The Response of R249 p53 Mutants to NSC319726 Treatment

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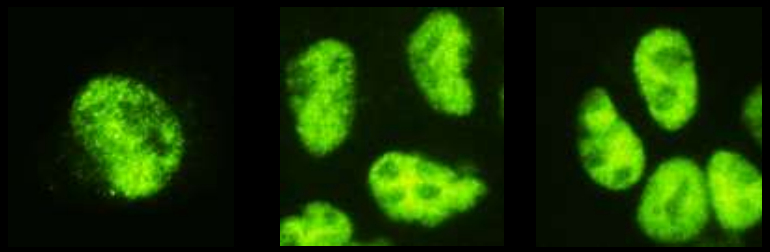
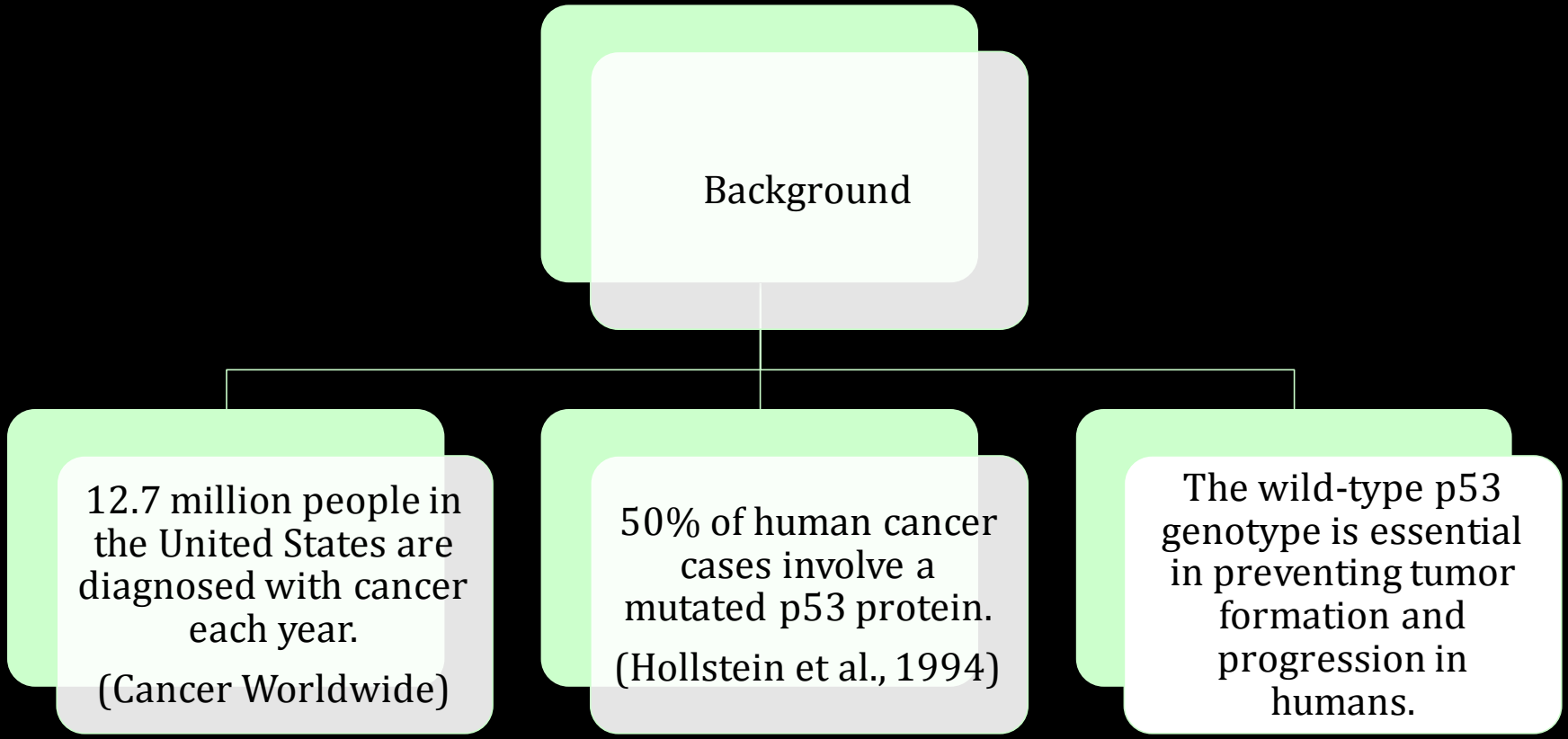


