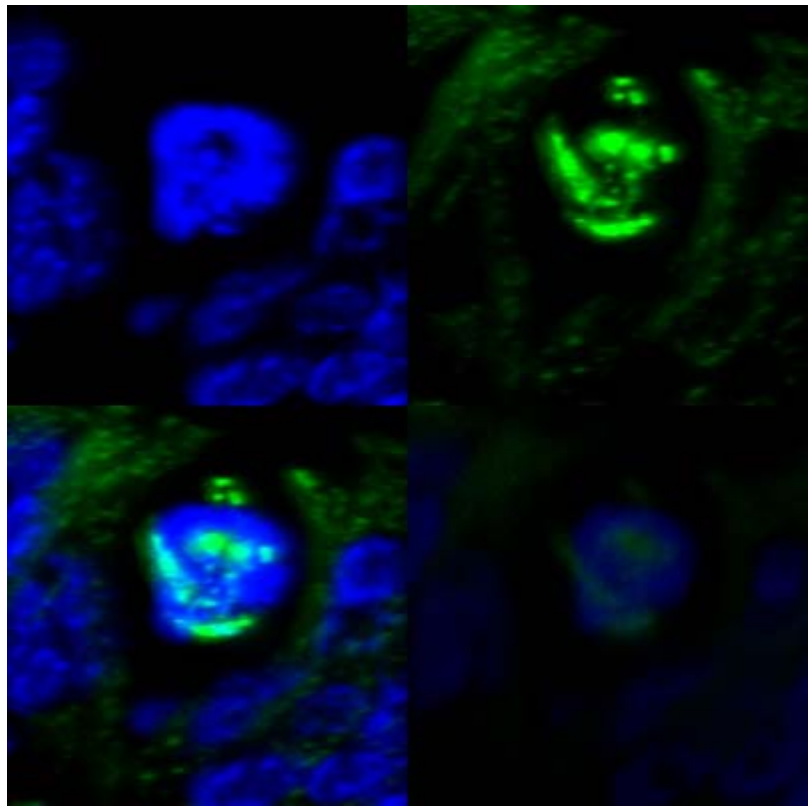



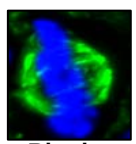

Spindle Morphology
- Spindle Bipolarity
- Chromosome Alignment

# Semi-automated method to measure spindle bipolarity and chromosome alignment

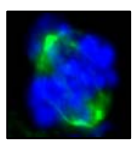




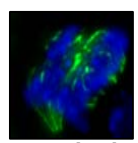
