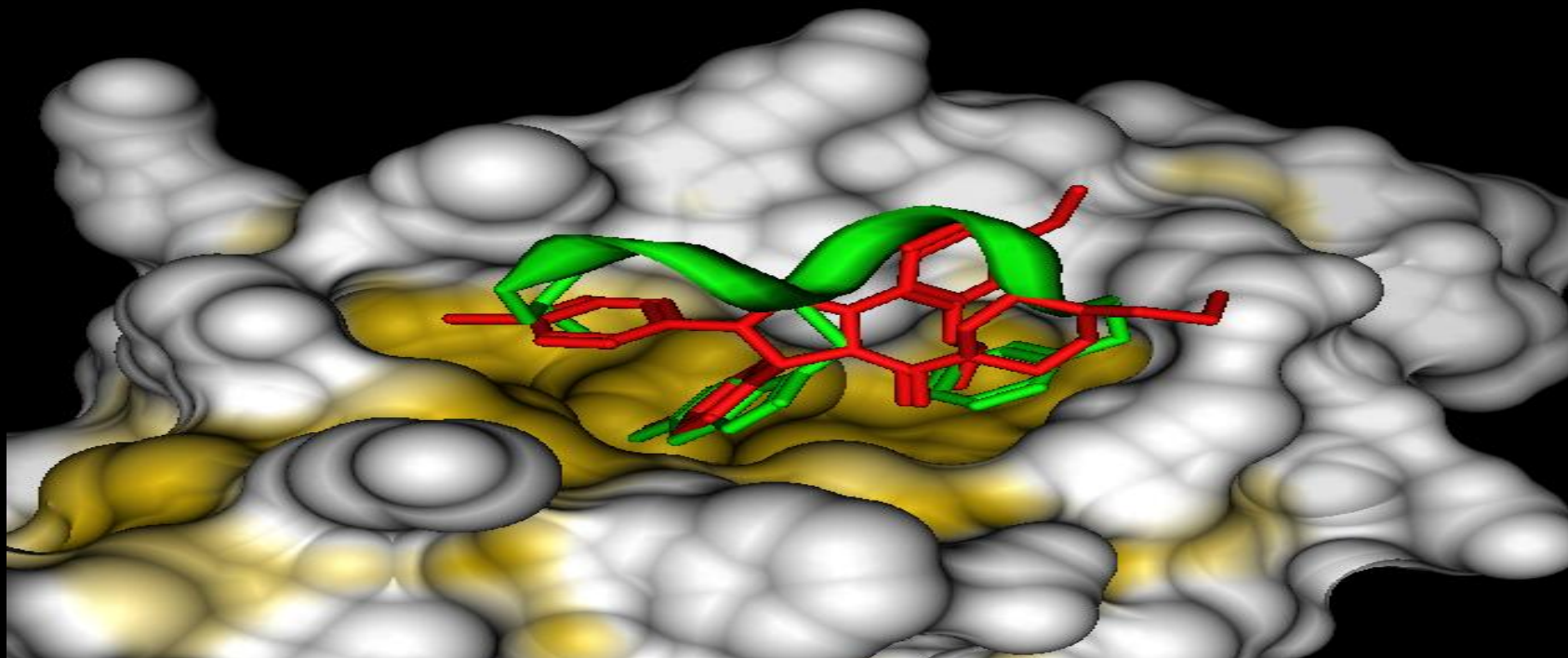


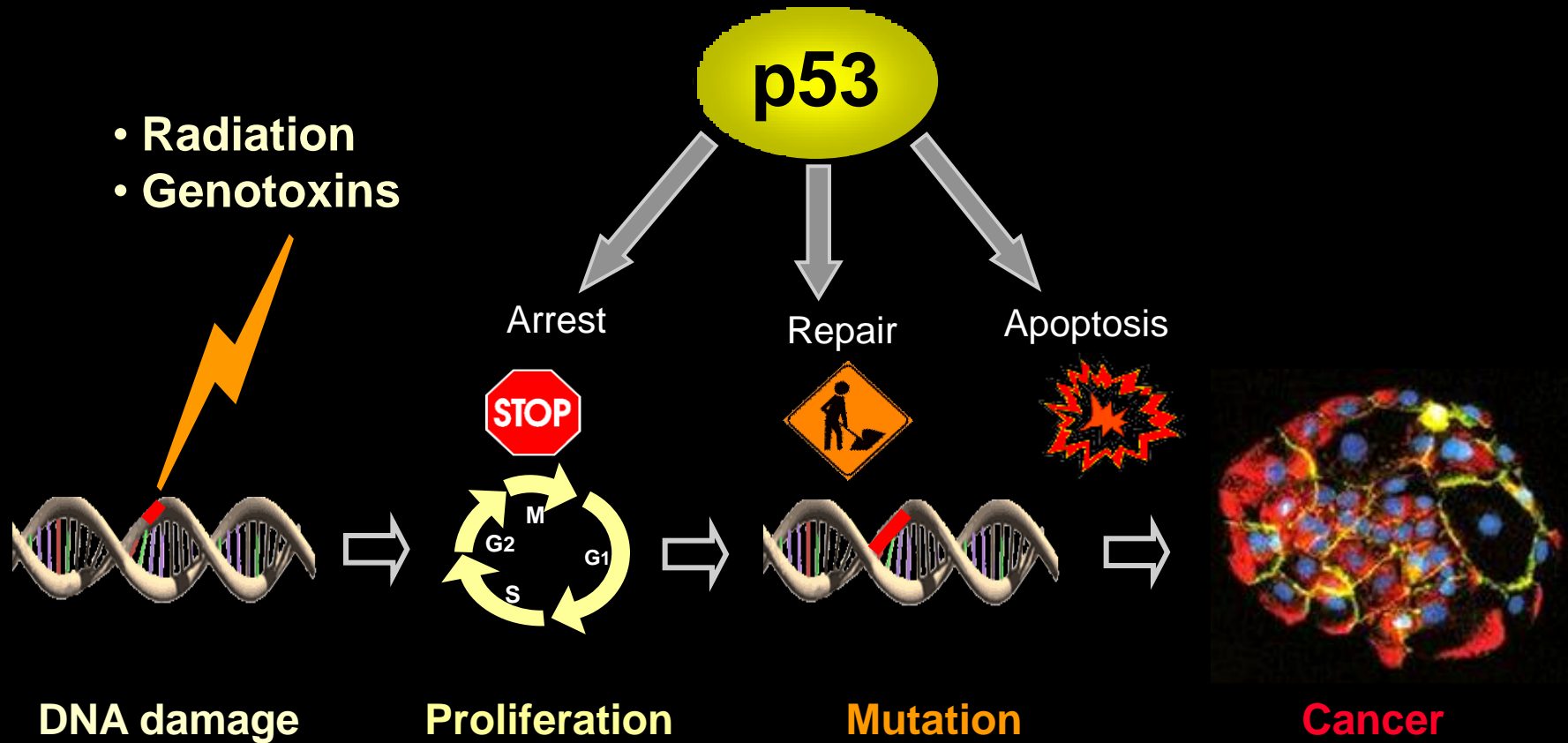
Patient Selection Strategies for the Development of MDM2 Inhibitors