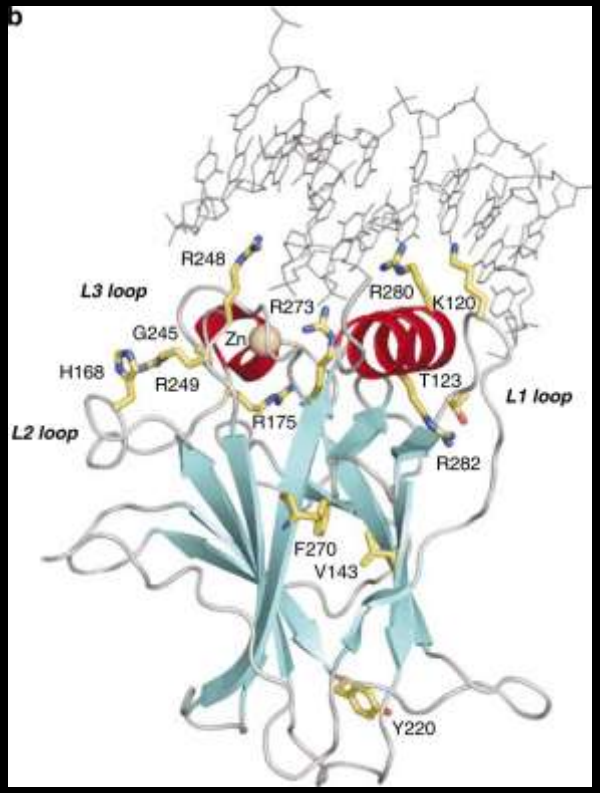
p53 and its Role in Cancer Progression



p53 in its Wild-Type State

Gain-of-function Theory

(Dittmer et al., 1993)



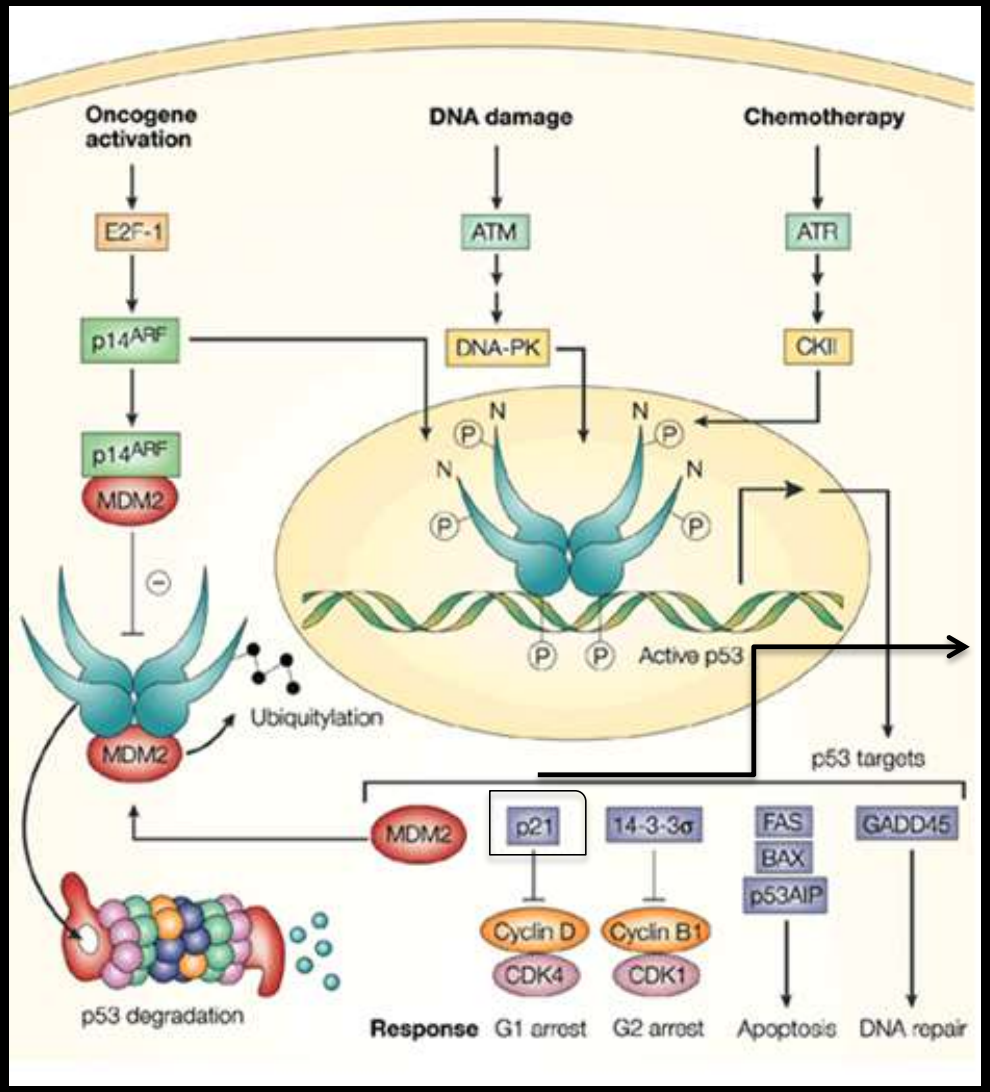
(Joerger and Fersht., 2007)

393 Amino Acids

(Baker et al., 1989)

The Role of Wild-Type p53 in the Cell

"Cellular Gatekeeper" and "Guardian of the Genome" (Lane, 1992)