**Score**



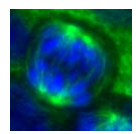
**Bipolar Aligned**



**Bipolar Not Aligned**



**Not Bipolar**



**No Call (telophase)**

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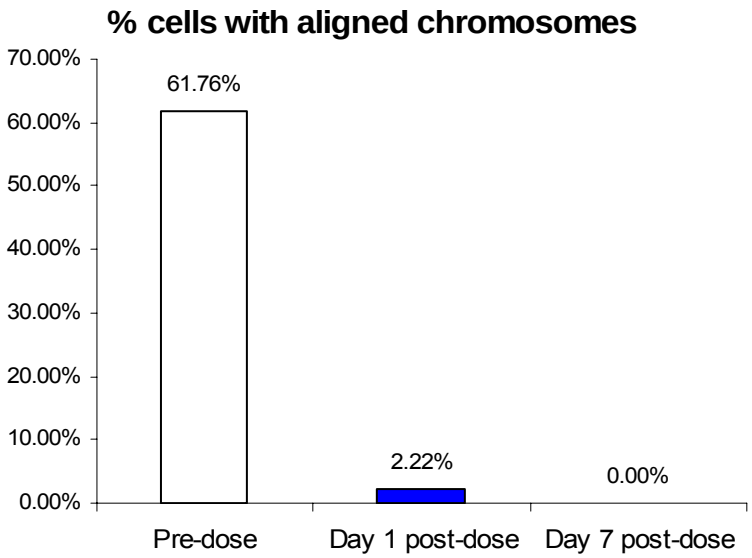
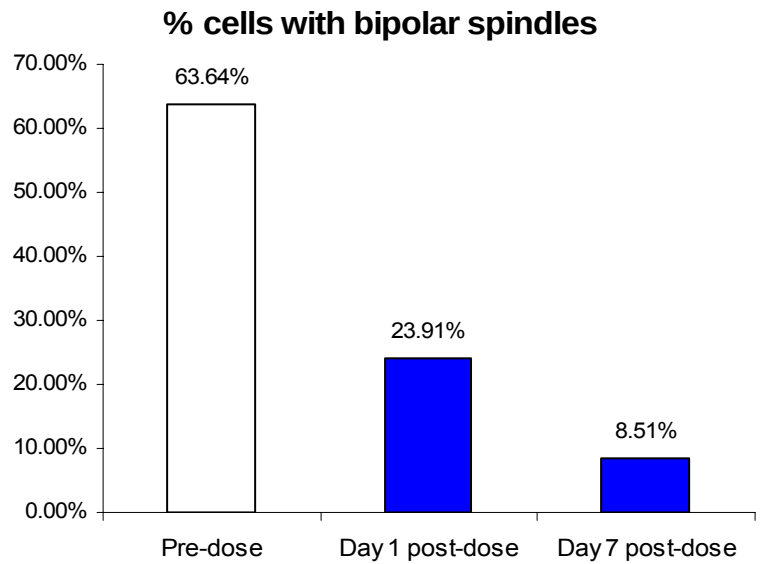
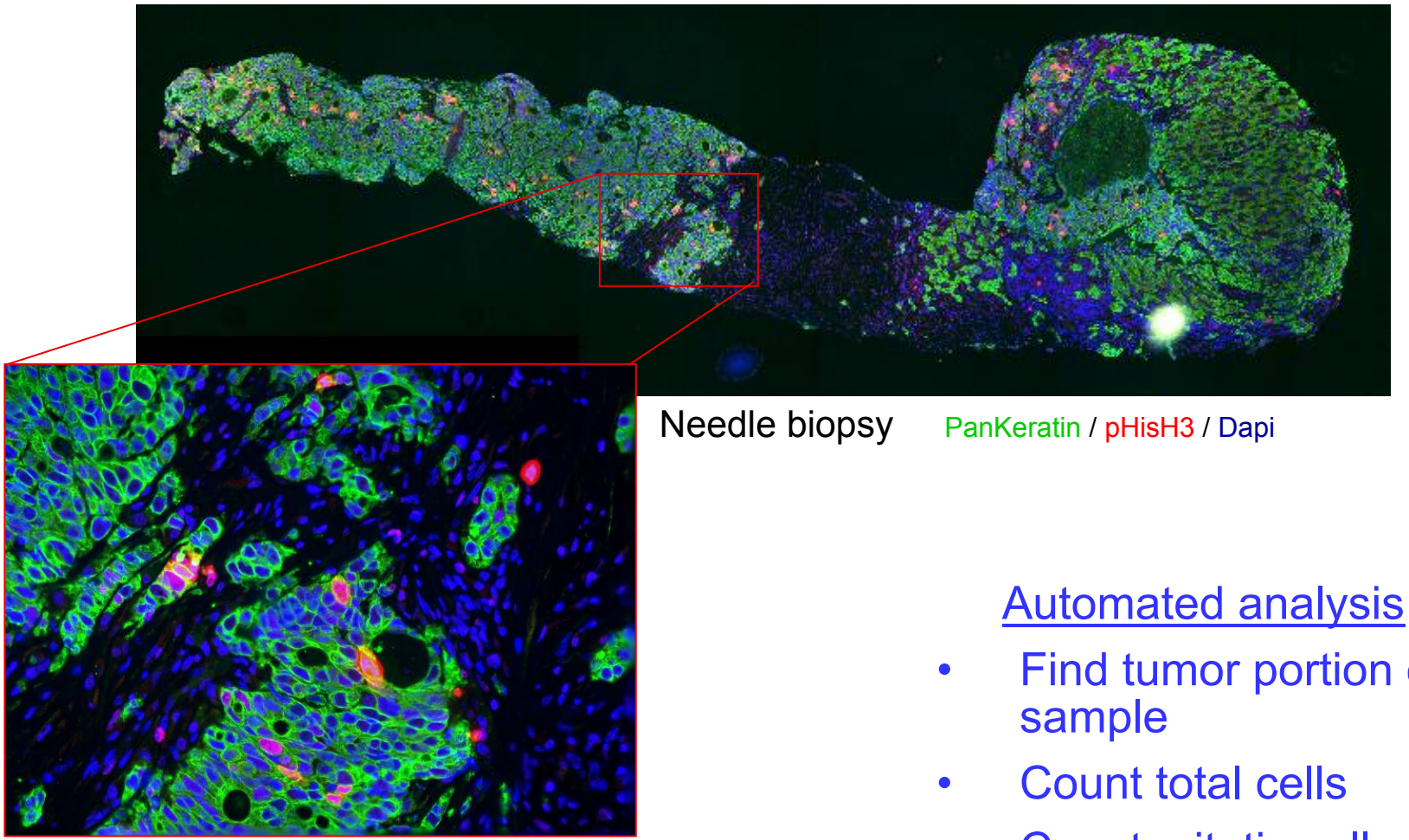
# PT 703 tumor biopsies