Robert Wasserman
May 17, 2010



The p53 tumor suppressor



p53: the most frequently altered protein in human cancer



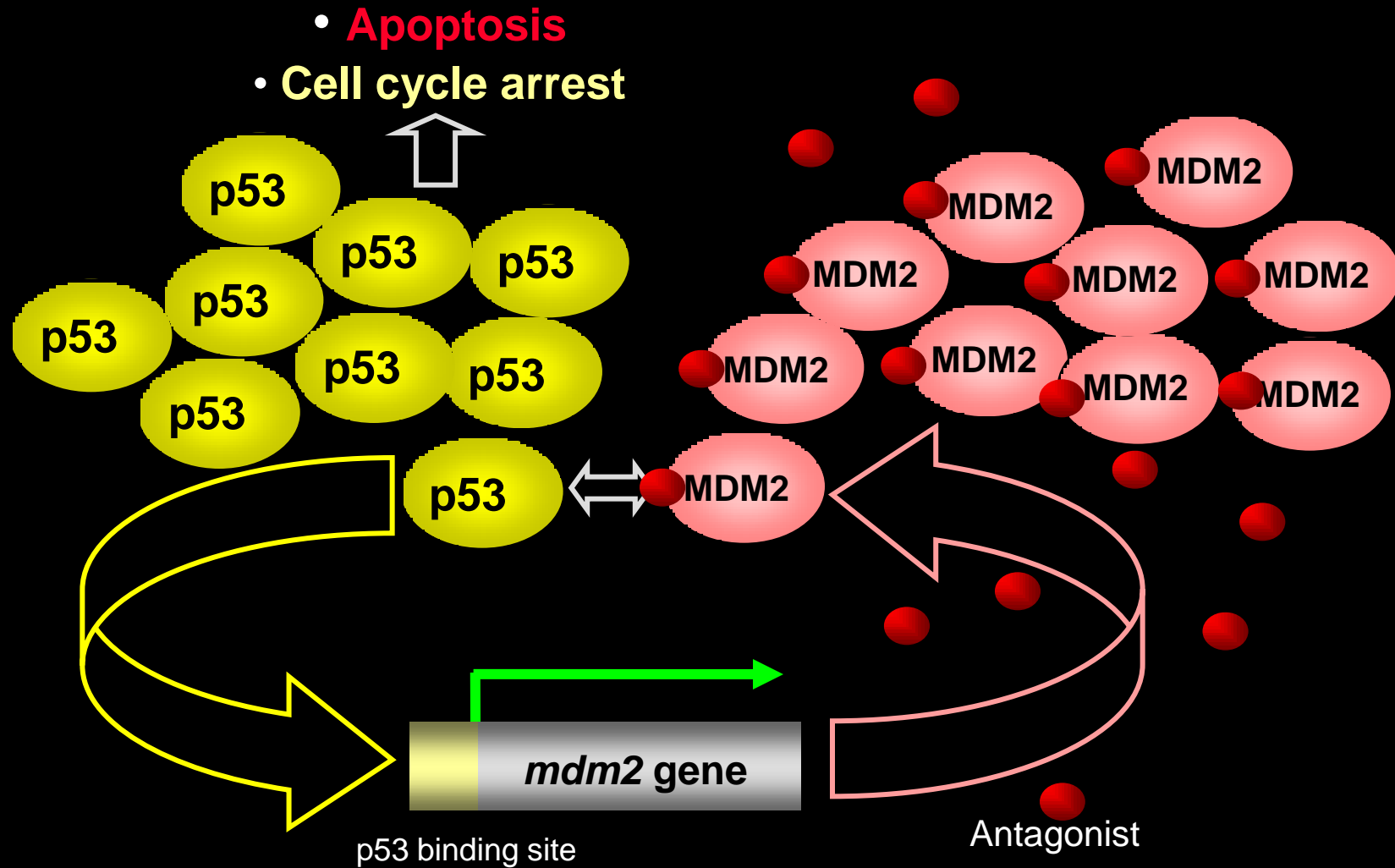
Mutated or deleted in 50% of all human tumors

