OHSU

DNA Repair, Replication, & Mutagenesis Research at OHSU

DNA Repair

BER: Amanda McCullough, R. Stephen Lloyd

NER: Amanda McCullough, R. Stephen Lloyd, Mitch Turker

Fanconi anemia: Maureen Hoatlin, Markus Grompe, Grover Bagby, Susan Olson

DNA Replication: Matt Thayer, R. Stephen Lloyd, Maureen Hoatlin

Mutagenesis: Mitch Turker, Amanda McCullough, R. Stephen Lloyd

DNA Damage Response Pathways: Amanda McCullough, Maureen Hoatlin, Matt Thayer

OREGON HEALTH & SCIENCE UNIVERSITY

Amanda McCullough, Molecular and Medical Genetics
mculloaATohsuDOTedu

Maureen Hoatlin, Biochemistry and Molecular Biology
hoatlinmATohsuDOTedu

R. Stephen Lloyd, Center for Research on Occupational and Environmental Toxicology
lloydstATohsuDOTedu

Matt Thayer, Biochemistry and Molecular Biology
thayermATohsuDOTedu

Mike Liskay, Molecular and Medical Genetics
liskaymATohsuDOTedu

Markus Grompe, Oregon Stem Cell Center
grompemATohsuDOTedu

Grover Bagby, Knight Hematology/Oncology Administration
groverATohsuDOTedu

Susan Olson, Molecular and Medical Genetics
olsonsuATohsuDOTedu

Mitch Turker, Center for Research on Occupational and Environmental Toxicology
turkermATohsuDOTedu

The McCullough Lab:
MMG, CROET, CANB mcculloaATohsuDOTedu

The major focus of the laboratory is to understand the fundamental mechanisms by which environmental exposures, endogenous chemicals and radiation lead to cancers, metabolic diseases, and aging

DNA Damage & Repair

DNA-Protein Crosslinks

Oxidative DNA Damage

UV-induced DNA Damage

Human polymorphic variations

Biochemical mechanisms

Cellular response pathways

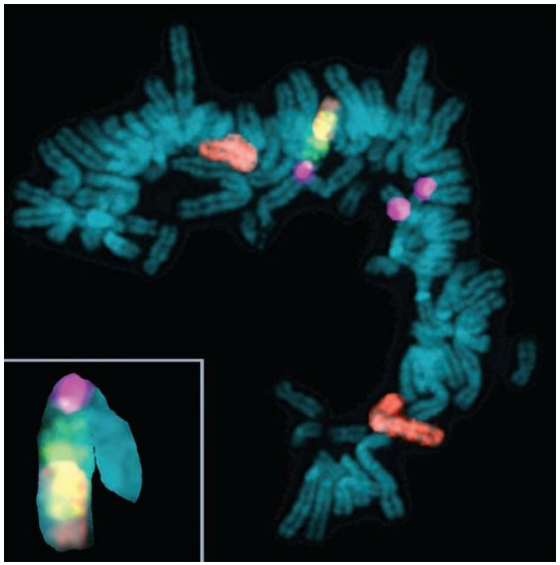
Genome-wide screens

Disease-associated mutations

Matt Thayer Laboratory

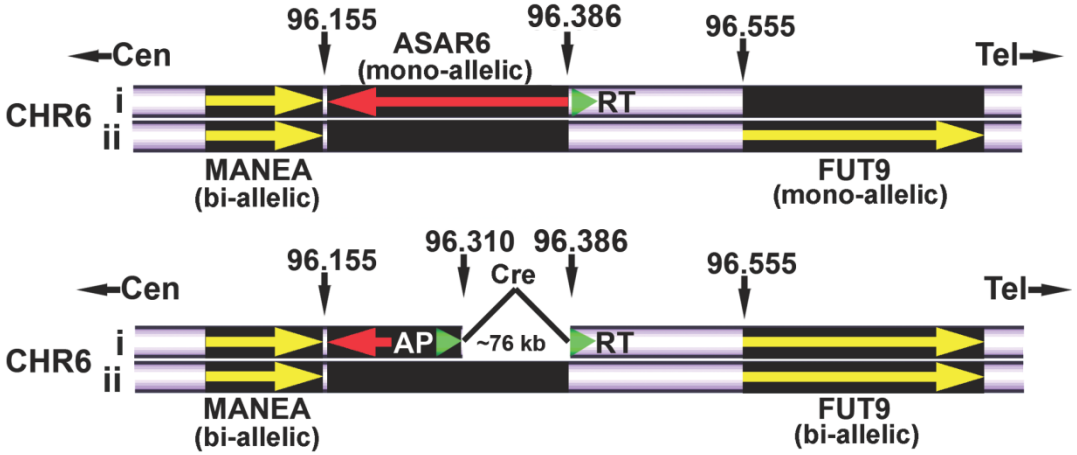
BMB, MMG, NGP, PMCB, CANB thayermATohsuDOTedu

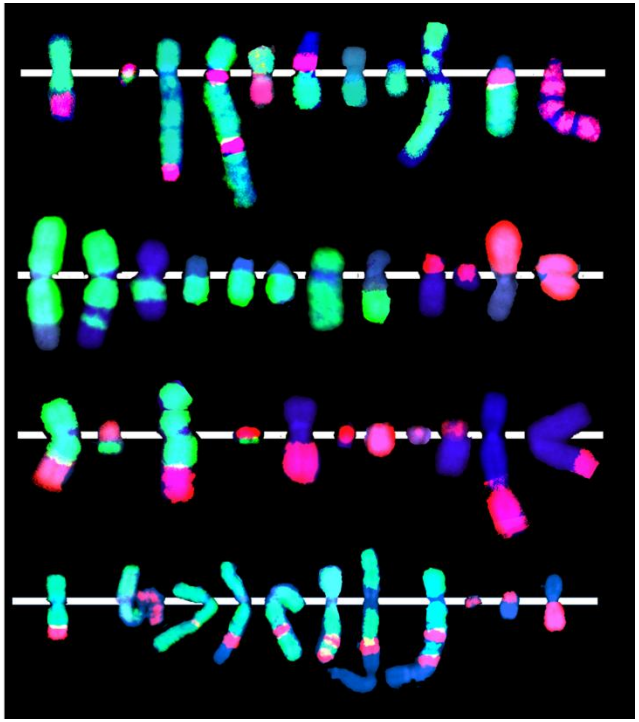
Chromosome Replication Timing



Disruption of discrete cis-acting loci result in delayed replication timing of the entire chromosome. BrdU (green) incorporation in a t(6;9); chromosome 6 (red), and the centromere of chromosome 9 (purple).

Disruption of



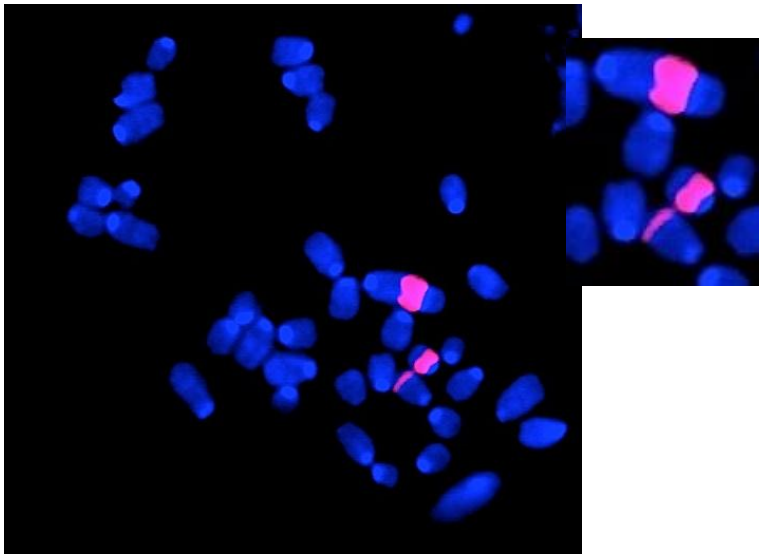


Cre/loxP-mediated disruption of the ASAR6 gene (encoding a large ncRNA) results in extremely late replication, activation of previously silent mono-allelic genes, and instability of human chromosome 6.

Mitch Turker Laboratory

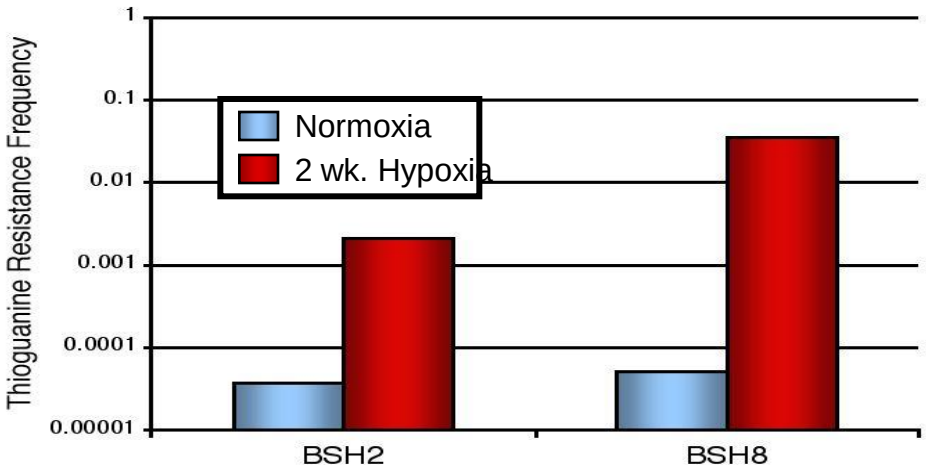
MMG, PMCB, CANB turkermATohsuDOTedu

Mutation Formation



Space radiation effects- Pink sections represent portions of mouse chromosome 8 in an *Aprt* mutant kidney cell that have inserted in other chromosomes. The *Aprt* mutant cell arose in the intact kidney due to shattering of chromosome 8.

Epigenetic Silencing



Epigenetic Silencing- Induction of epigenetic silencing of the human *BRCA1* promoter by hypoxia in mouse cells.

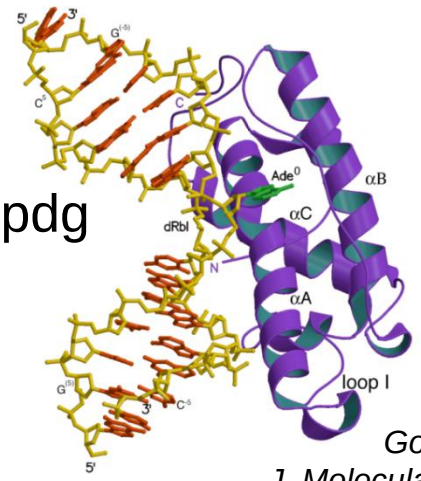
Overview of Lloyd Lab Research Investigations

MMG, PMCB, CANB lloydstATohsuDOTedu

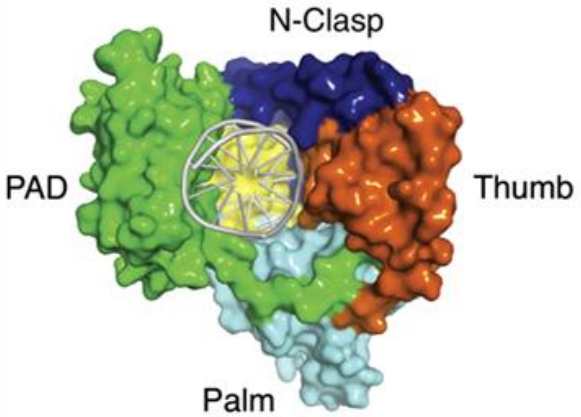
DNA Damage Resulting from Occupational & Environmental Exposures

Repair **Tolerance** **Replication & Mutagenesis**

T4-pdg



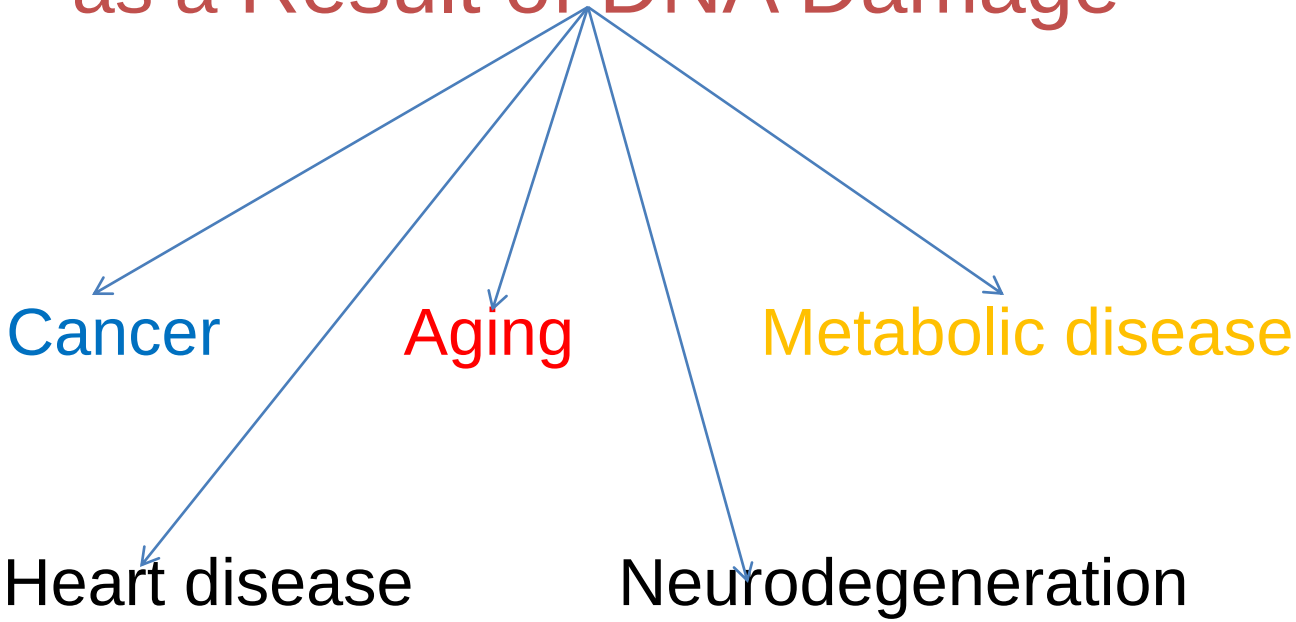
Golan et al, 2006
J. Molecular Biology 362, 241-258