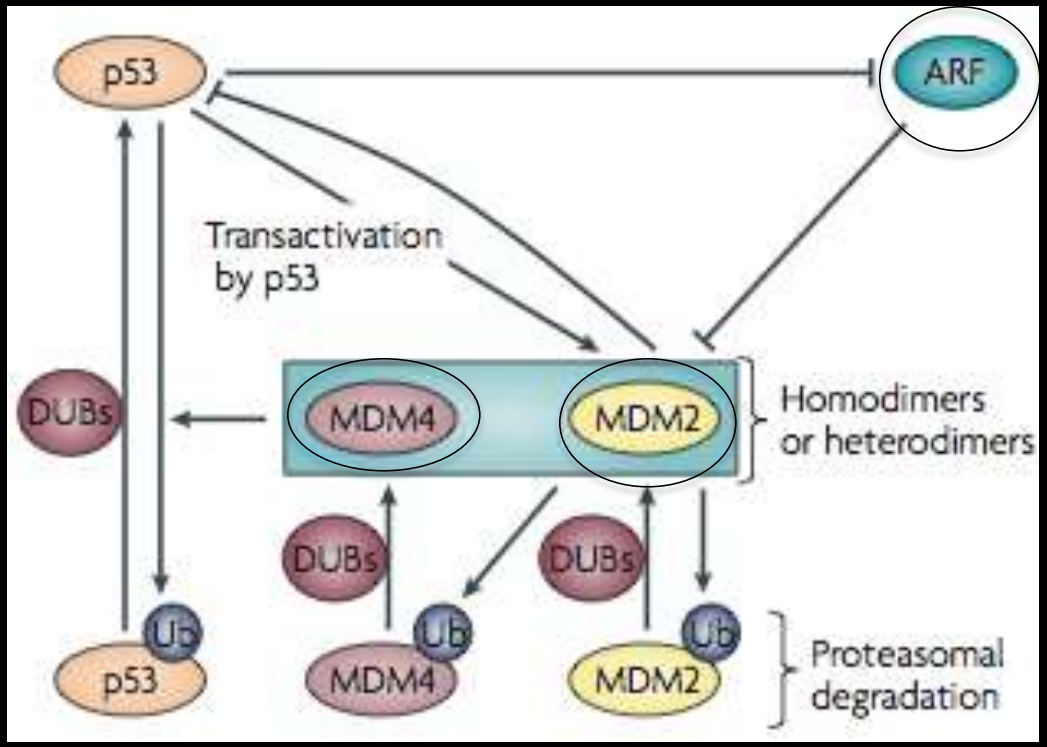


Transcription factor

(Bullock and Fersht., 2001)

The Tight Regulation of p53 in the Cell

E3 Ubiquitin Ligase
Negative Regulators



Positive
Regulator

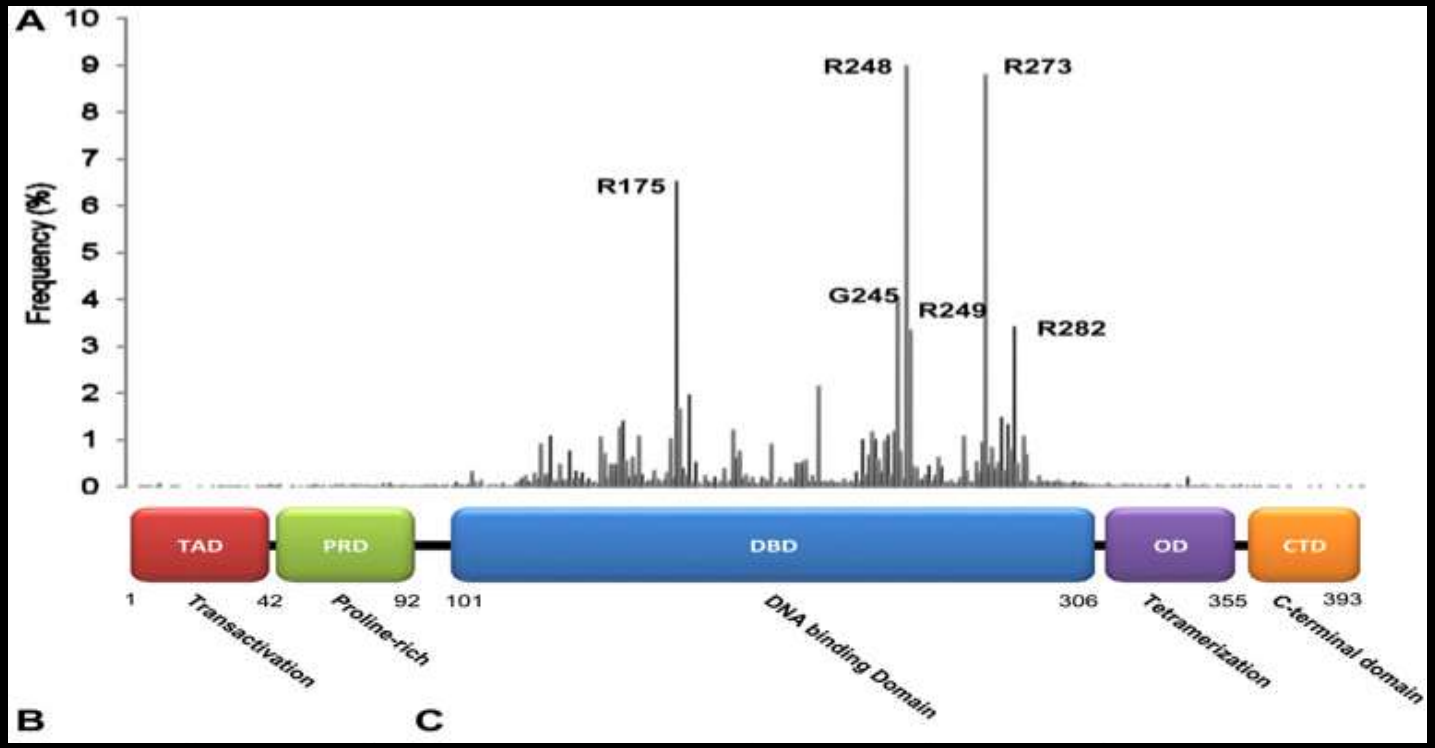
(Brown, 2009)