- Inactive as a transcription factor
- Inadequate response to stress

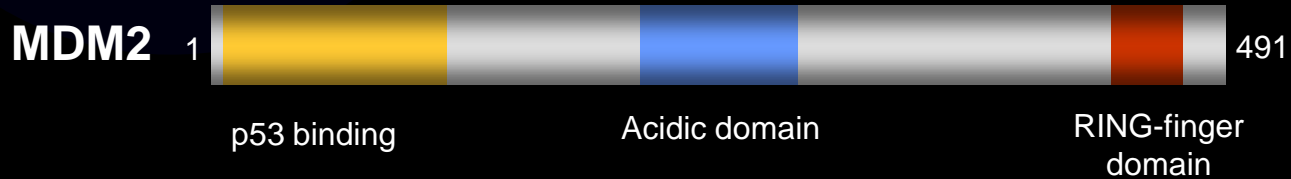
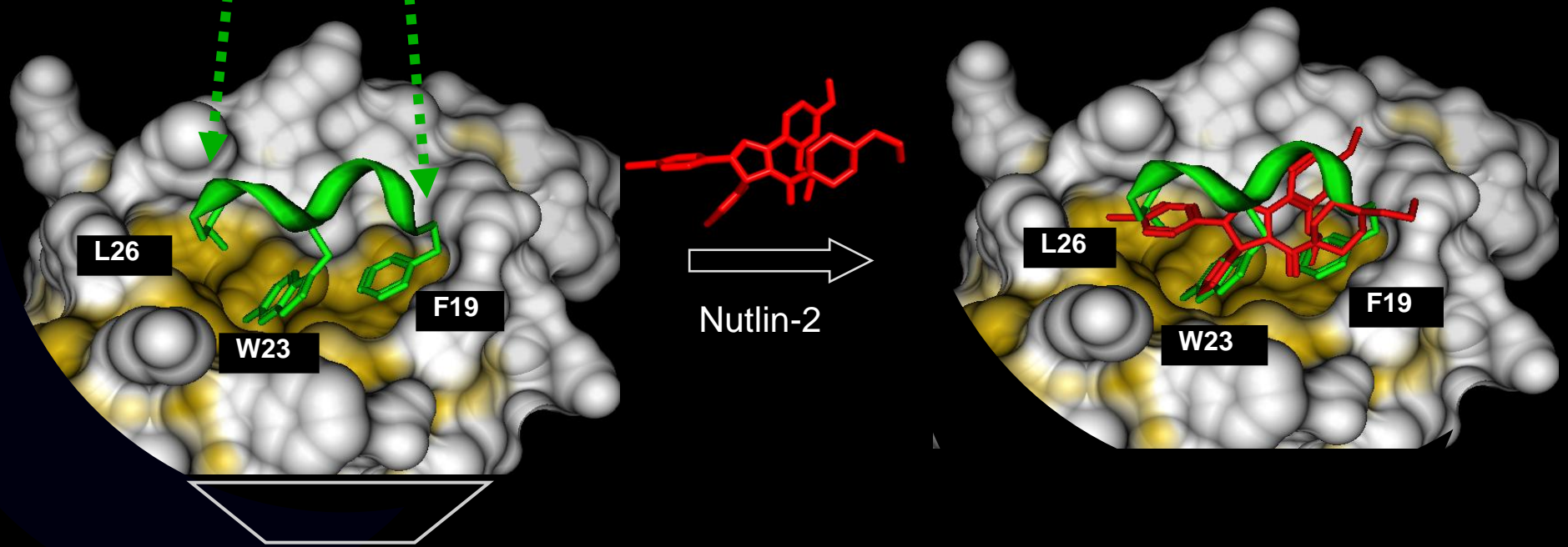
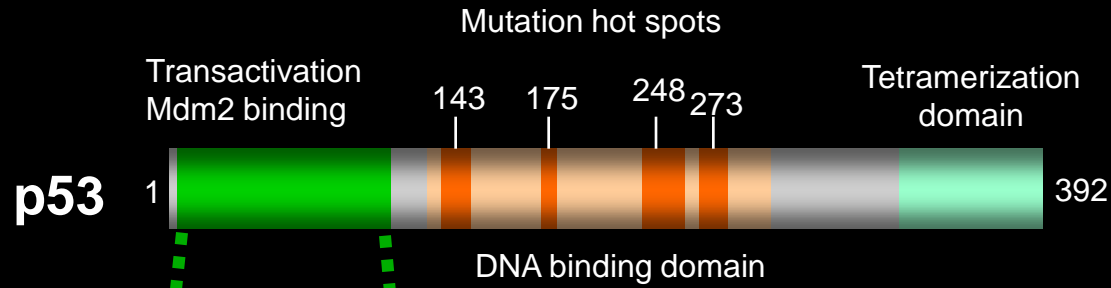
Half of human tumors retain wild-type p53 but can be disabled by other mechanisms