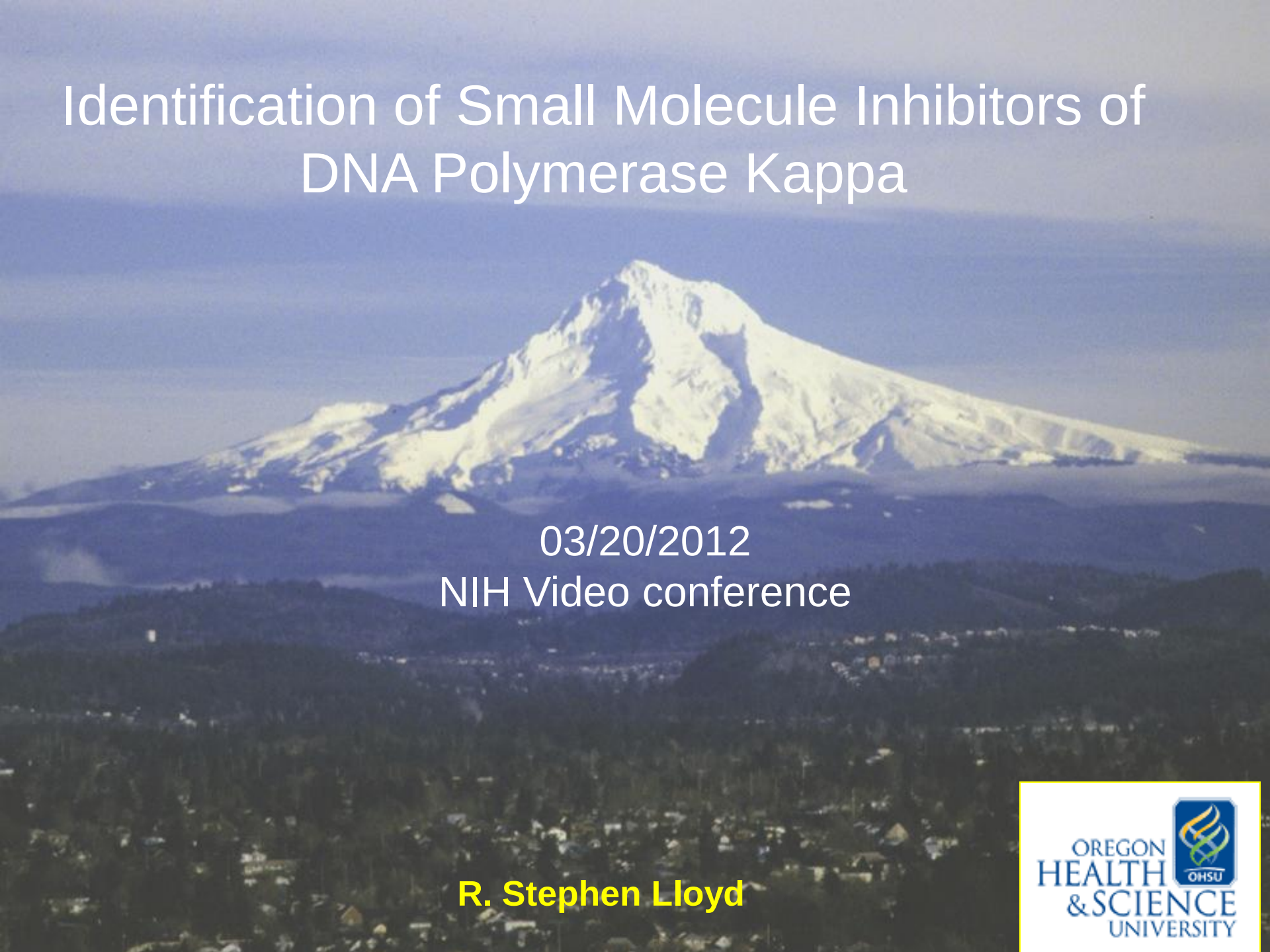
Pol κ

Lone et al., Mol Cell (2007), 25: 601-614

Disease Endpoints as a Result of DNA Damage



Identification of Small Molecule Inhibitors of DNA Polymerase Kappa



03/20/2012
NIH Video conference

R. Stephen Lloyd

OREGON HEALTH & SCIENCE UNIVERSITY
OHSU

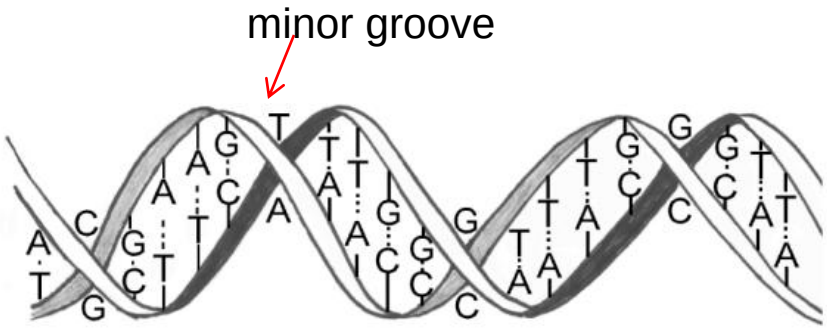
DNA polymerase kappa (pol κ)

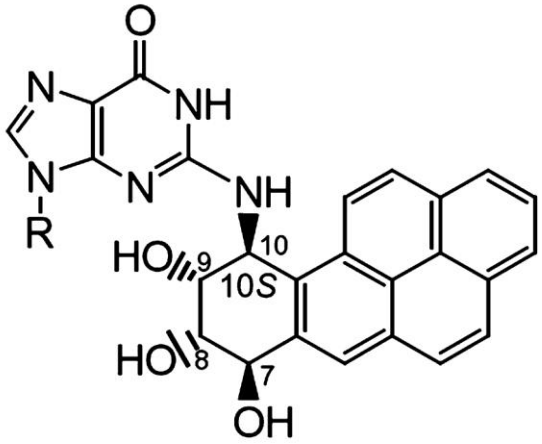
Copies non-damaged DNA with low fidelity

Proficient in the replication bypass of minor groove N^2-dG lesions:

Benzo[*a*]pyrene-induced lesions

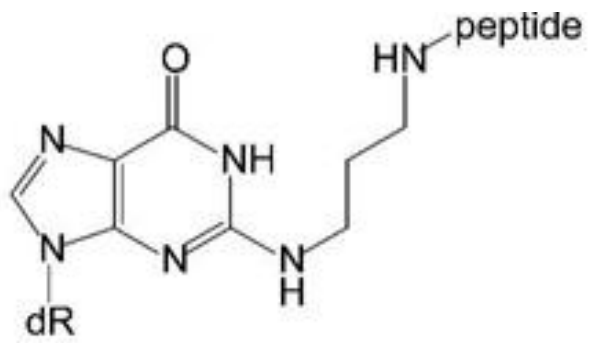
Acrolein-induced lesions





(+)-*trans*-[BP]-N^2-dG

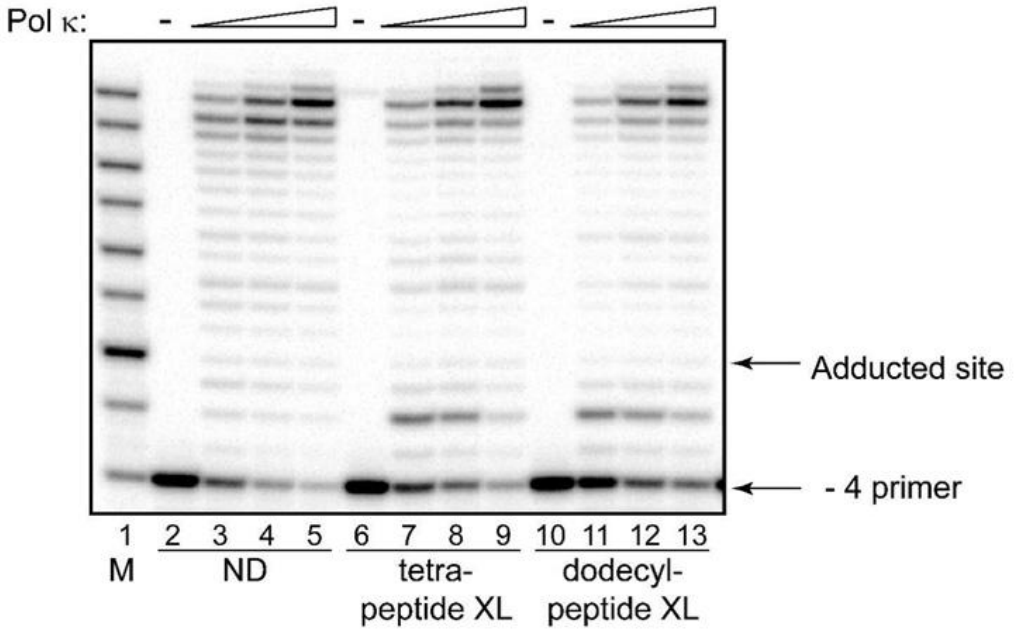
Replication bypass of acrolein-mediated N^2-dG DNA−peptide cross-link

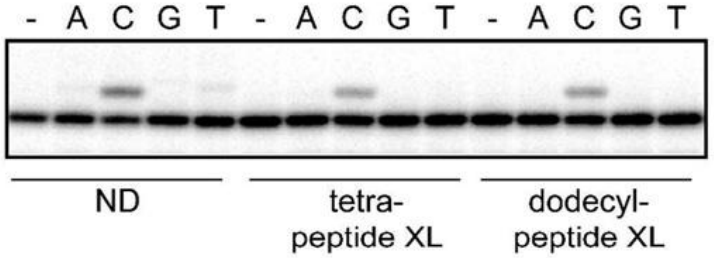


γ-HOPdG-mediated cross-link

Lys-Trp-Lys-Lys

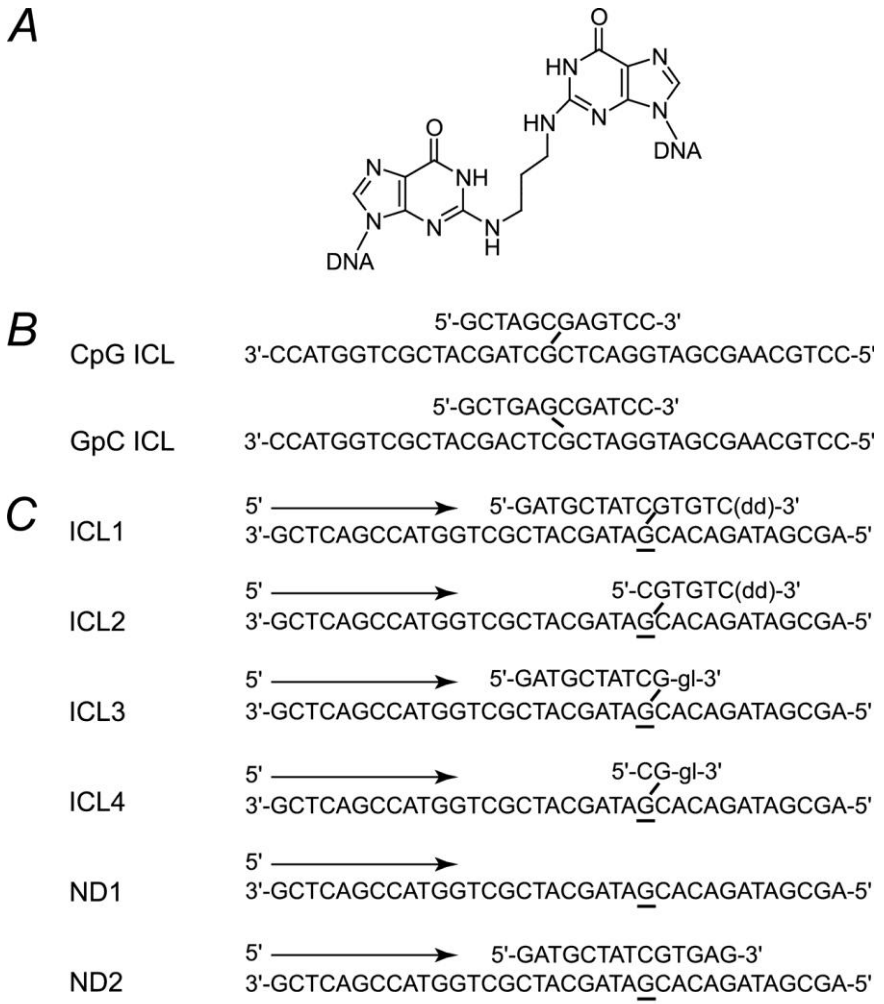
Lys-Phe-His-Glu-Lys-His-His-Ser-His-Arg-Gly-Tyr

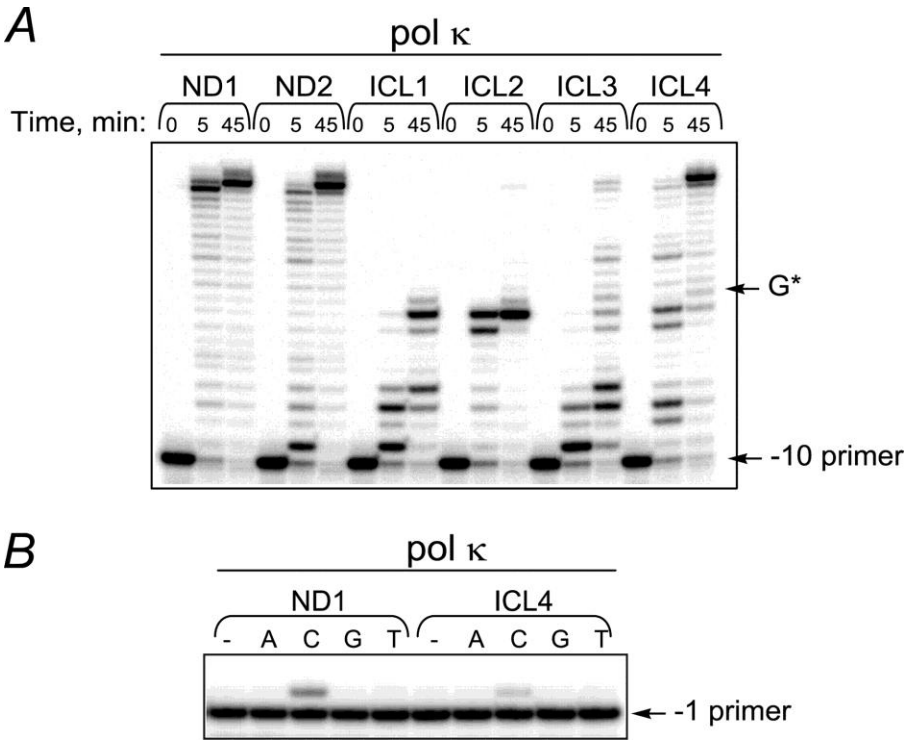




Minko *et al.* (2008) *Chem. Res. Toxicol.*

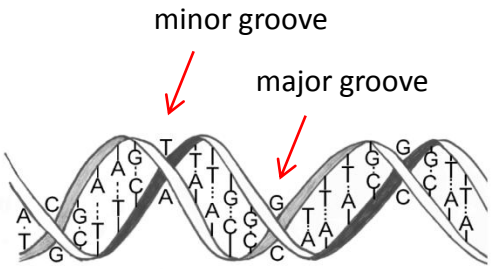
Replication bypass of acrolein-mediated N^2-dG interstrand cross-link