The Mutated Guardian

1/3 p53 mutants are missense mutants

DNA Contact Conformational

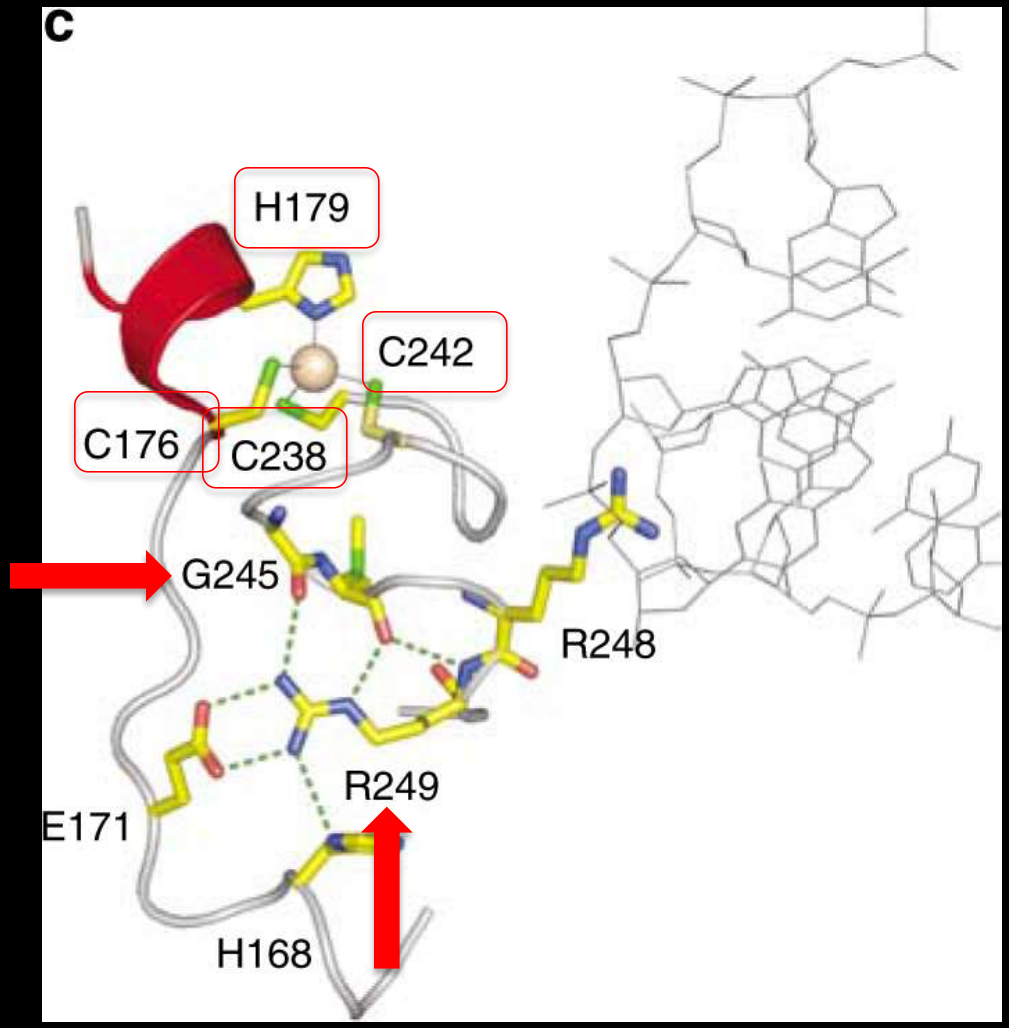
(Cho et al., 1994)



(Freed-Pastor and Prives, 2009)

Drug-Related p53-Targeted Therapy

Personalized Medicine



(Joerger and Fersht., 2007)

NSC319726
Thiosemicarbazone
Zinc
Metallochaperone
(Yu et al., 2012)