- MDM2 is overproduced in many human tumors and effectively impairs p53 function

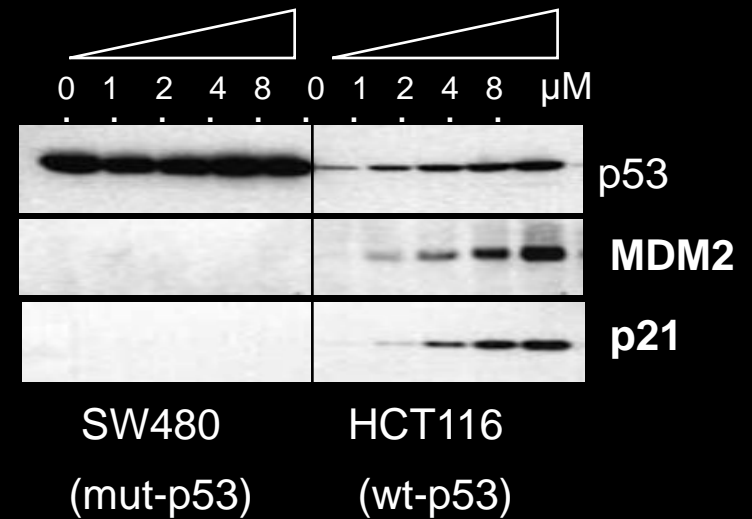
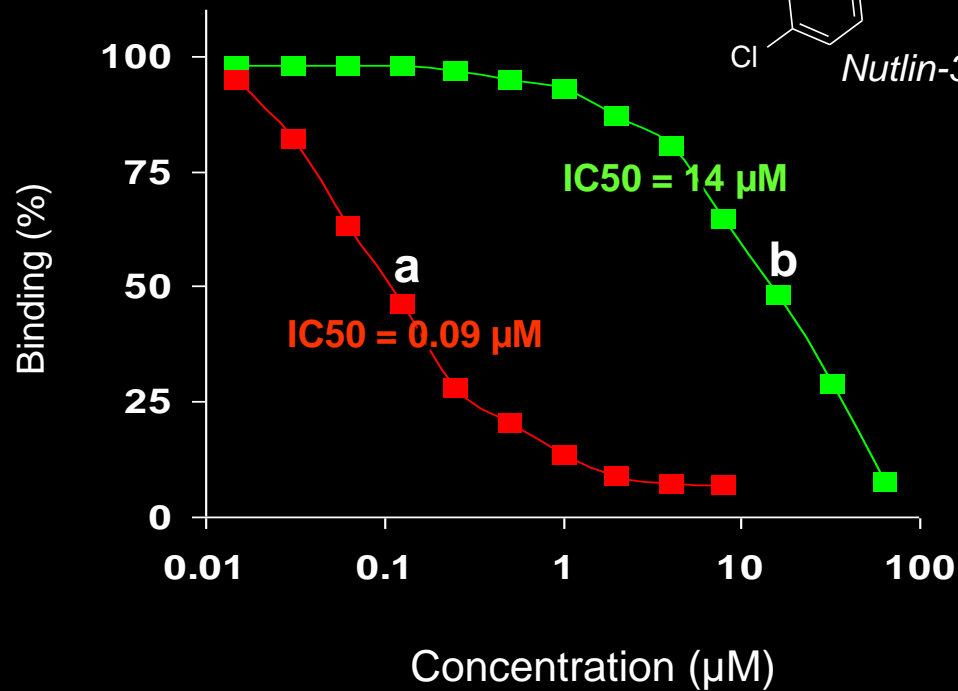
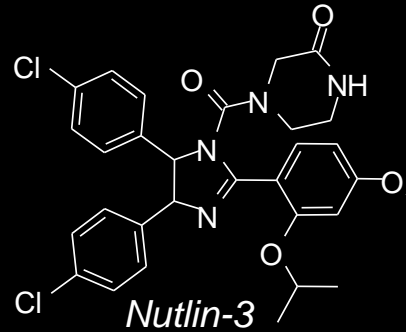
MDM2 antagonist can activate p53