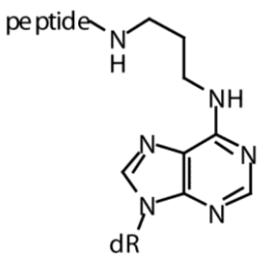




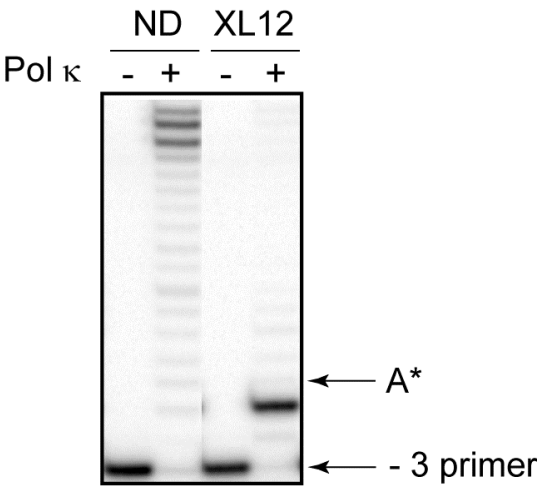
Minko *et al.* (2008) *J. Biol. Chem.*

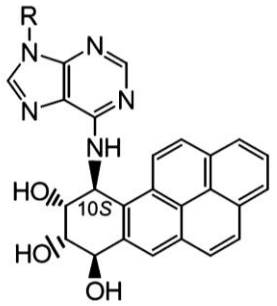
Pol κ cannot bypass major groove N^6-dA lesions



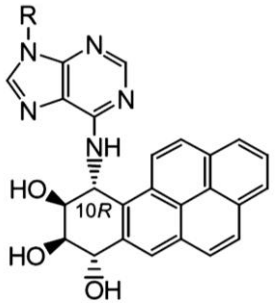


N^6-dA peptide cross-link

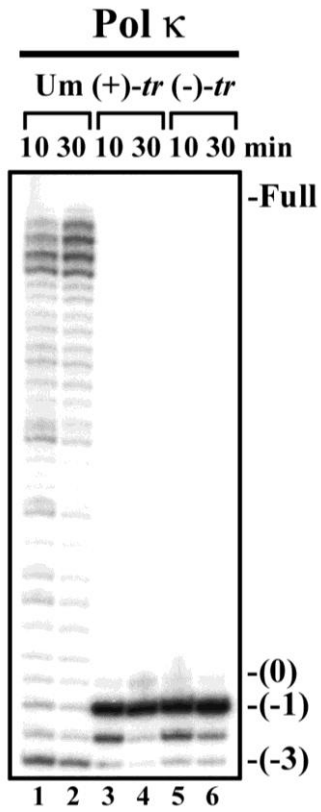




(+)-*trans*-[BP]-N^6-dA



(-)-*trans*-[BP]-N^6-dA

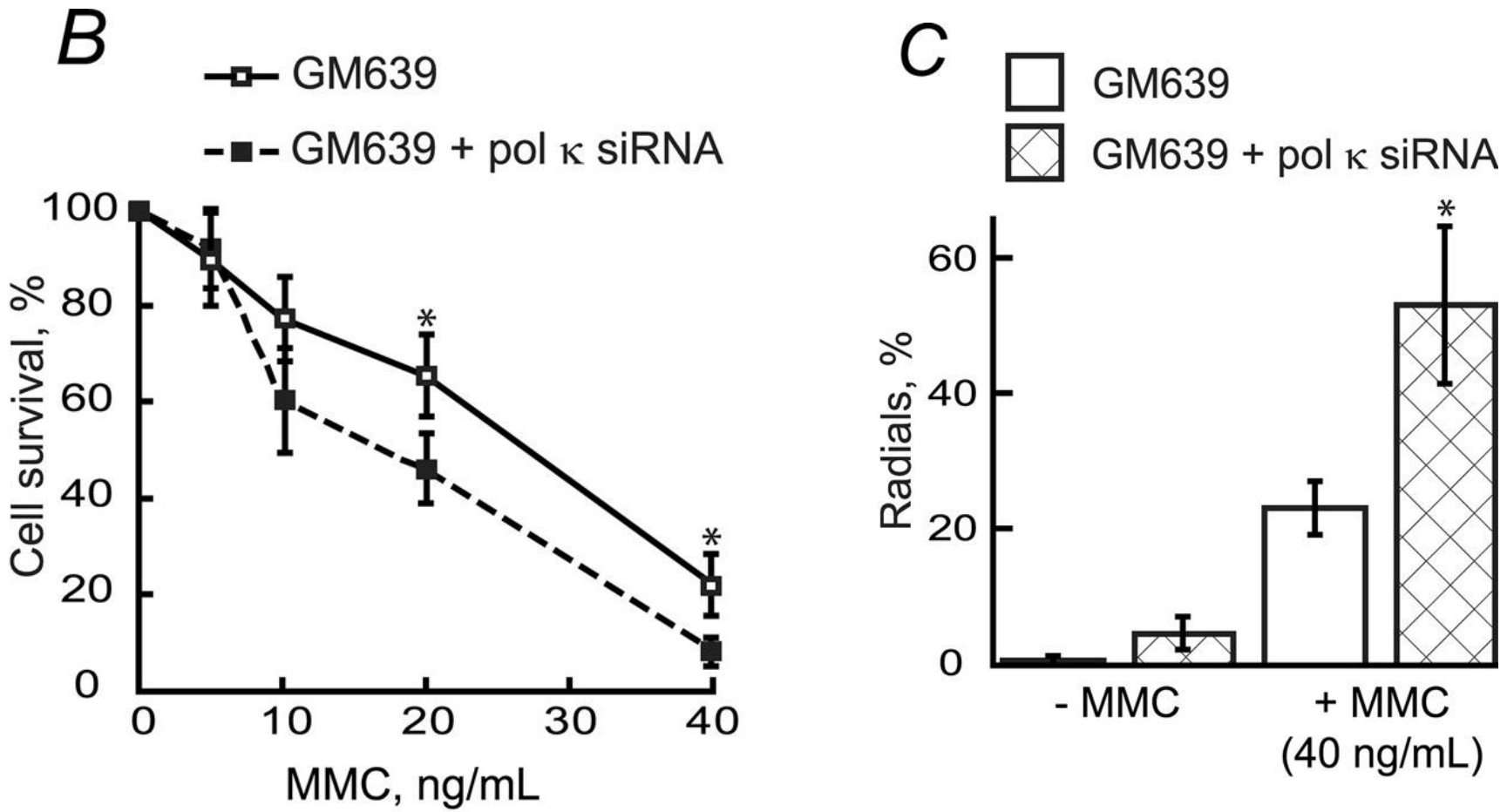


Yamanaka *et al.* (2011) *J. Bacteriol.* and Rechkobilit *et al.* (2002) *J. Biol. Chem.*

A biological role for polymerase κ ?

- Is there a potential role for polymerase κ in mediating cellular responses to DNA damaging agents?
- Is there evidence to associate polymerase κ with a specific cancer?

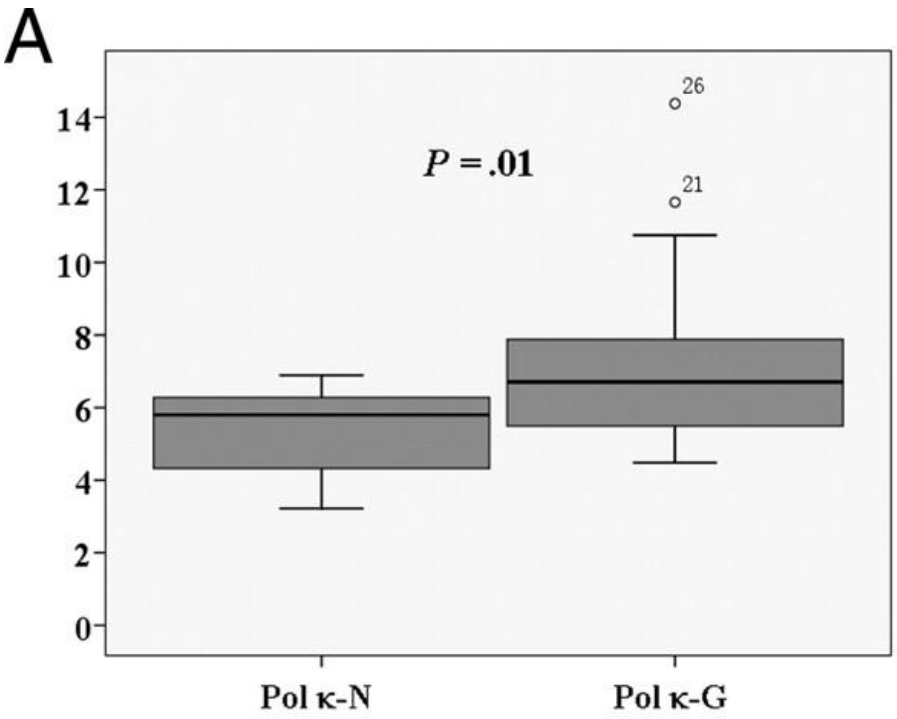
Role of pol κ in processing of mitomycin C-induced N^2-dG interstrand cross-links



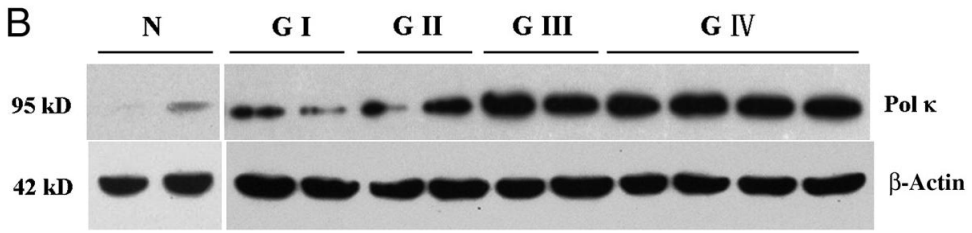
Minko *et al*. (2008) *J. Biol. Chem.*

Pol κ is upregulated in gliomas

mRNA

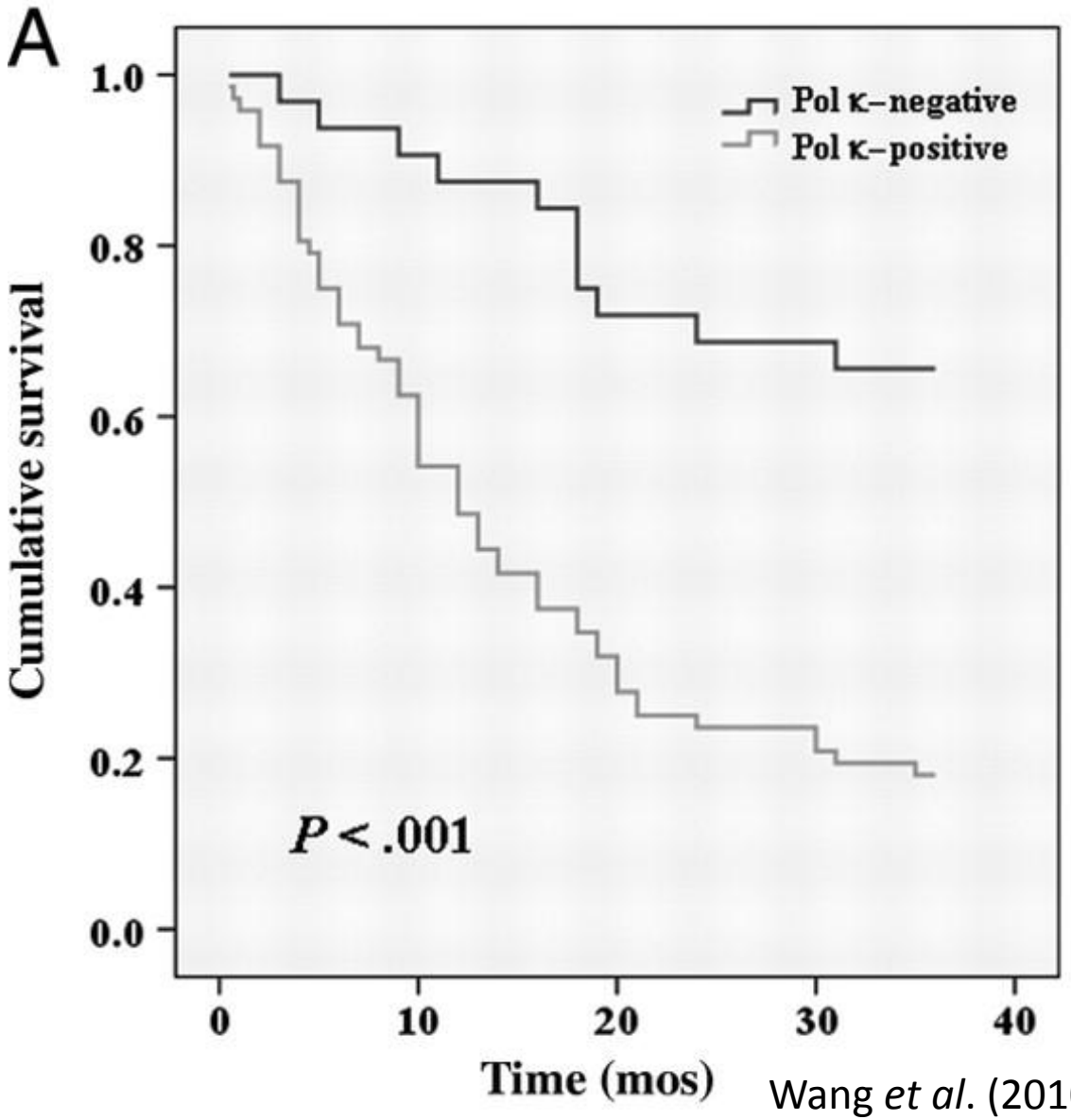


protein



Wang *et al*. (2010) *Neuro-Oncology*

Pol κ is a prognostic factor in gliomas



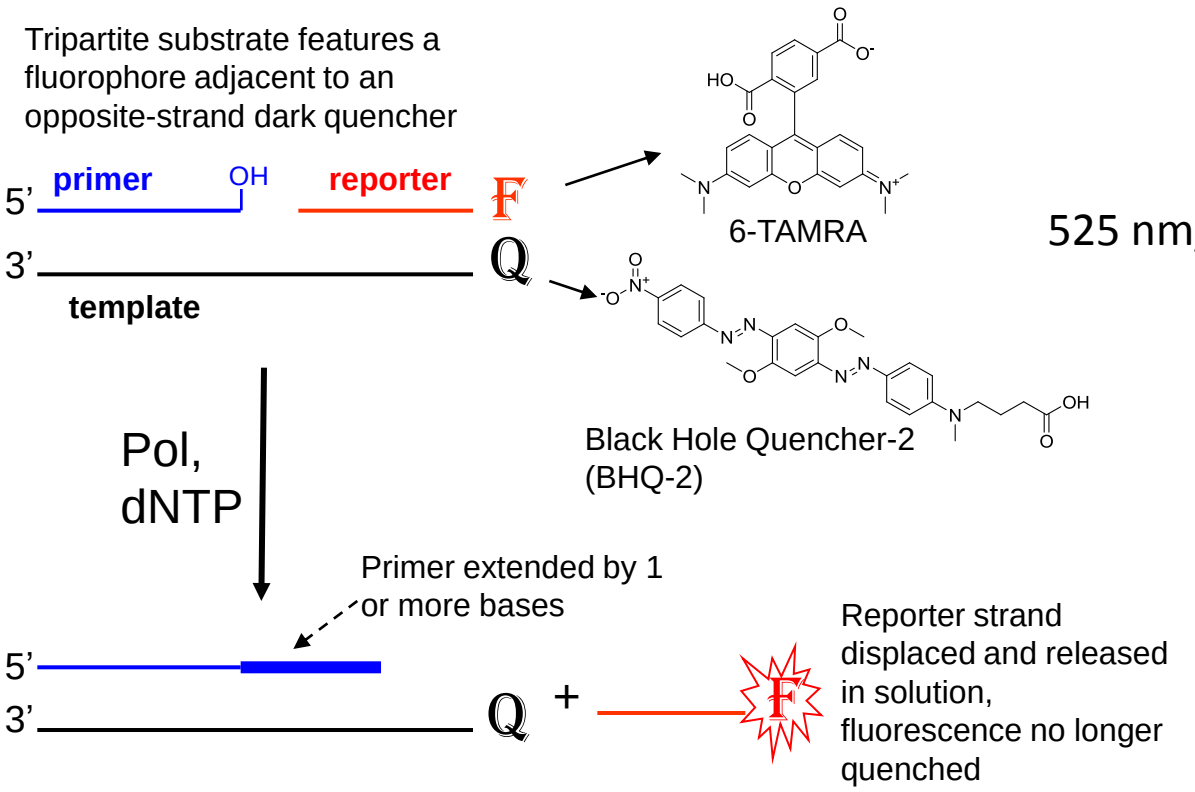
Wang *et al*. (2010) Neuro-Oncology

Hypotheses

- Small molecule inhibitors of pol κ may be useful for treatment of cancer with dysregulated pol κ expression