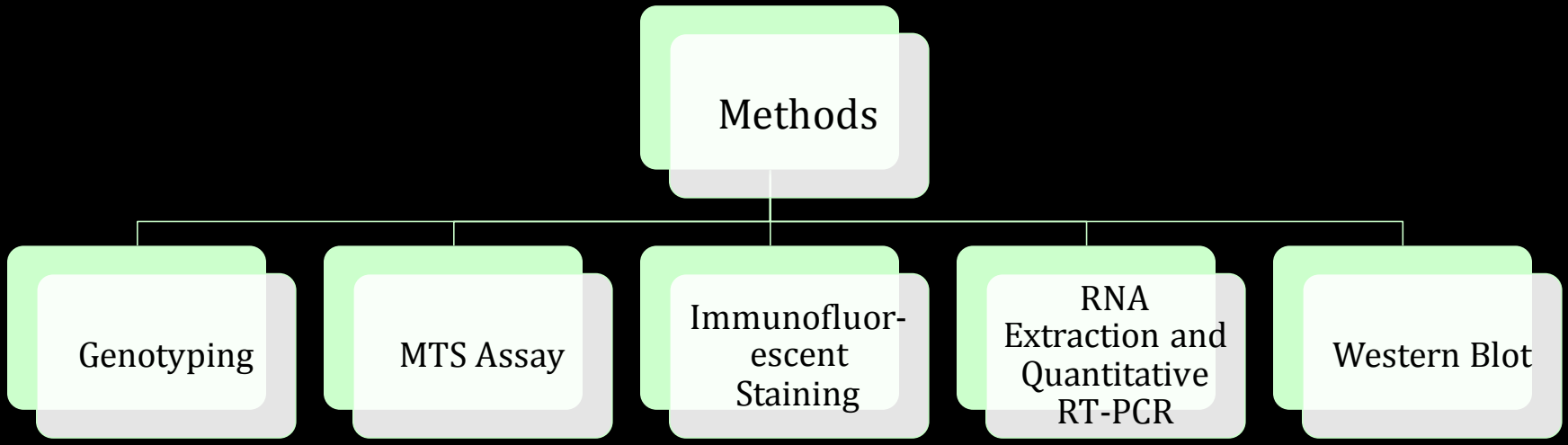
Research Questions and Hypotheses

- Research Question: Is the R249 codon of p53 involved in zinc binding? If so, will it experience restored wild-type structure and function post NSC319726 drug treatment?

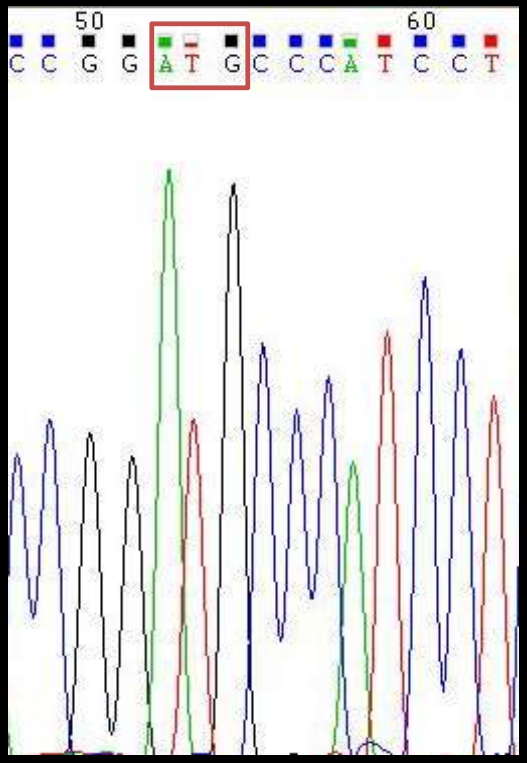
- H_1: Wild-type p53 function will not be reactivated in p53 R249 mutants upon exposure to NSC319726.

- H_0: Wild-type p53 function will be reactivated in p53 R249 mutants upon exposure to NSC319726.

Methods

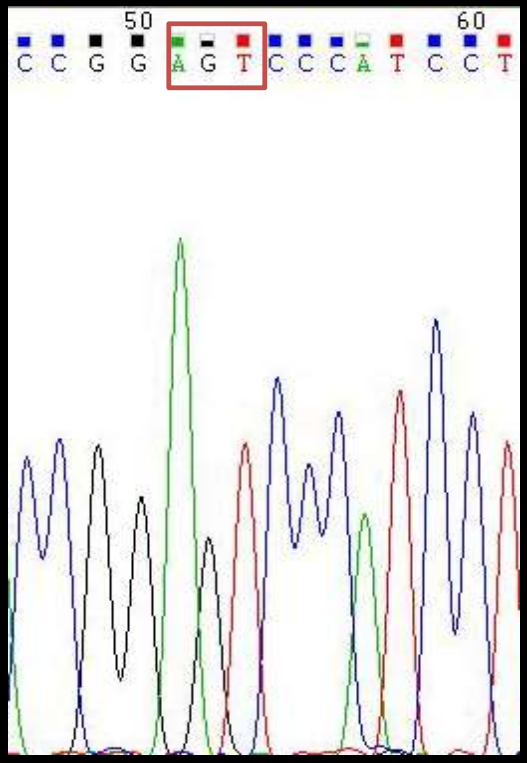

Methods
Genotyping
MTS Assay
Immunofluor-escent Staining
RNA Extraction and Quantitative RT-PCR
Western Blot