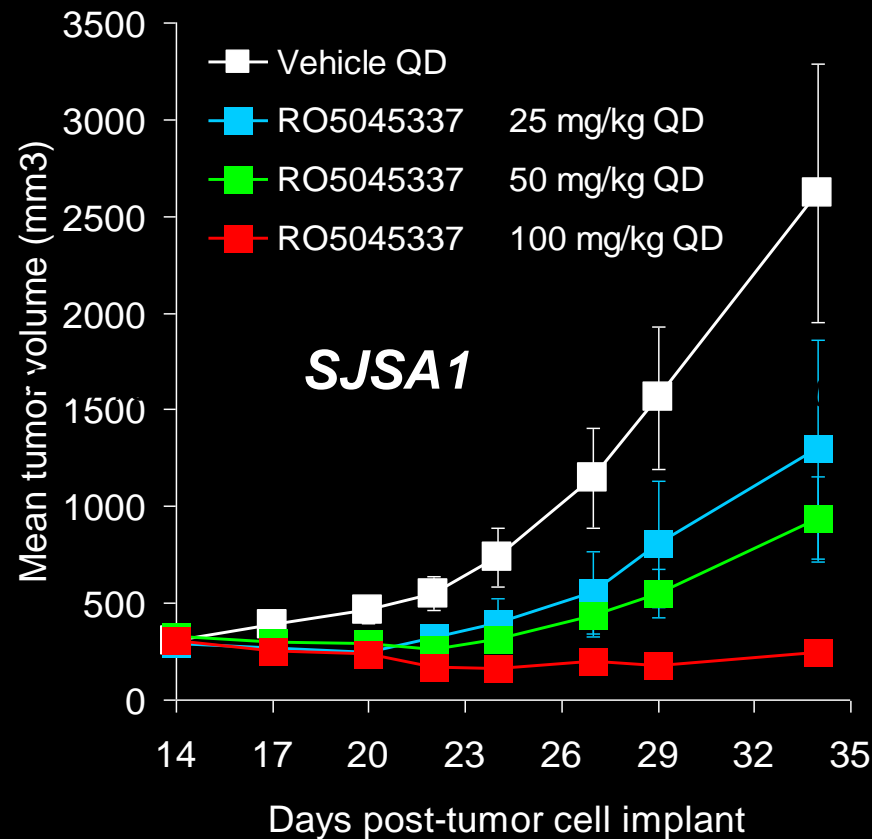
Nutlins inhibit p53-MDM2 binding



Nutlins release p53 from MDM2

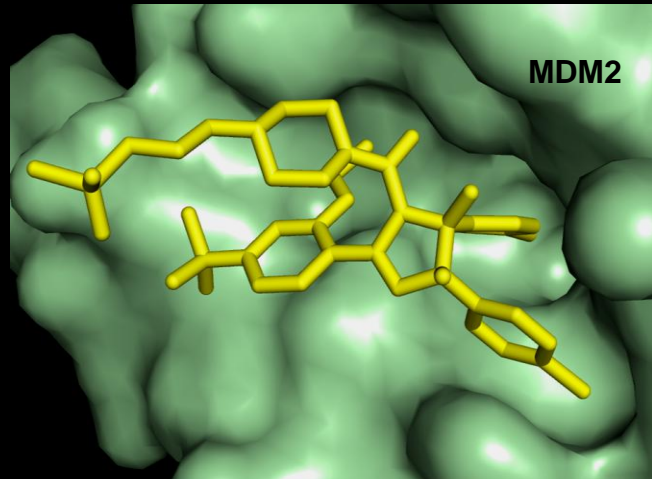


Highly efficacious against osteosarcoma xenografts overexpressing MDM2

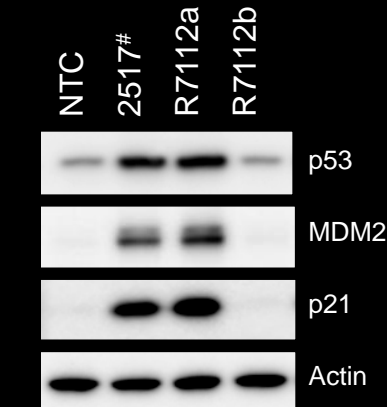
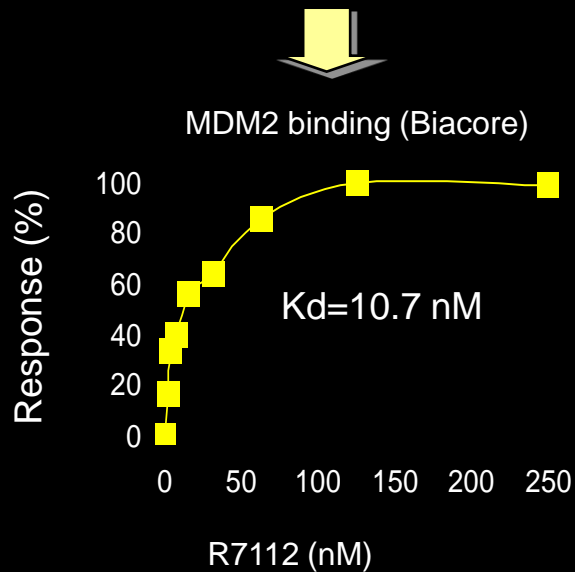


- No bodyweight changes. MTD not reached in Nu/Nu mice
- No treatment-related histopathology changes

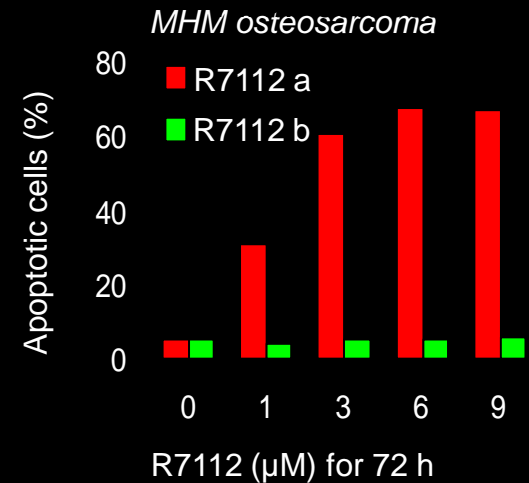
RG7112 is a potent MDM2 inhibitor



- Binds MDM2 in the p53 binding pocket
- Stabilizes and accumulates p53
- Induces p53 target genes
- Blocks cell cycle progression in G1 and G2
- Inhibits cancer cell growth
- 30-fold selective for wild-type vs. mutant p53 cells
- Induces p53-dependent apoptosis



HCT116 cells: 5 μ M for 24 h



Clinical Ph I strategy



- **Two Ph I studies**
- **Dosing schedule = Day 1 – 10 Q 4 wks**
- SOLID TUMOR STUDY (relapsed/refractory solid tumors)
 - sarcomas (p53wt, mdm2 amplified)
- LEUKEMIA STUDY (relapsed/refractory acute leukemias and CLL)
 - (p53wt, mdm2 overexpression/amplification)
- **Preselection of patients in Phase I expansion Phase planned**

MDM2 Antagonist Biomarker Program



Predictive Markers

p53 wt versus mutant

MDM2 amplification and over-expression

→ in tumors

PD marker

MIC-1 (GDF15)