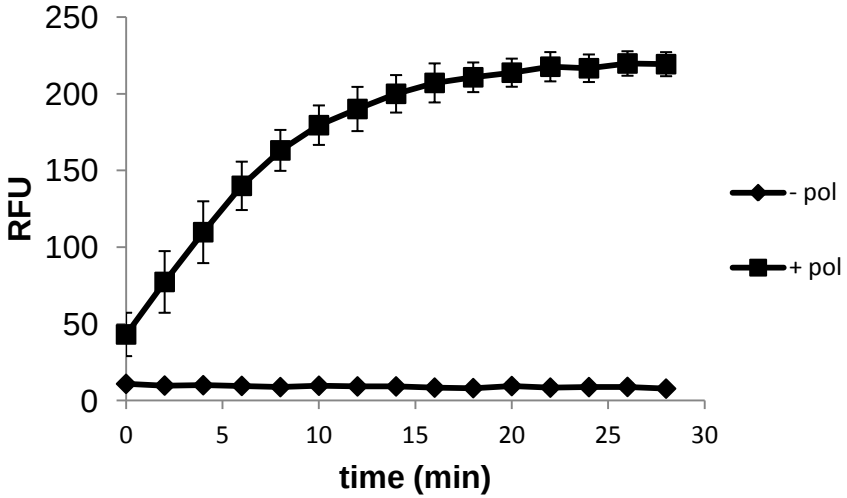
- Small molecule inhibitors of pol κ may render tumor cells more susceptible to the cytotoxic effects of specific chemotherapeutic agents

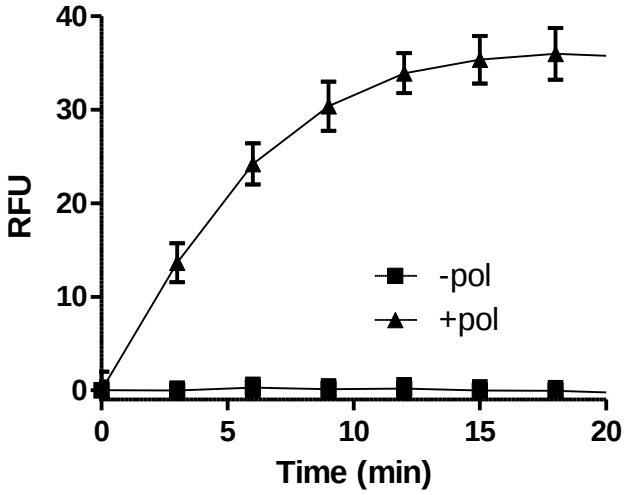
Fluorescence-based strand displacement assay



Dorjsuren *et al*. (2009) *Nucleic Acids Res.*

Fluorescence-based strand displacement assay





384-well plate
Rxn volume = 10 μl

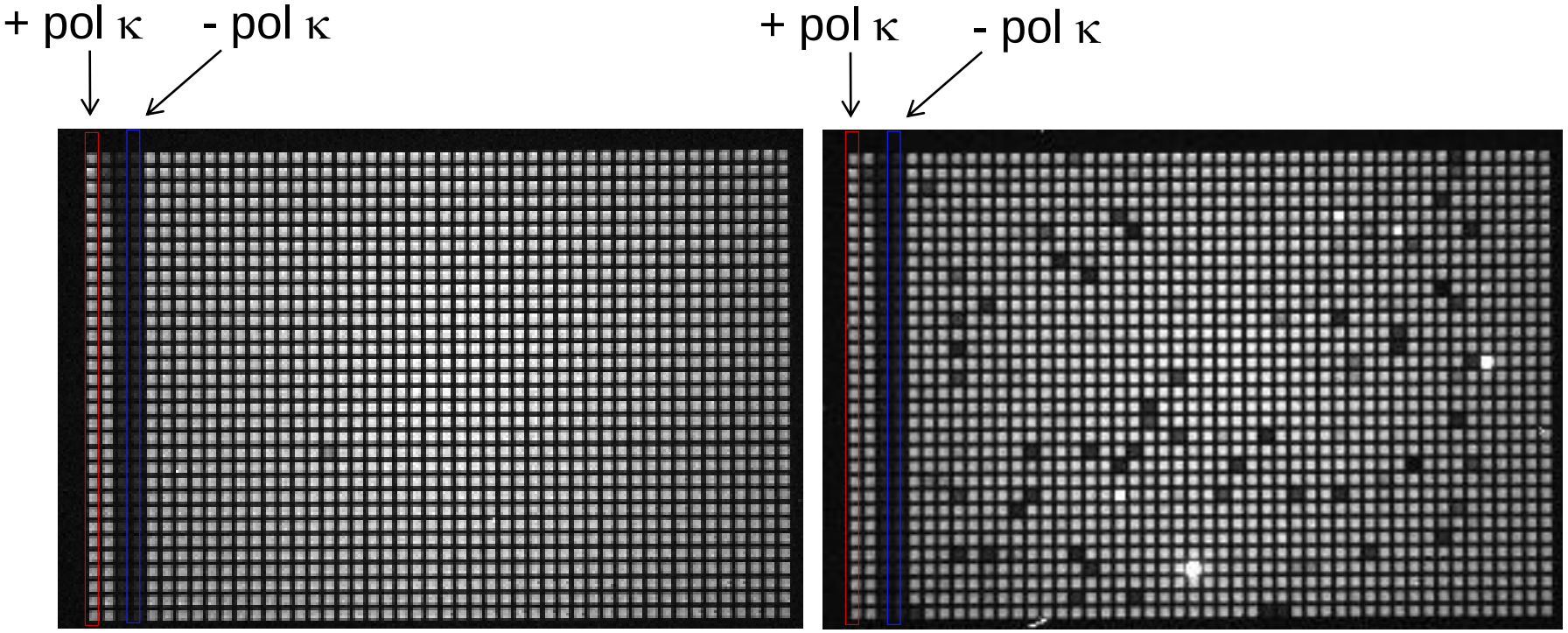
1536-well plate
Rxn volume = 4 μl

In collaboration with Dr. Anton Simeonov's group at NIH Chemical Genomics Center (NCGC)

Pilot HTS

Pilot screens were conducted with several compound libraries including the Library of Pharmacologically Active Compounds (LOPAC^{1280}) - total of ~15,000 compounds

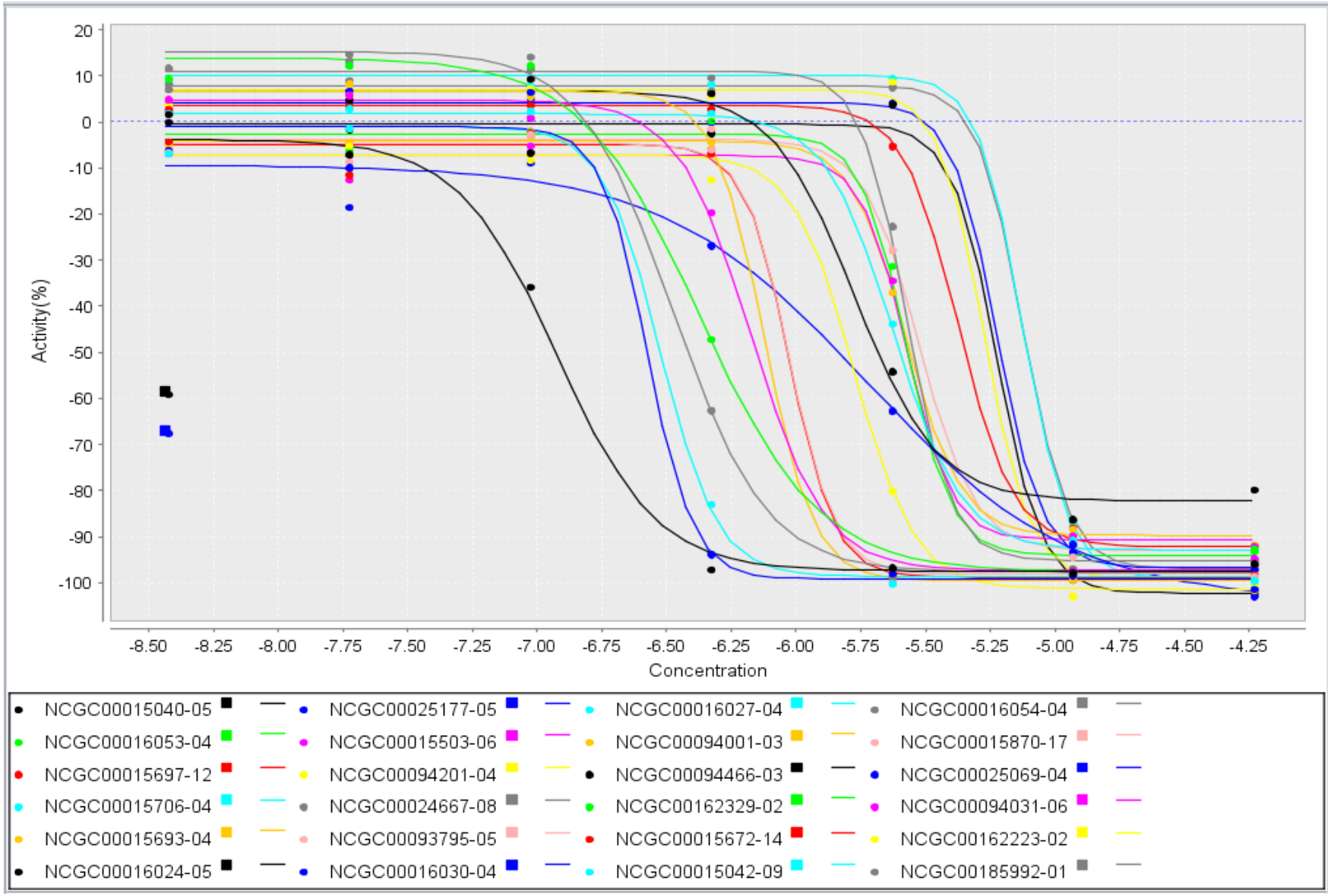
1536-well assay plate image



Lowest compound concentration plate

Highest compound concentration plate

Summary of results from HTS



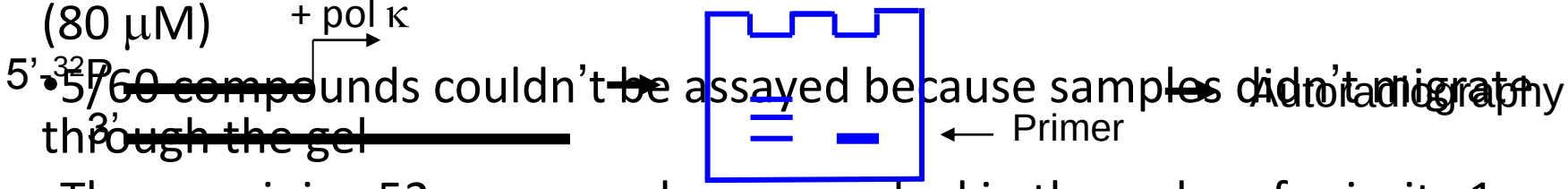
Results of pilot HTS/secondary assay

Pilot HTS

- ~ 500 hits were identified
- 60 top hits were selected for orthogonal confirmatory secondary assays

Secondary Assay-radioactive gel-based primer extension assay

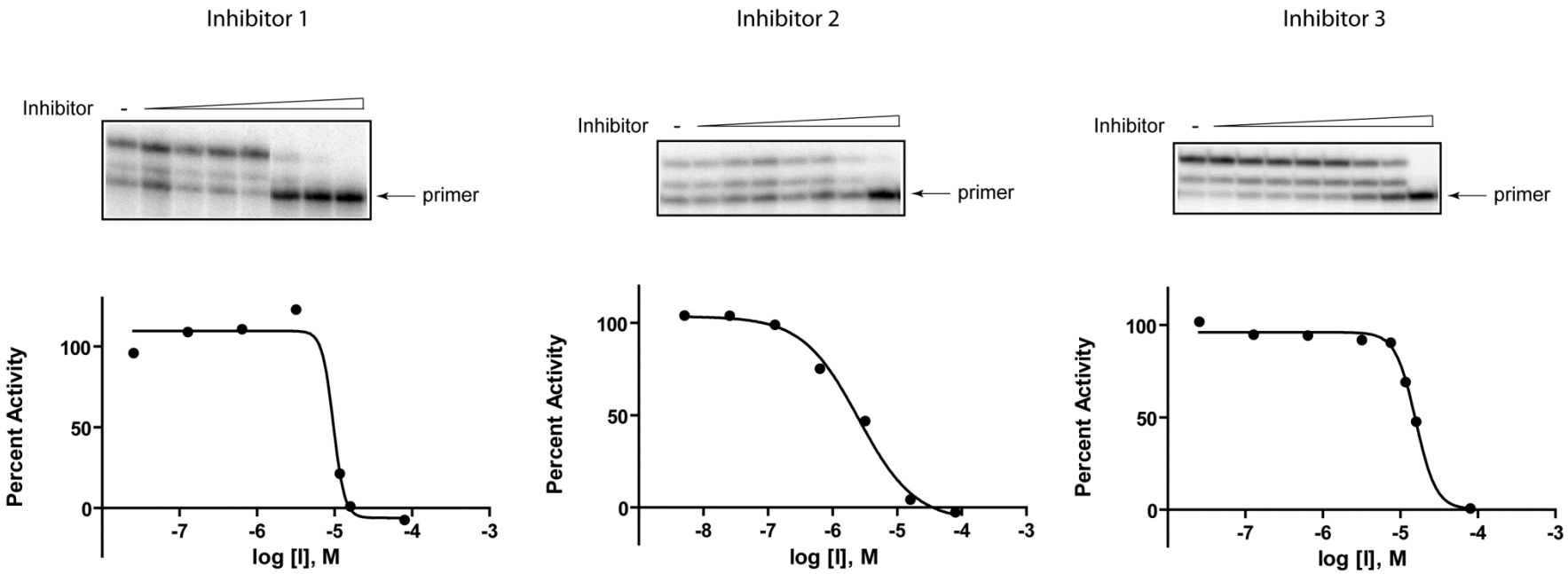
- 3/60 compounds did not inhibit pol κ at highest concentration tested (80 μM)
- 5/60 compounds couldn't be assayed because samples didn't migrate through the gel
- The remaining 52 compounds were ranked in the order of priority 1-



Criteria for compound prioritization

- Does it contain problematic functional group?
- Does it exhibit high quality dose-response curve in HTS?
- Is it a FDA-approved drug?
- Is it optimizable by medicinal chemistry?