Genotyping Confirms the p53 Status of the Cell Lines



Hs700T Exon 7

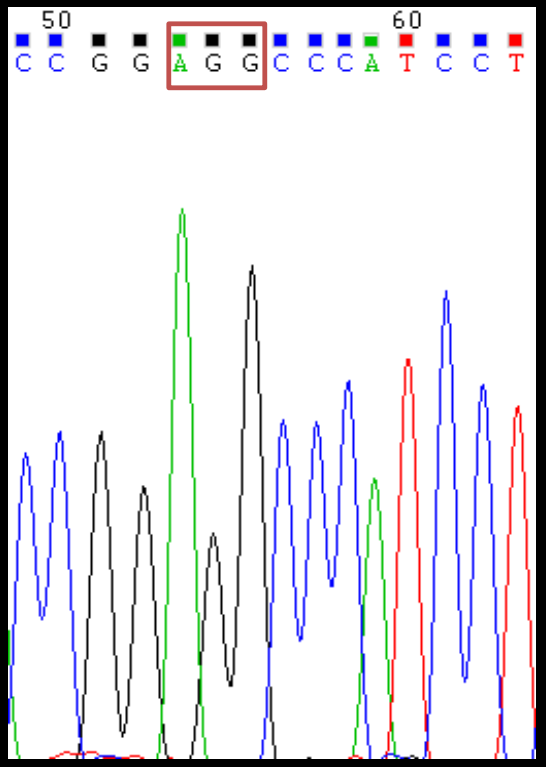
Amino Acid 249 Genotype ATG Methionine **R249M mutant**