## Aligned chromosomes, bipolar spindles



# Measure Aurora A pathway modulation in clinical tumor needle biopsies



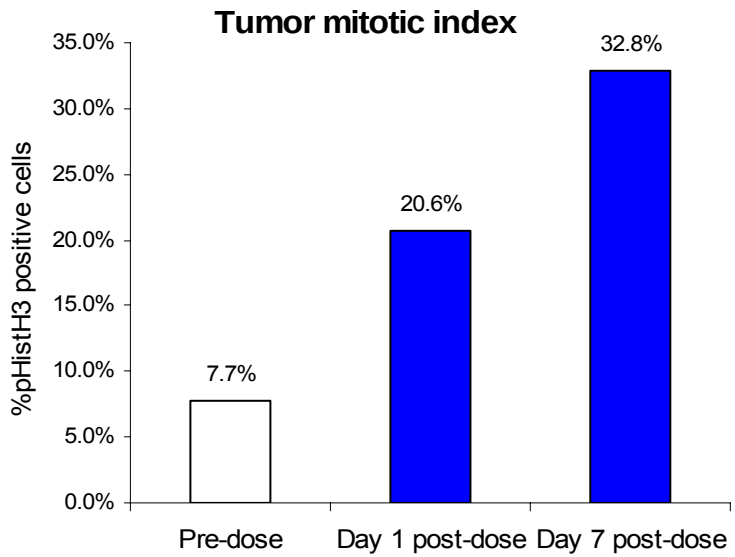
Needle biopsy PanKeratin / pHisH3 / Dapi

Automated analysis

- Find tumor portion of sample
- Count total cells
- Count mitotic cells (tumor only)