Summary of radioactive gel-based assays using non-damaged DNAs



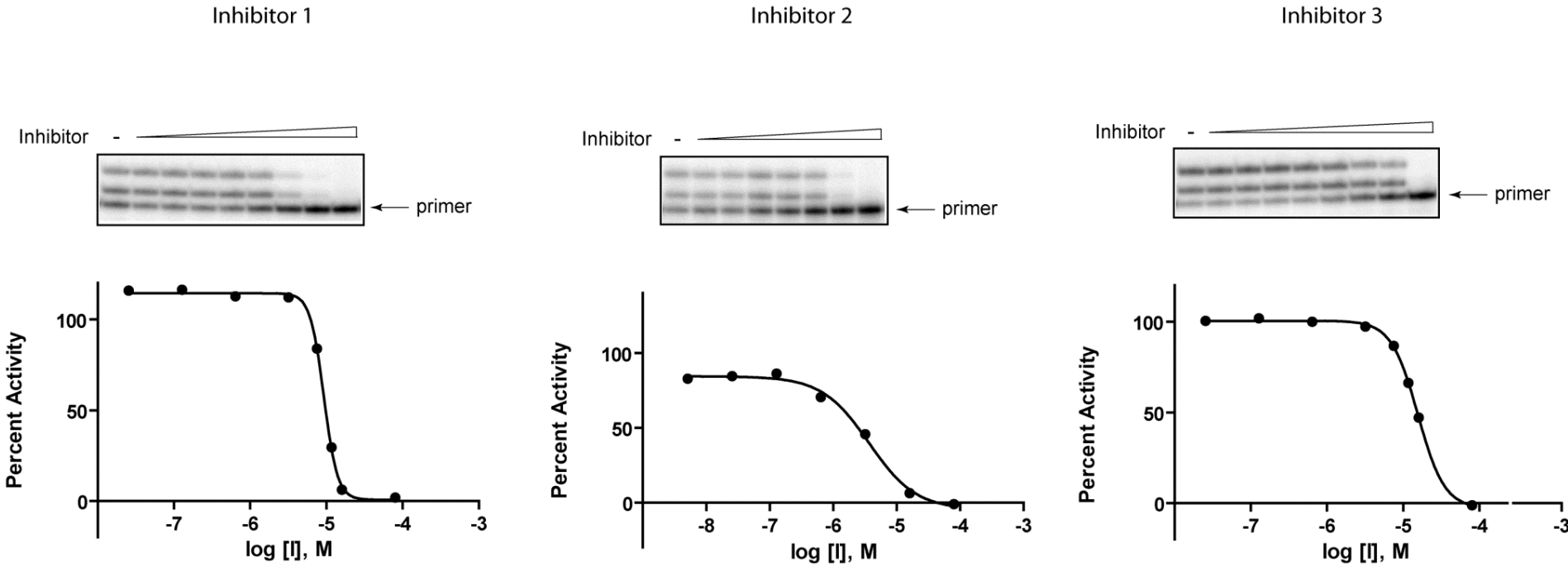
A representative data from three independent experiments are shown on the graph

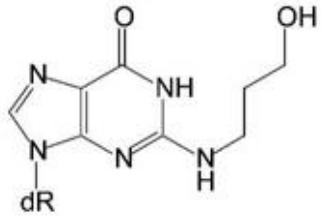
Radiolabel gel-based DNA polymerase assay with high priority hits

sample name	HTS IC_{50} (μM)	gel assay IC_{50} (μM)
hit 1	8.44	3.4 +/-0.9
hit 2	29.93	13 +/-1.7
hit 3	28.18	9.2+/-0.5
hit 4	25.12	20
hit 5	31.62	8.9 +/-1.1
hit 6	5.32	2.7 +/-0.8
hit 7	25.12	18 +/-6.5

IC_{50}, specificity, availability

DNA polymerase assays with lead inhibitors using DNAs containing acrolein-derived ring-opened form of γ-HOPdG

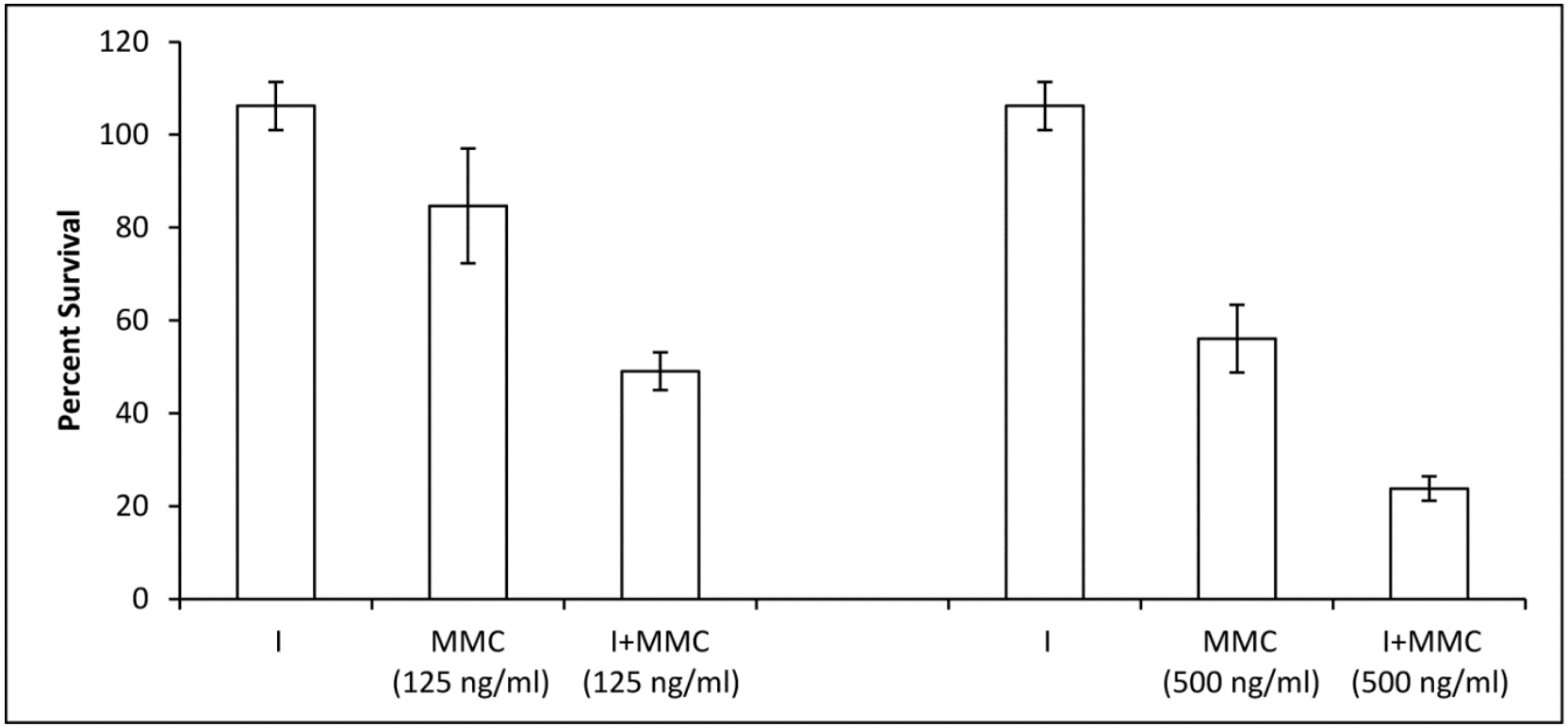




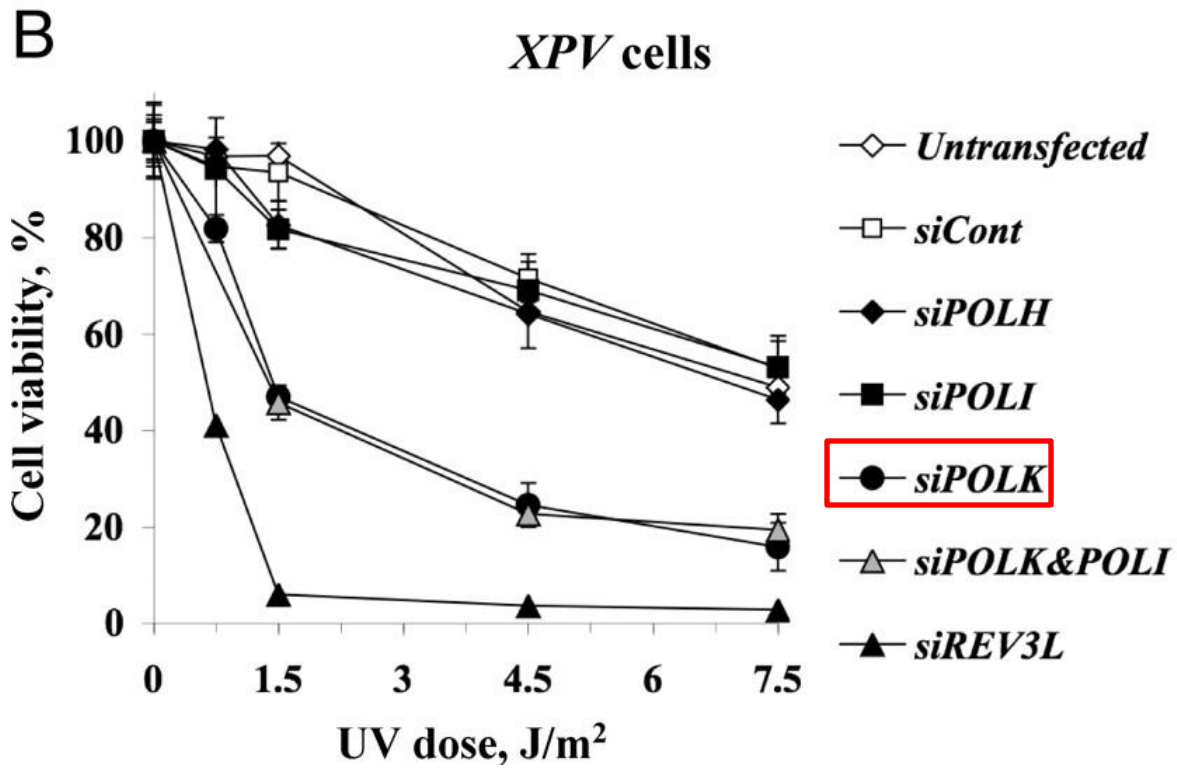
Representative data from three independent experiments are shown

Inhibitor 1 potentiated cytotoxicity of MMC in human fibroblast cells

- Hypothesis: Human fibroblast cells + inhibitor are more sensitive to MMC than human fibroblast cells - inhibitor



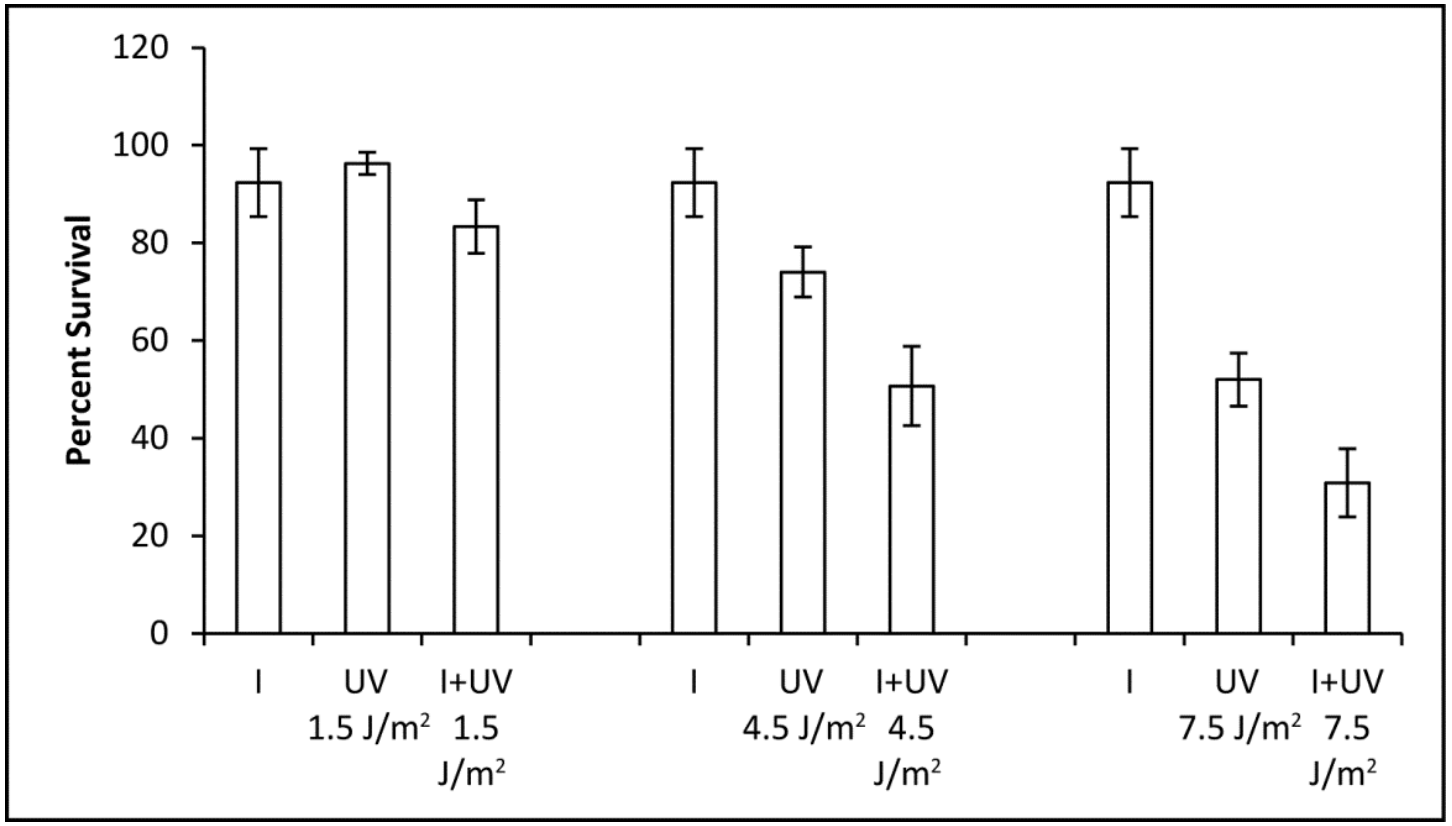
Pol κ-depleted Xeroderma Pigmentosum Variant (XPV) cells are sensitive to UV