H460 Exon 7

Wild Type p53

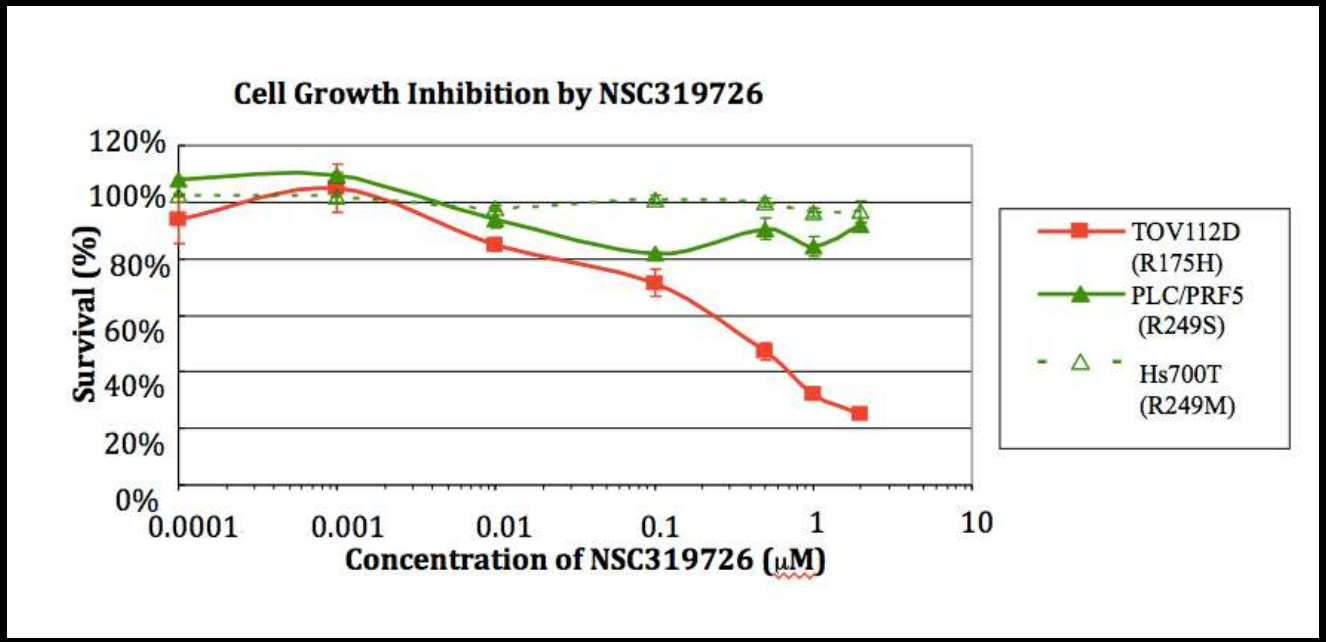
Amino Acid 249 Genotype AGG Arginine



PLC/PRF5 Exon 7

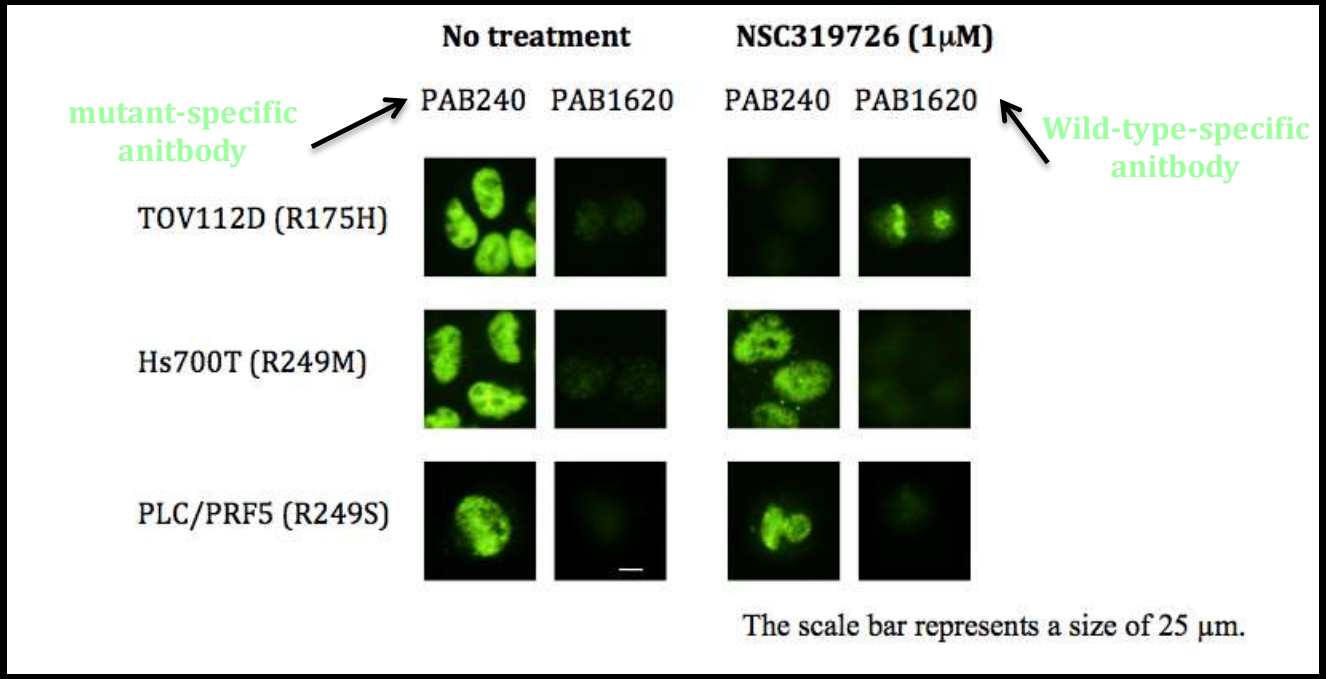
Amino Acid 249 Genotype AGT Serine **R249S mutant**

NSC319726 does not Inhibit Cell Growth in R249 Mutants



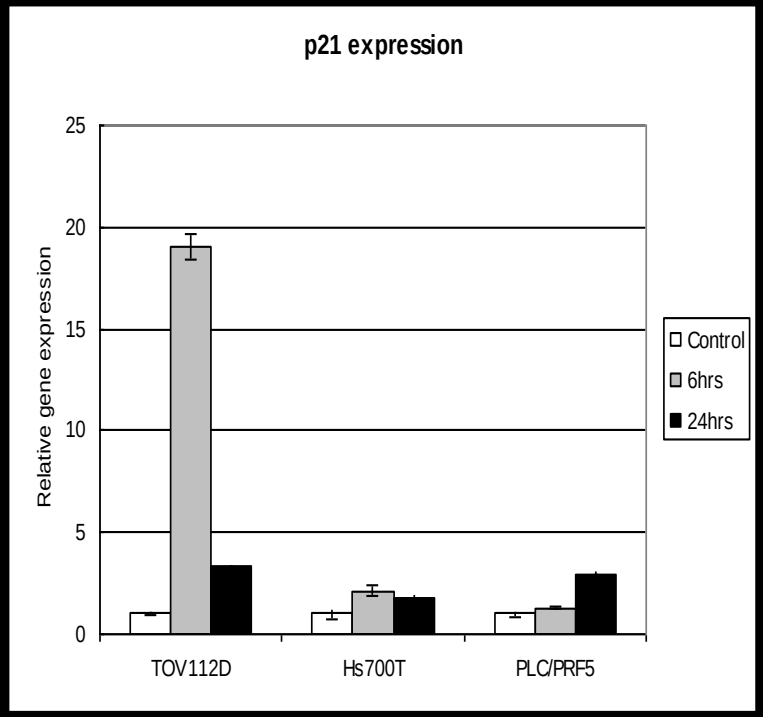
- Evident growth inhibition for TOV112D cell lines
  - IC_{50}= 0.4 μl (Yu, 2012)
- PLC/PRF5 and Hs700T survival % remained stable despite slight fluctuation

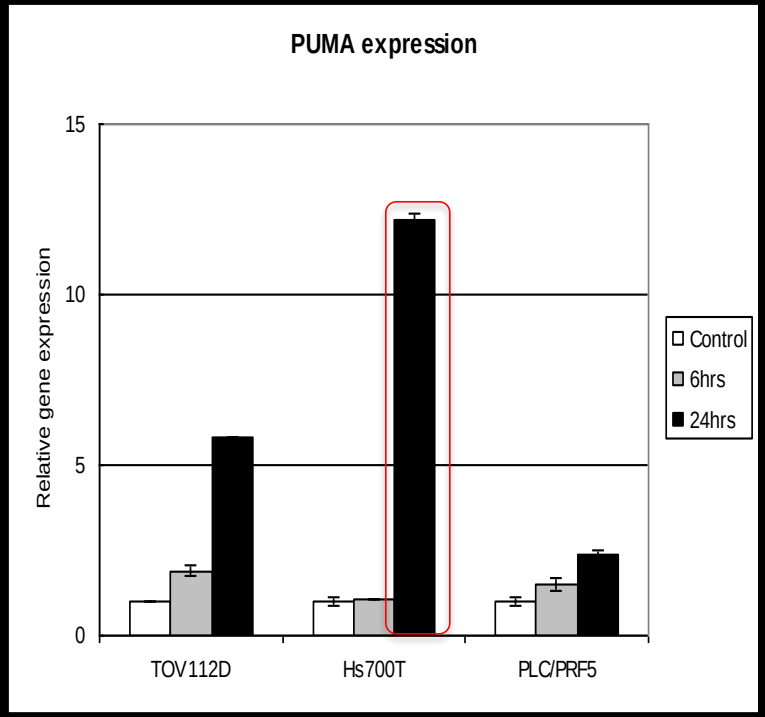
NSC319726 Restores Wild-Type Conformation in R175H but not in R249 Mutant Proteins