# PT 703 tumor biopsies

## Aligned chromosomes, bipolar spindles, mitotic index

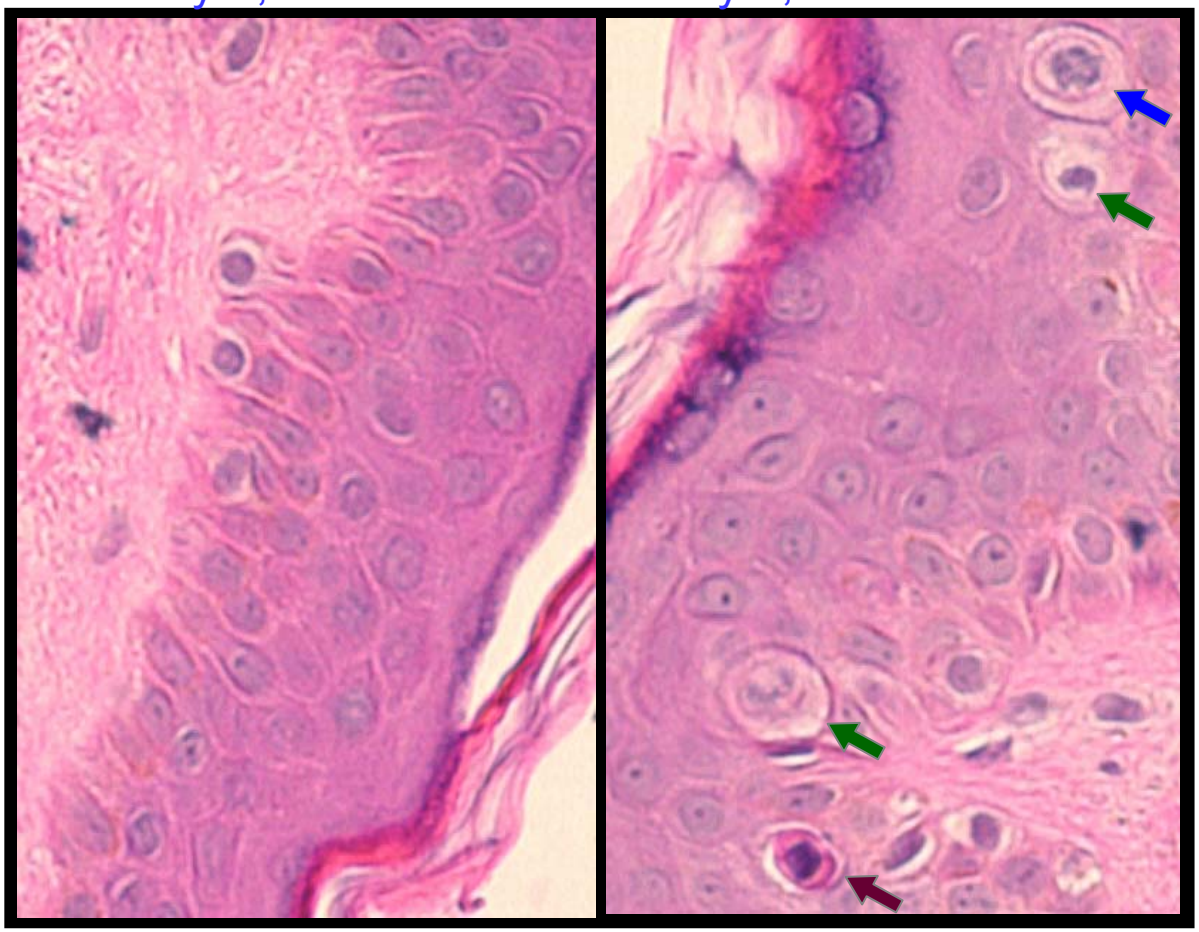


# PT 703, a case study

## Skin hematoxylin & eosin stain

Day 1; Pre-dose

Day 7; 6 Hr Post-dose



Mitotic

Mitotic / early apoptotic

Apoptotic

<u>Apoptotic index</u>
<u>(Apoptotic cells / mm BEL)</u>