Ziv *et al.* (2009) *Proc. Nat. Acad. Sci.*

Inhibitor 1 potentiated cytotoxicity of UV in XPV cells

- Hypothesis: XPV cells + inhibitor are more sensitive to UV than XPV cells - inhibitor



Current status

- In collaboration with NCGC, completed screen of ~400,000 molecules at 5 concentrations; similar screens have been carried out for polymerases η and ι by Dr. Roger Woodgate (NCI) and β by Dr. Samuel Wilson (NIEHS); comparative inhibition analyses
- Co-crystallization trials with lead inhibitors and polymerases κ and Dpo4
- Design of a cell biology-based HTS for polymerase κ

Acknowledgements

Kinrin Yamanaka
Dr. Irina Minko

Collaborators

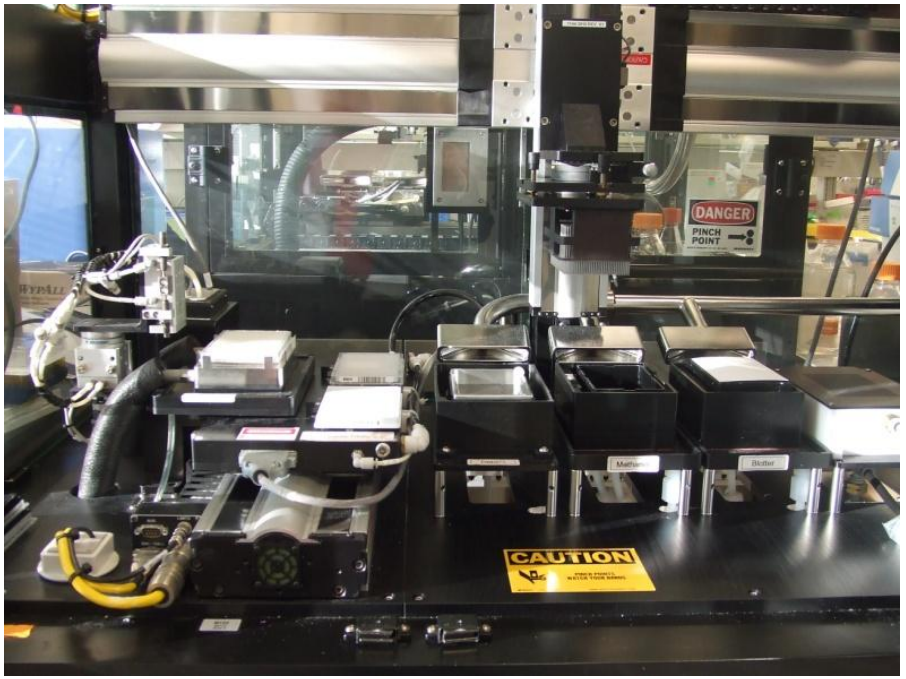
OHSU
Dr. Amanda McCullough
Dr. Robb Moses
Dr. Susan Olson

NIH Chemical Genomics Center
Dr. Anton Simeonov
Dr. George Dorjsuren
Dr. Ajit Jadhav
Dr. David Maloney

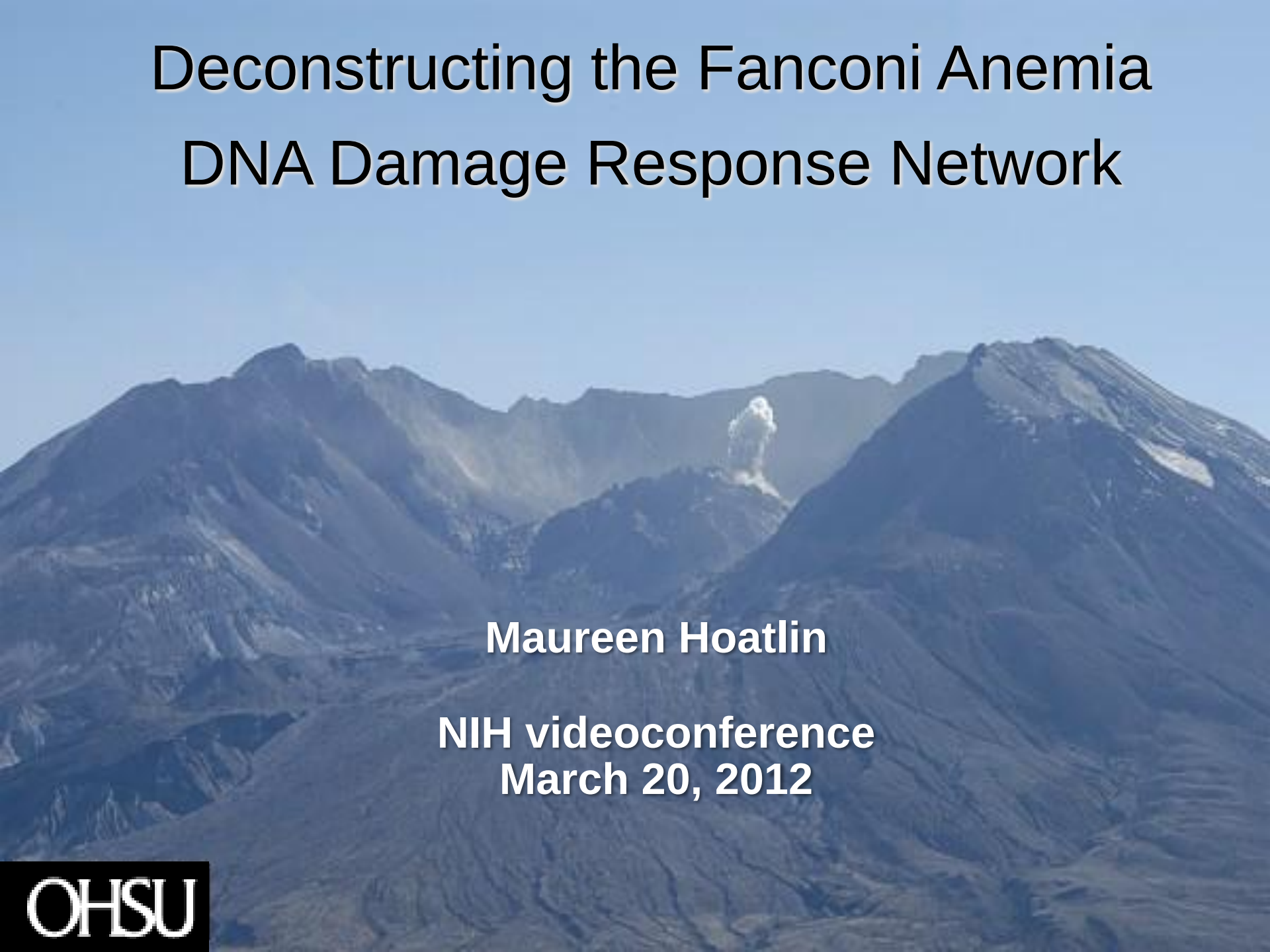
Vanderbilt University
Dr. Carmelo Rizzo – Oligonucleotide chemistry
Dr. Martin Egli - DNA polymerase κ
Dr. Michael Stone – DNA structural analyses

University of Arkansas of Medical Sciences
Dr. Robert Eoff - DNA polymerase κ

University of California San Francisco
Dr. James Cleaver - XPV



Deconstructing the Fanconi Anemia DNA Damage Response Network



Maureen Hoatlin

NIH videoconference
March 20, 2012

OHSU

The Hoatlin Lab
BMB, MMG, PMCB, CANB hoatlinmATohsuDOTedu

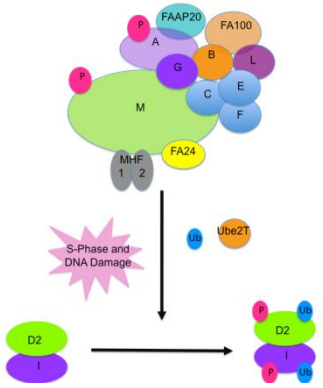
The major focus of the laboratory is to understand the underlying molecular defects in Fanconi anemia in order to improve treatments for FA patients, as well as identify new treatments for certain cancers that occur in the general population

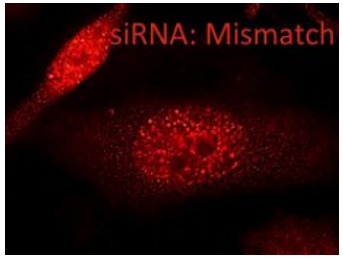
Fanconi anemia

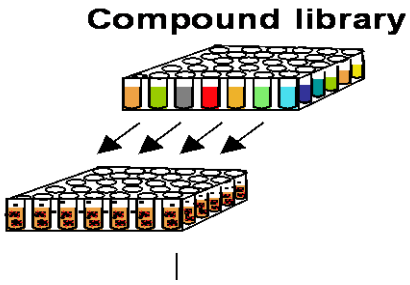
Discovery of new proteins in the FA pathway

Analysis of FA protein function

Assay for Small molecule inhibitors/activators of the FA pathway







Fanconi Anemia

Rare, multi-gene autosomal or X-linked disorder

Clinical features:

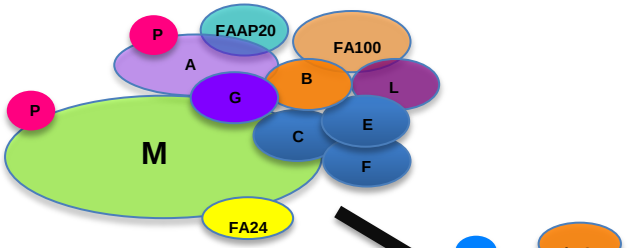
- Developmental defects
- Bone marrow failure (median onset 7 yrs, survival 16 yrs)
- Cancer predisposition (AML, solid tumors)

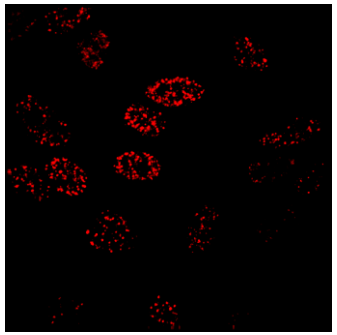
Cellular phenotype:

- Spontaneous cytogenetic abnormalities: chromosome breakage and radials
- Hypersensitive to DNA interstrand crosslinking agents (Mitomycin C, cisplatin)

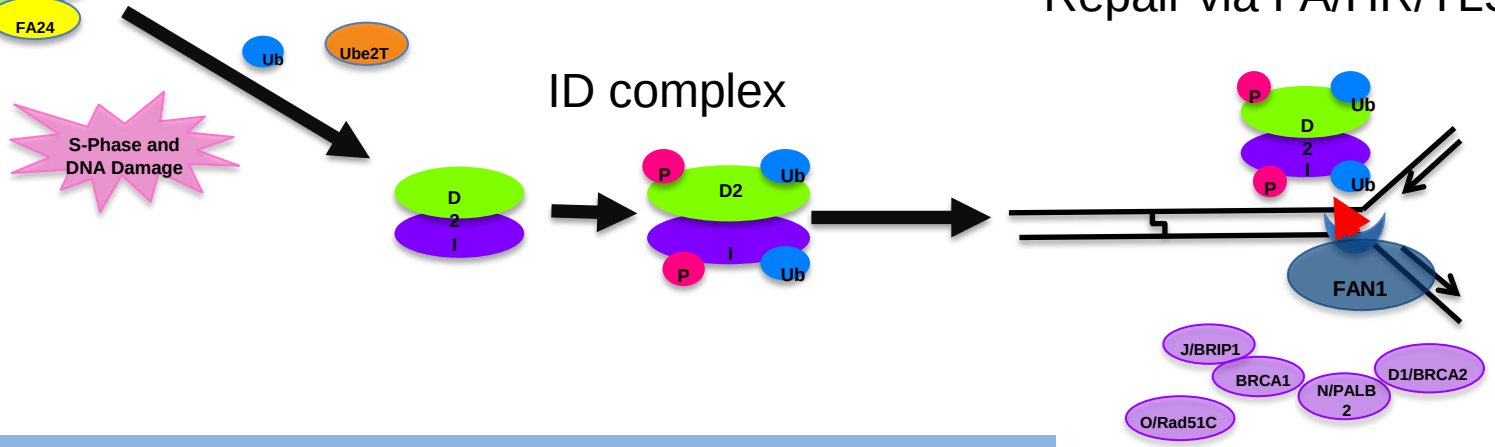
Fanconi Anemia Pathway-2012

FA core complex-E3 Ubiquitin ligase





Repair via FA/HR/TLS



Challenges

- Complexity
- Functional interdependence
- More FA proteins, and FA-associated proteins, to be discovered

Targeting the DNA Damage Response

- May improve therapeutic index
  - Cancer cells often have defective DNA repair pathways
  - Synthetic lethality (PARP inhibitors)