- R175H, R249M, R249S conformational mutants
- Only TOV112D wild-type p53 conformation restored by NSC319726 drug treatment

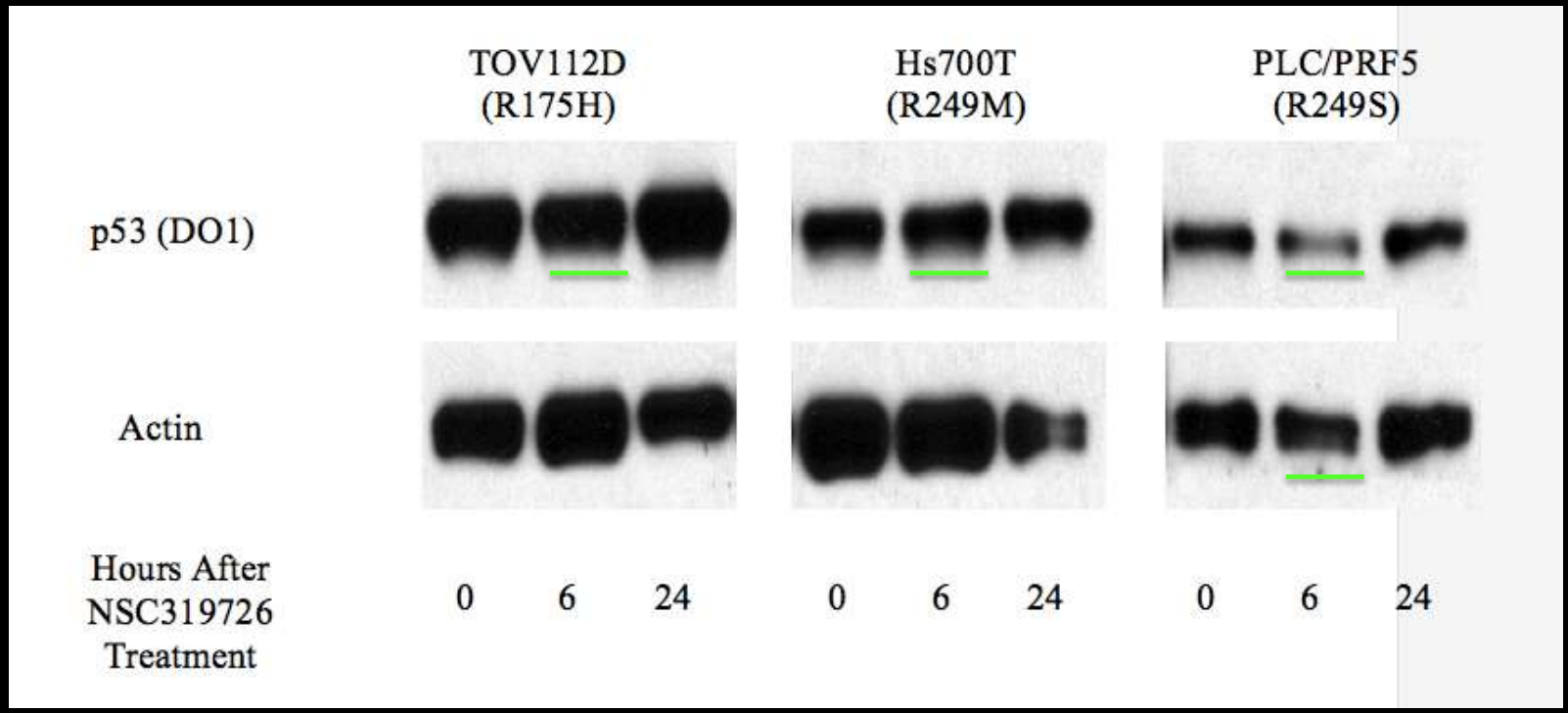
NSC319726 does not Restore p53 Transcriptional Function in R249 p53 Mutants





- Up-regulation of PUMA in Hs700T NSC319726-dependent but p53-independent

NSC319726 does not Change p53 Protein Levels (Stability) in R249 Mutants



- H_1 confirmed

Discussion/ Conclusion

- It was confirmed that wild-type p53 structure and function is rescued in TOV112D cell line after NSC319726 treatment.

- Unlike other hotspot mutants (C176F, C242S, G245S), R249 p53 mutants do not receive restored wild-type p53 structure and function post NSC319726 drug treatment.

Acknowledgements

I would like to thank my mentors, Dr. Darren Carpizo and Dr. Xin Yu at the Cancer Institute of New Jersey for their time and dedication and Mr. Michael Inglis and Mrs. Melissa Carnahan for their endless wisdom and support.

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Major Findings

H_1 was confirmed. Thus, it was concluded that wild-type p53 structure and function is rescued in TOV112D cell line after NSC319726 treatment.