Day 1; Pre-dose = 0.00

Day 1; 6 Hr Post-dose = 0.13

Day 7; 6 Hr Post-dose = 1.96

Day 7; 24 Hr Post-dose = 3.31

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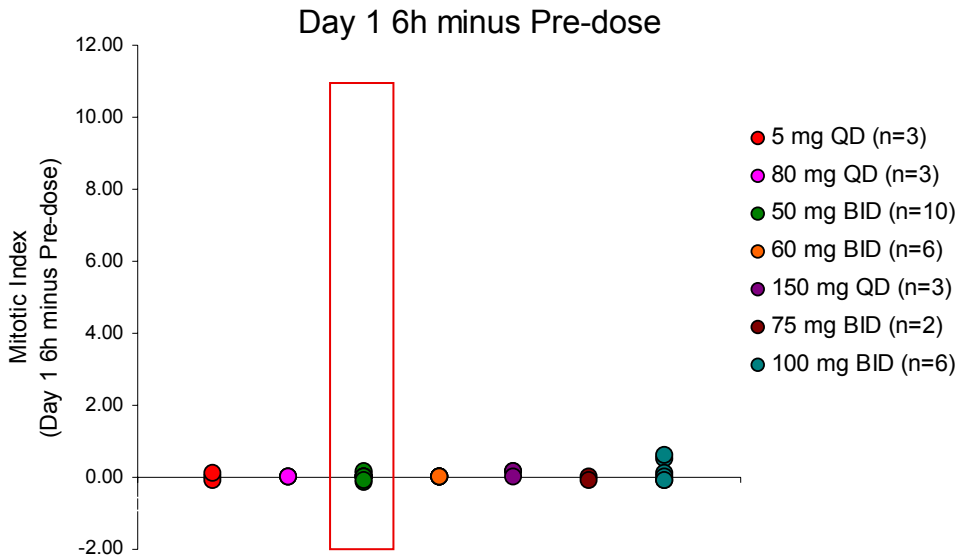
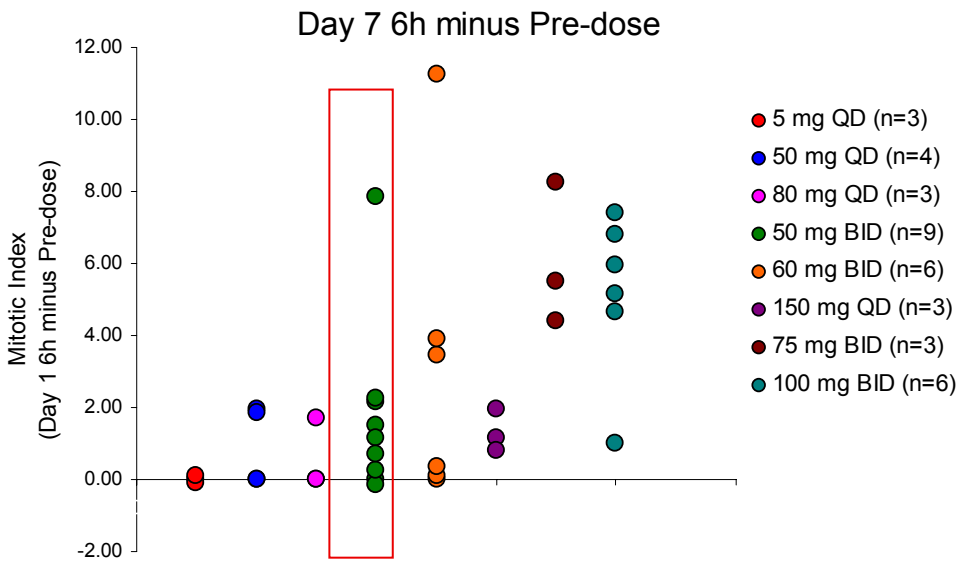
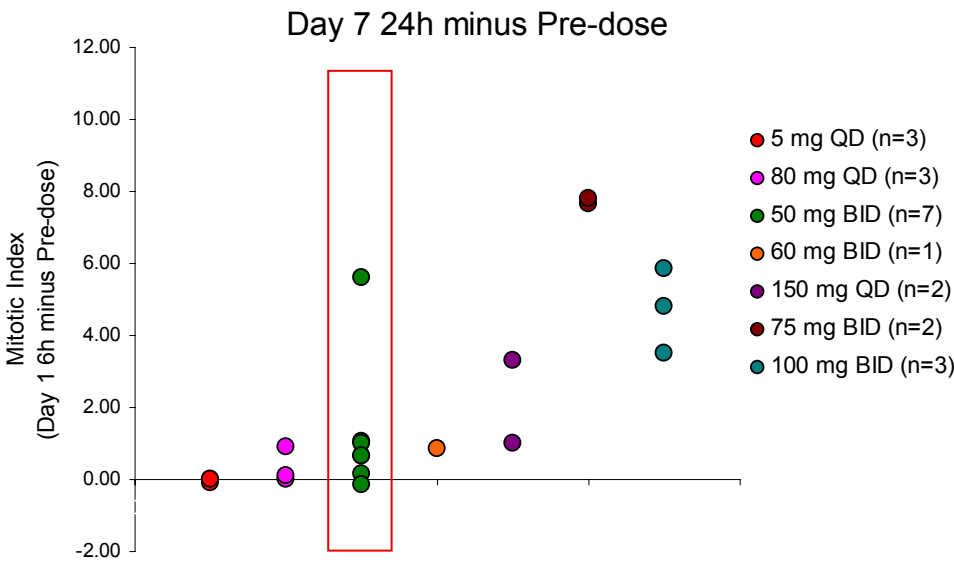
# MLN8237 skin apoptotic index (14002)