➢ FA is novel, relatively unexplored arm of the DNA damage response

Experimental Strategy

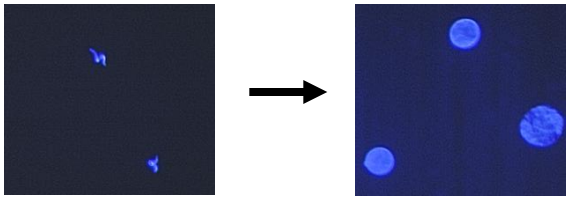
Eggs

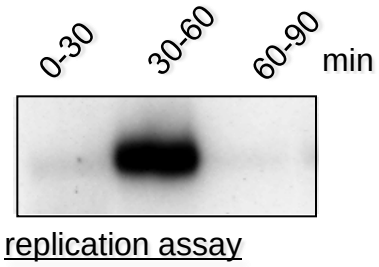
10,000 g

extract

- **Add DNA substrates**
- **Add chemicals**
- Deplete proteins

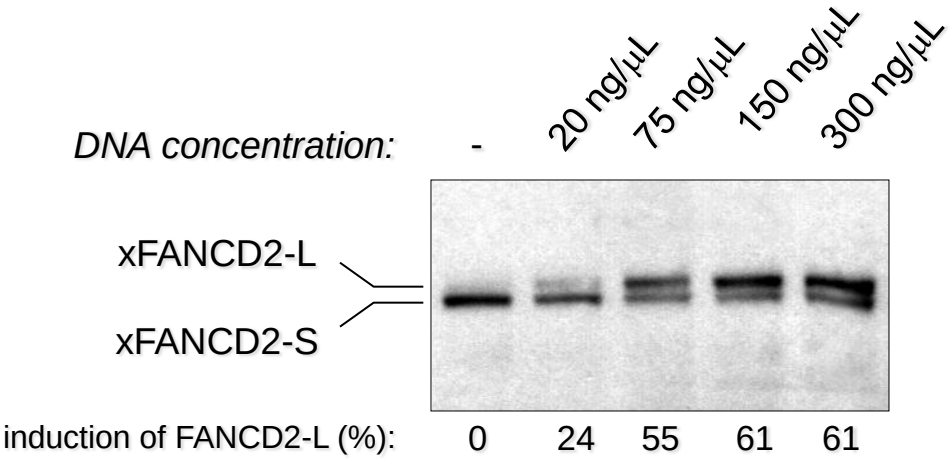
Capable of in vitro replication

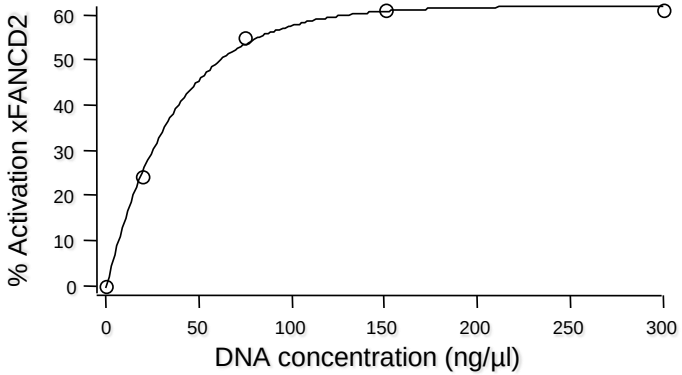




replication assay

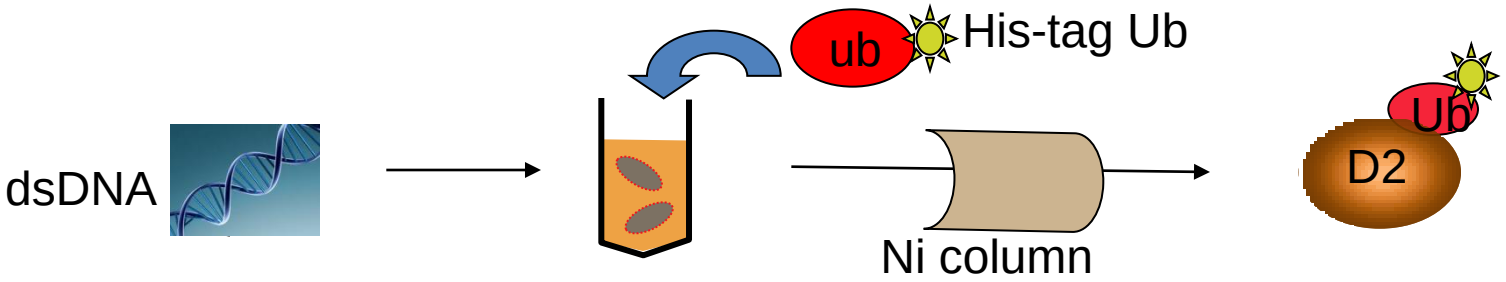
DNA-dependent Stimulation of the FA Pathway

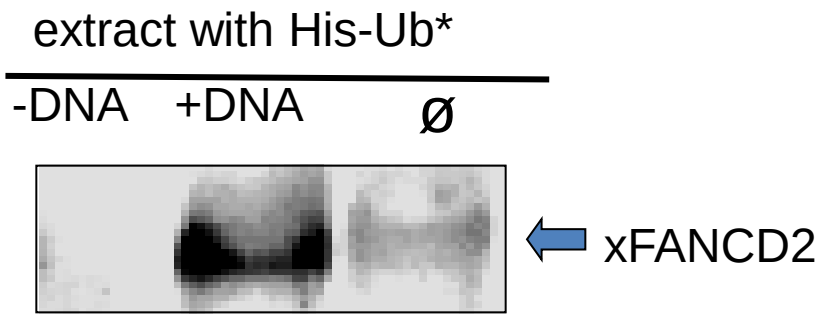




Sobeck et al, MCB 2007; Landais et al, Int J Cancer, 2009

DNA stimulates FANCD2 monoubiquitylation in egg extracts

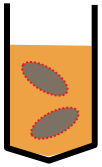




Chemical Library Screening Strategy in Extracts

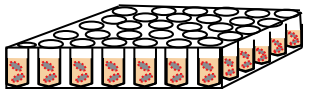
DNA substrate

+ candidate drug



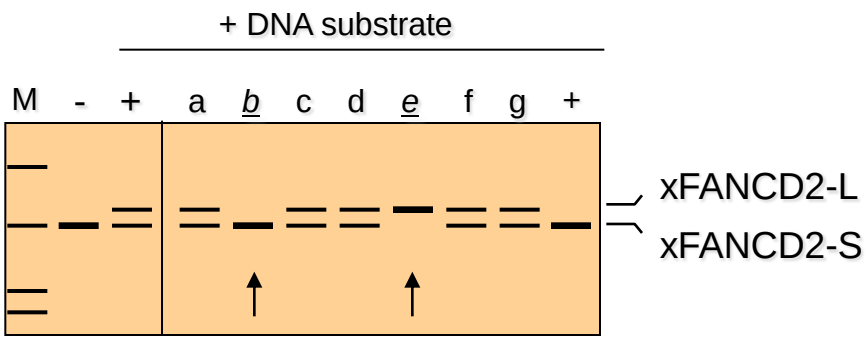
Cell-free Extract

Academic throughput screen



Compound library

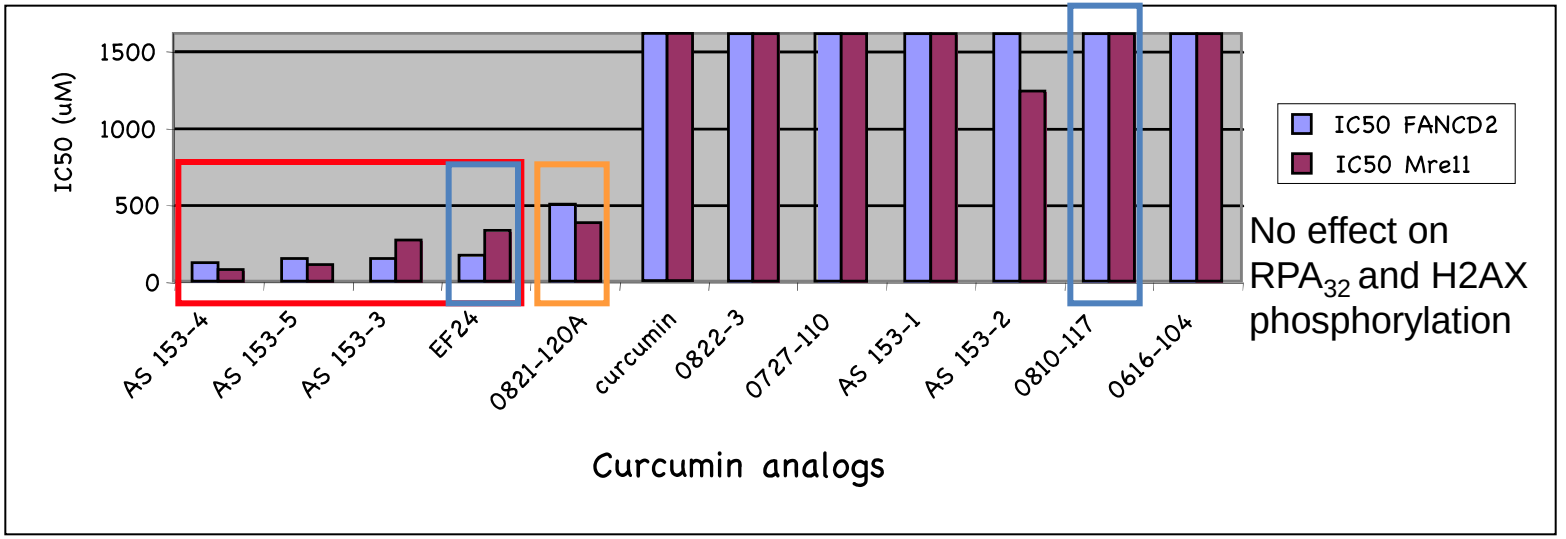
Immunoblot Analysis

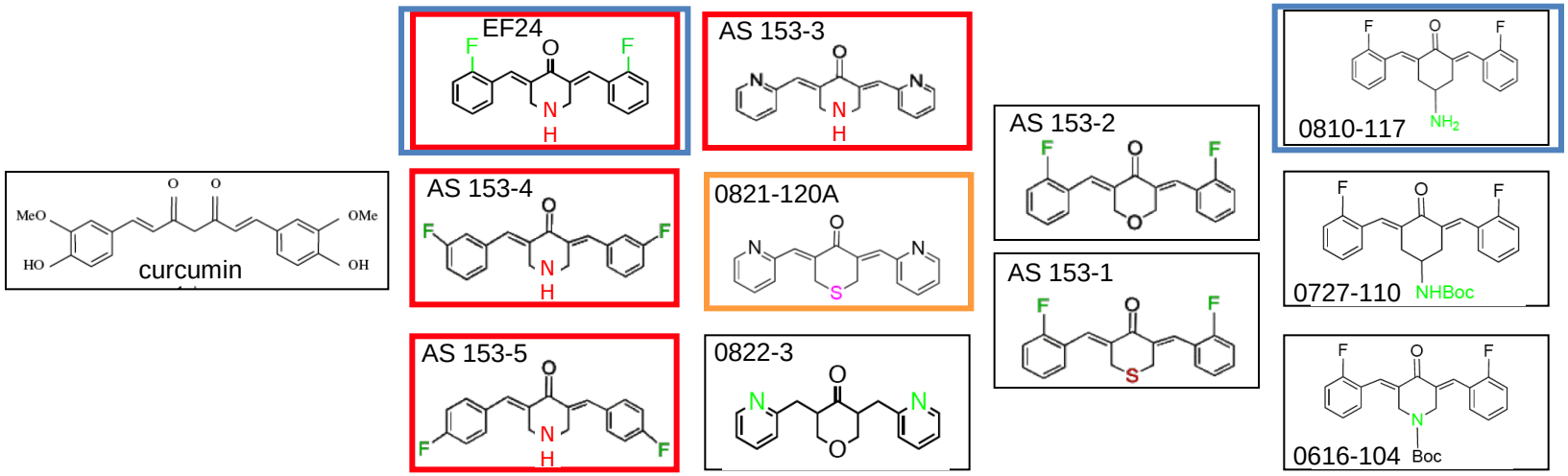


Assay via FANCD2 activation (L), or non activation (S only)

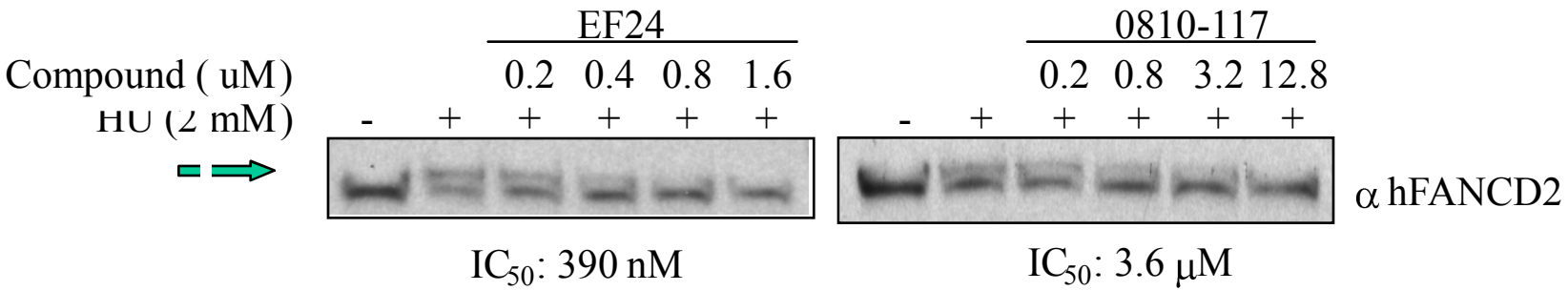
Outcome:
Inhibition of FANCD2-Ub?

Inhibition of FANCD2-Ub in Extracts by Curcumin Analogs





Curcumin analogs inhibit HU-induced FANCD2 monoubiquitylation in Hela cells



Prediction: inhibiting the FA pathway in cells that have defects in genes That are synthetic lethal with FA will be selectively toxic

Concept of Synthetic Lethality

Mutation	Phenotype
Gene A	Alive
Gene B	Alive
Gene A Gene B	Dead

A and B act redundantly or in a parallel pathway

Synthetic lethality occurs when two otherwise nonlethal mutations together result in an inviable cell

Synthetic Lethality and Fanconi Anemia

Table 1
The 10 siRNA oligonucleotide targets that are most selectively toxic to FA pathway–deficient EUFA326 cells compared with the corrected EUFA326G cell line

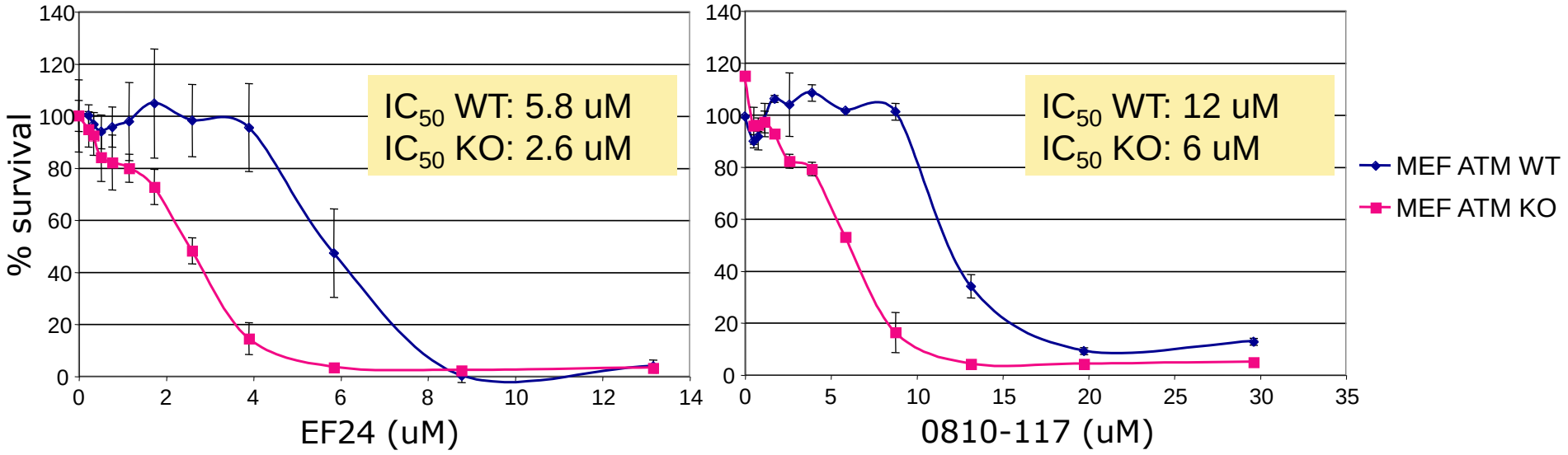
Rank	Gene target	Function	EUFA326/ EUFA326G viability
1	*TREX2*	DNA exonuclease	0.59 ± 0.09
2	*ADPRT (PARP1)*	BER	0.64 ± 0.10
3	*PLK1*	Cell cycle progression	0.68 ± 0.18
4	*UBE2B (RAD6)*	Switching of DNA polymerases	0.70 ± 0.14
5	*CDK7*	Transcription	0.73 ± 0.11
6	*TP53BP1*	Sensor of DSBs; activates ATM	0.73 ± 0.10
7	*ATM*	DSB response kinase	0.73 ± 0.05
8	*NEIL1*	BER	0.74 ± 0.09
9	*RAD54B*	HR	0.75 ± 0.08
10	*NBS1*	Sensor of DSBs; activates ATM	0.76 ± 0.20
Control	GFP/LacZ	Control	0.97 ± 0.06