**Positive values are in a direction consistent with Aurora A inhibition*

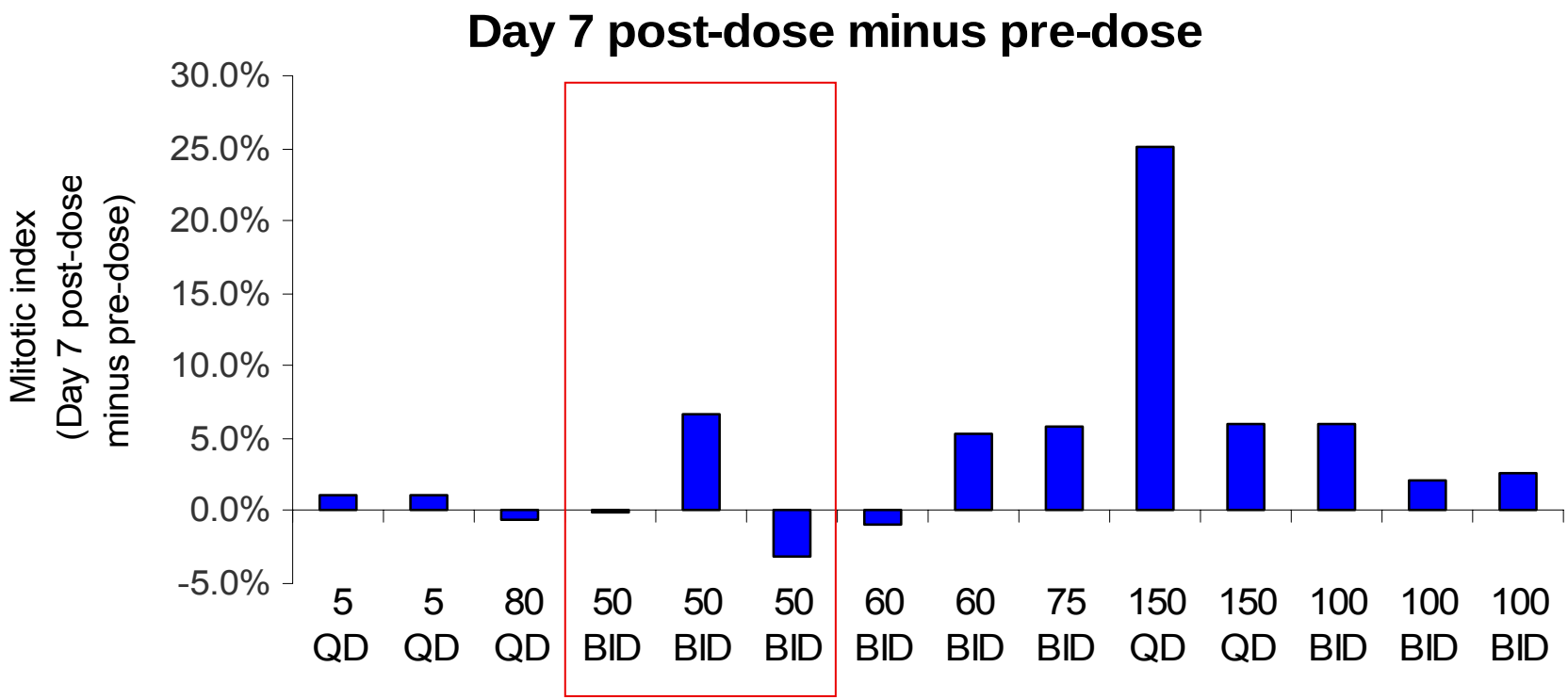


# MLN8237

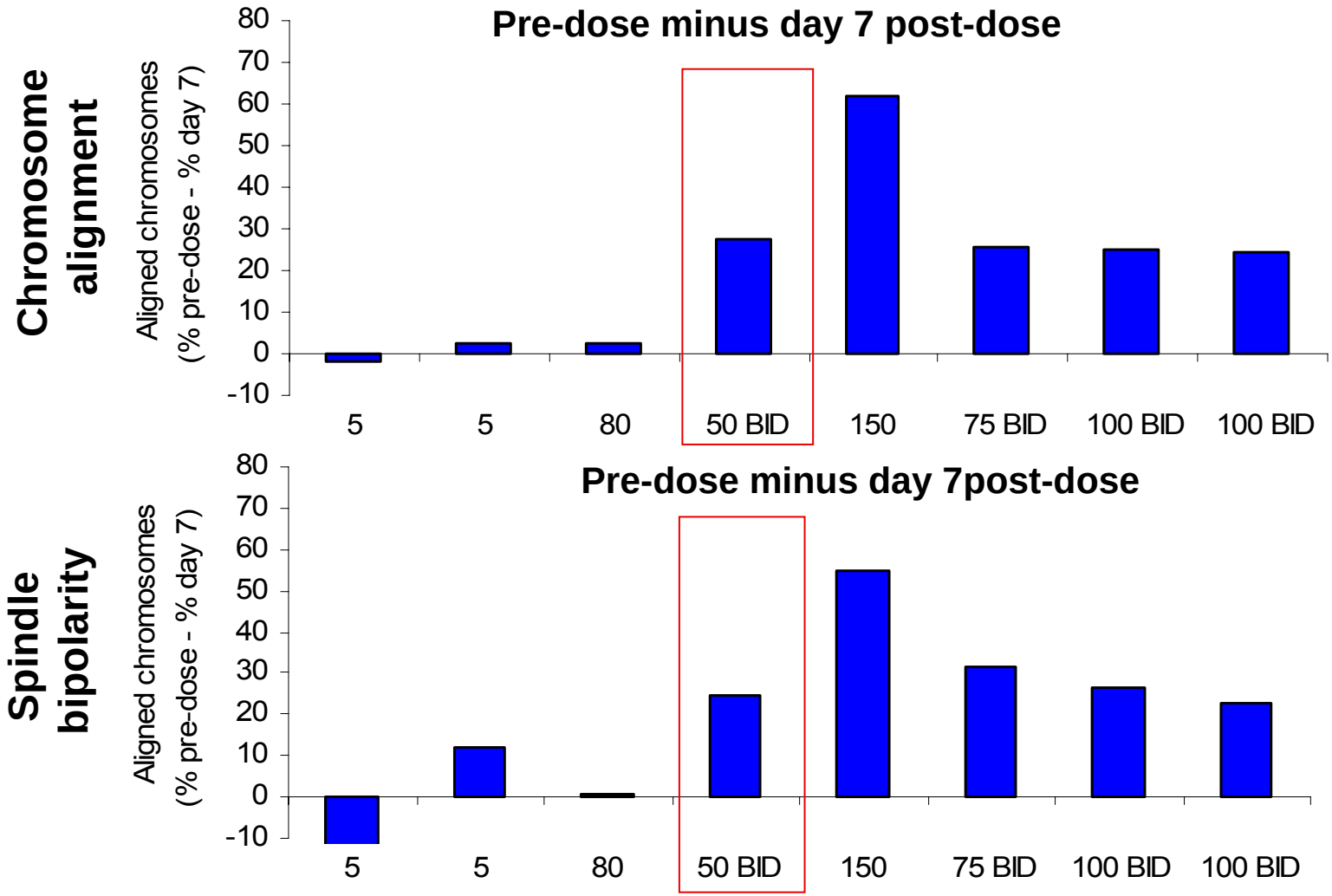
## Tumor mitotic index




***Positive values are in a direction consistent with Aurora A inhibition***

# MLN8237

## Chromosome alignment / spindle bipolarity

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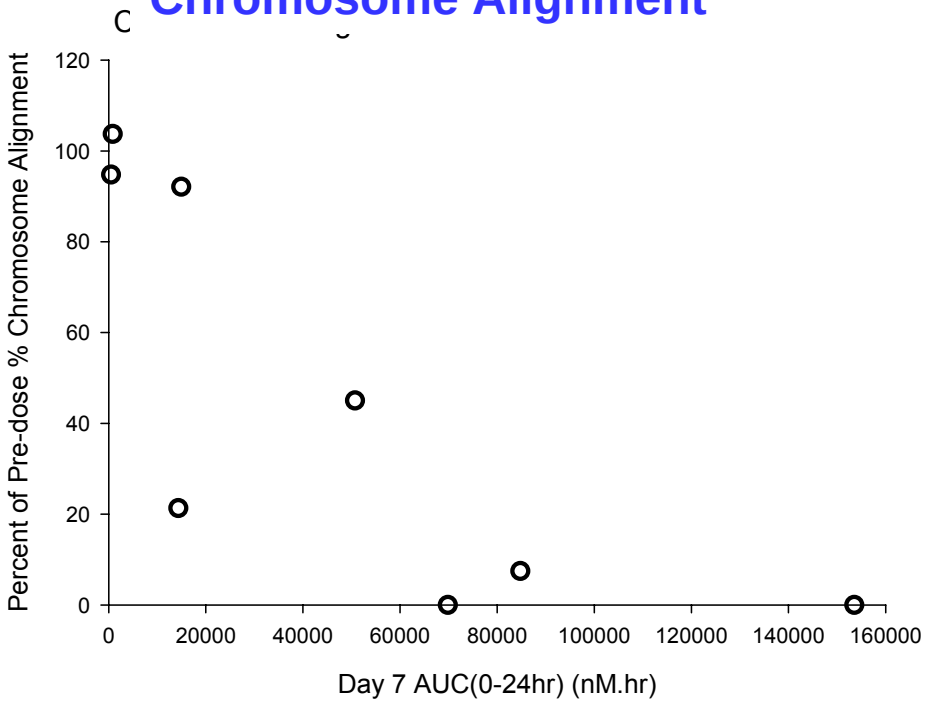
**Positive values are in a direction consistent with Aurora A inhibition*