Kennedy et al, J. Clin. Invest. 2007

Example: ATM deficiency (mutations, deletions, lower expression) in hematological malignancies

Malignancy	% of patients	Source
Mantle cell lymphoma	43%	Fang et al, PNAS 2003
Other lymphomas	10%	Fang et al, PNAS 2003
Chronic lymphocytic leukemia	34%	Starostik et al, Cancer Res. 2003
Acute lymphoblastic leukemia	28%	Haidar et al, Cancer 2003

Differential toxicity of curcumin analogs in isogenic ATM-proficient and ATM- (KO) MEF cells



- EF24 and 0810-117 are twofold more toxic in ATM KO than in WT cells, consistent with a synthetic lethal effect

- EF24 is active at twofold lower concentrations than 0810-117

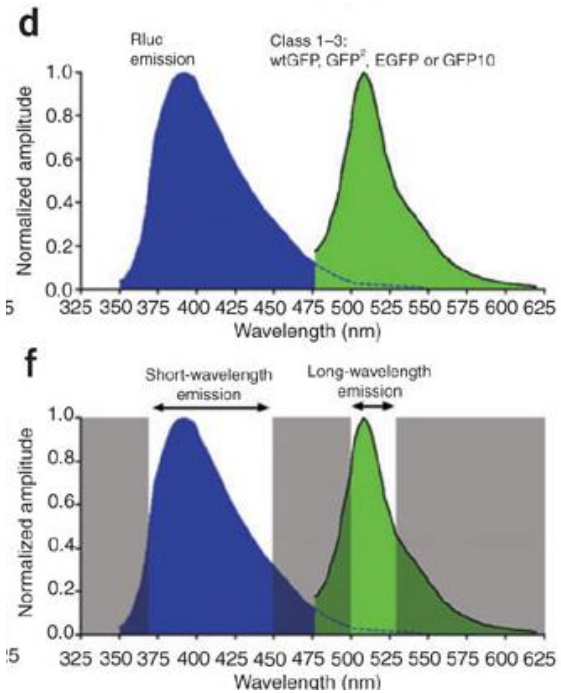
Converting Ubiquitination Assay to High Throughput Screen

Bioluminescence Resonance Energy Transfer: BRET^2

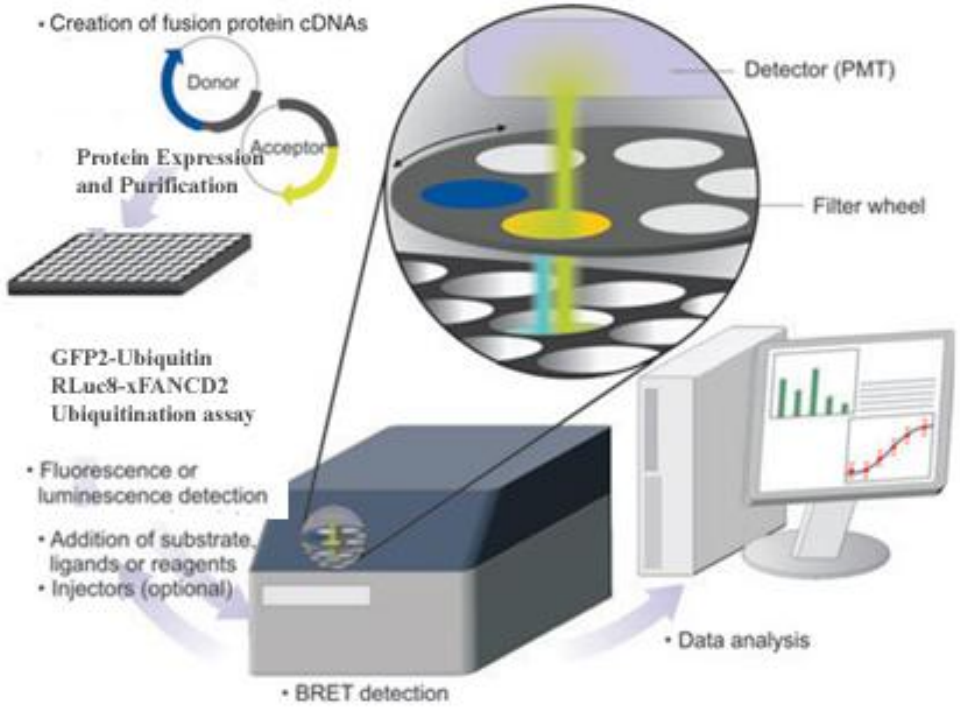
Donor: RLuc8-xFANCD2
Acceptor: GFP2-Ubiquitin

Spectrum Separation

Filter Setup



Experimental Setup



Figures adapted from Pleger & Eidne, Nature Methods 3, 165-174 (2006)

Acknowledgements

Current Lab Members

Chelsea Jenkins
Michael Wallisch
Jenny Kan
Eric Brown
Poulami Mitra

Past Lab Members

Igor Landais (VGTI)
Alex Sobeck (U of Minn)
Matt McCarroll (OHSU)
Hannah Cho
Stacie Stone (Novartis)
Alexis LaChapelle
Mac Schober (Georgetown)
Victor Wong (Yale)

Collaborators

Wiedong Wang- NIA/NIH
Hans Joenje- Free University and Medical Center, Amsterdam
Jan Christian- University of Utah
Jean Gautier- Columbia University
Karlene Cimprich- Stanford
Jim Synder- Emory

National Cancer Institute
at the National Institutes of Health

OTRADI

Fanconi Anemia
RESEARCH FUND, INC.

OREGON HEALTH & SCIENCE UNIVERSITY

OHSU Rare Disorders Research Consortium (RDRC)

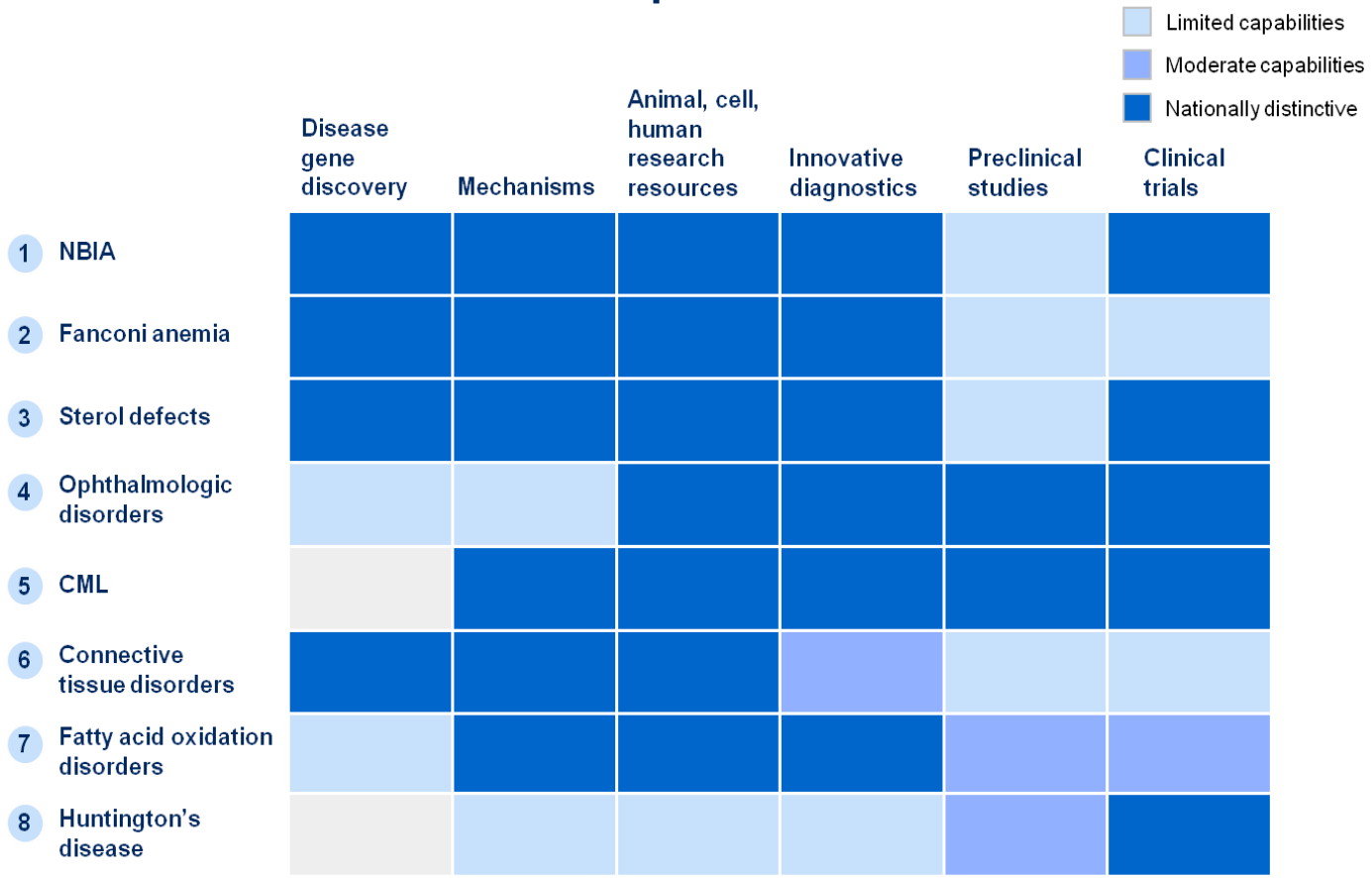
Rare Disease Research Consortium

- The OHSU Rare Disorders Research Consortium (RDRC) creates a local hub for rare disorders research by promoting interaction and sharing of expertise among the diverse investigators on campus.
- Supported by the OHSU Human Genetics Initiative and the Oregon Clinical & Translational Research Institute
- Approximately 150 staff and faculty involved with the consortium or rare disorders with over $35 million in research funding
- Ability to span the entire translational pipeline
- Unique resources: Animal models, international patient referrals, cell lines, databases, specialized techniques
- Chairs:
  - **Susan Hayflick, MD,** Molecular & Medical Genetics
  - **Maureen Hoatlin, PhD, MBA,** Biochemistry and Molecular Biology
- Abhijit Banerjee, Ph.D., M.B.A. Director of Business Development, OHSU banerjeaATohsuDOTedu

Rare Disorders Research Strengths

- OHSU researchers study more than 90 rare disorders

1 A disease-level look at OHSU's capabilities in rare disease research

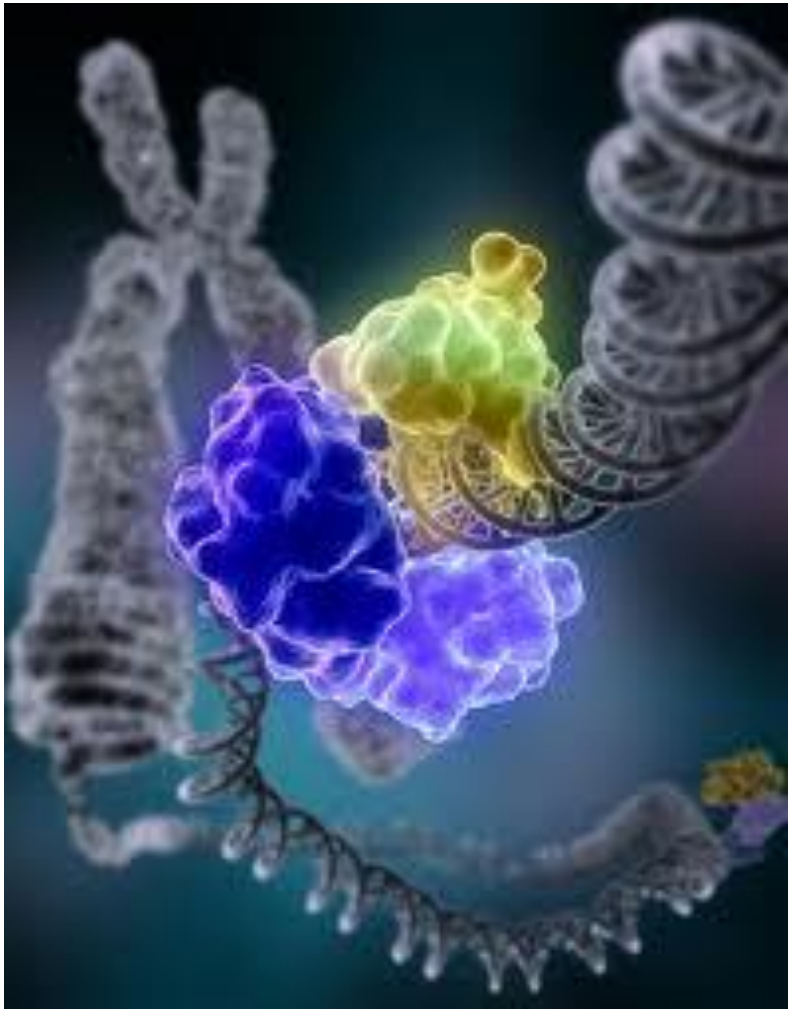


- OHSU has significant capabilities in at least 8 rare disease areas, with pockets of strength in several others
- A similar effort will need to be taken for CNS and additional areas of focus

OHSU Research Relevant to Rare Cancer Susceptibility and DNA Repair Disorders

Markus Grompe
Grover Bagby
Maureen Hoatlin
Kim-Hien Dao
Peter Kurre
Carolyn Sue Richards
Susan Olson
Amanda McCullough
R. Stephen Lloyd
Mitch Turker
Mike Liskay
Matt Thayer

…and more



RDRC Website

http://www.ohsu.edu/xd/research/clinical-research/hgi/consortium/

OREGON HEALTH & SCIENCE UNIVERSITY