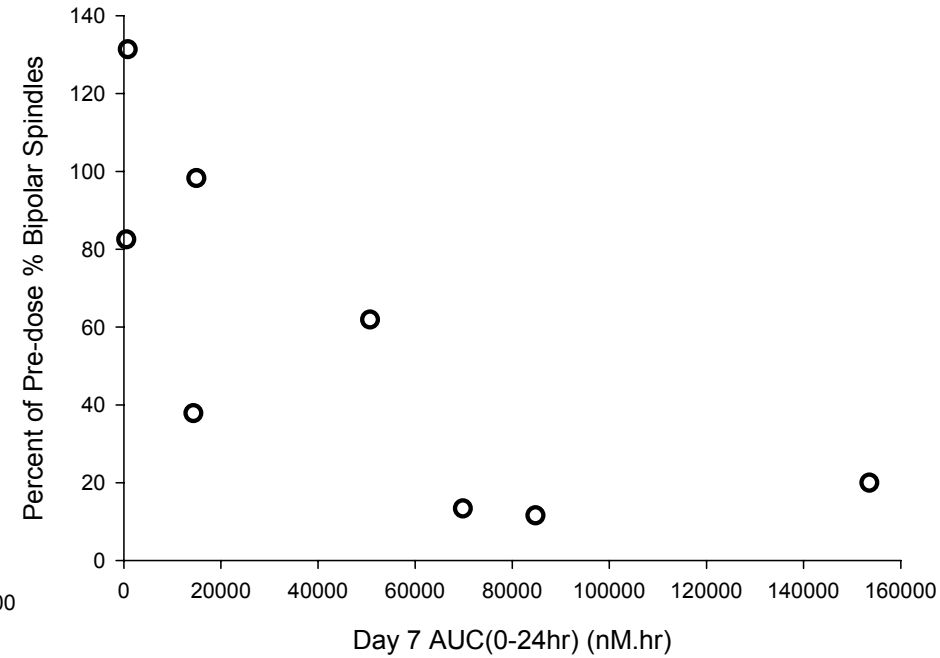
# Preliminary PK-PD relationship

## Emerging results from serial tumor biopsies

### Chromosome Alignment




### Spindle Bipolarity




- Eight patients with steady-state PK and tumor biopsy measurements
- Proof of mechanism - evidence for an exposure-related decrease in chromosome alignment and spindle bipolarity in mitotic cells

# How has the PK/PD data guided future decisions?

- Demonstrated proof of mechanism – MLN8237 inhibits Aurora A in patients
  - Clinical responses likely related to Aurora A inhibition
  - Use of pHistH3 as marker of mitotic accumulation confirmed selectivity for Aurora A relative to Aurora B in patients
  - Allows for rational drug development based on Aurora A mechanism
    - Combination selection, response marker identification
- Demonstrated that RP2D (50 mg BIDx7d) results in biologically active exposures
  - Same assays applied to MLN8054 demonstrated that biologically active exposures achieved at doses greater than the MTD (defined by somnolence)
- PD data informing future decisions
  - Guide dose and schedule decisions for combination studies

# Challenges / Unmet needs

- LIMs integration
- Simplify workflow for 3rd party integration
  - Slide scanners -> Image analysis platforms
- Infrastructure
  - Image management
  - Storage / Backup / Maintenance
- Cost
  - Premium for initial investment, maintenance, and “add-ons”

# Summary

- Developed and leveraged imaging technologies
- Tissue-based assays and technologies
  - Drive medicinal chemistry
  - Assess pharmacodynamic response in preclinical in vivo models
  - Assess pharmacodynamic response in variety of clinical tissues, in use in Phase 1 clinical trials

# Acknowledgements


- Molecular and Cellular Oncology
  - Jeff Escedy
  - Natalie Roy D'Amore
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  - Vaishali Shinde
  - Yu Yang
  - Brad Stringer


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