secreted, p53 downstream indicator
of p53 activation

→ in serum

Candidate response biomarkers

Differences in apoptosis induction

→ differences in response

→ preclinical Gx approaches

Explorative PD markers

→ in peripheral leukemic cells

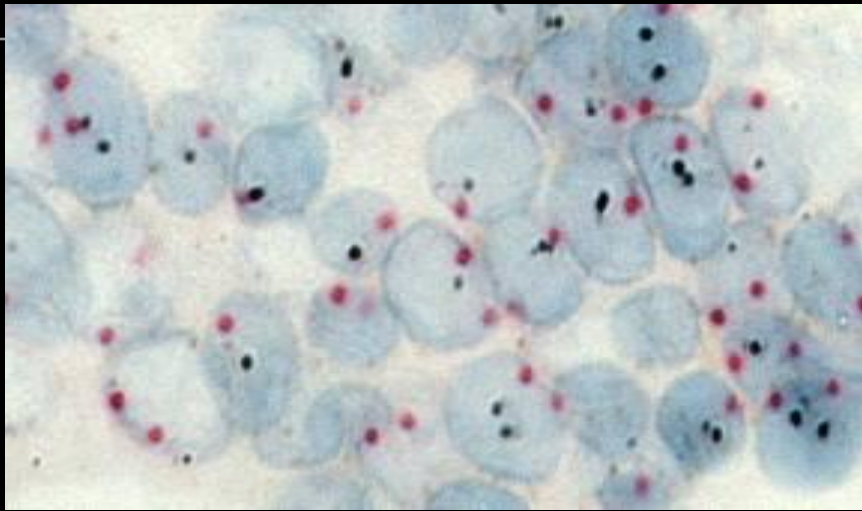
apoptosis, cell cycle,

p53 target genes

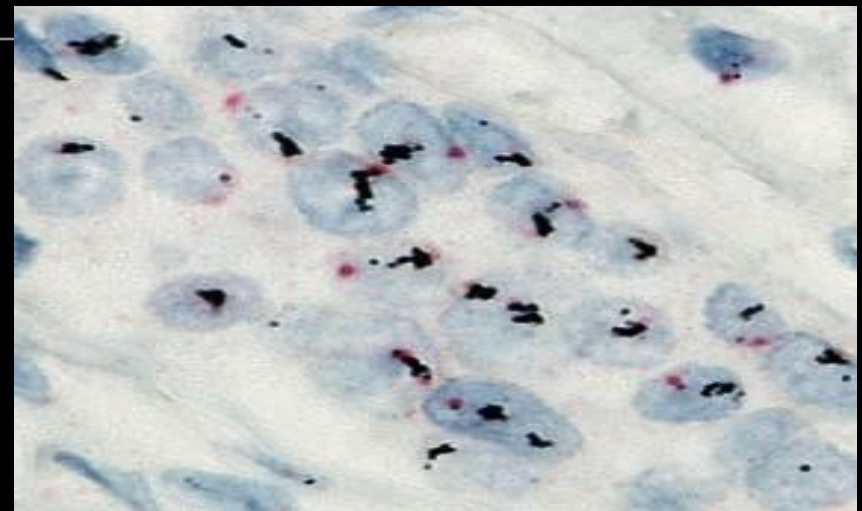
→ in solid tumors: PET imaging

INFORM MDM2 Dual Probe Assay

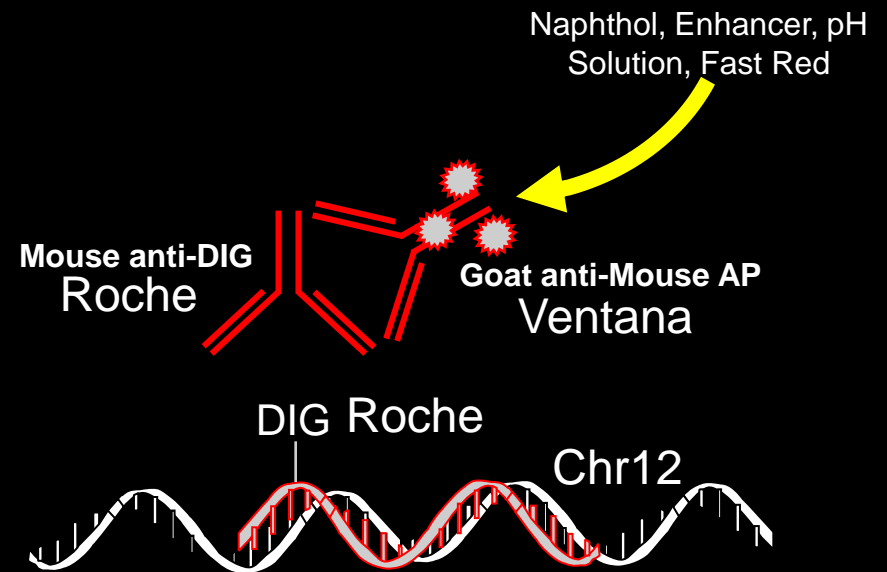
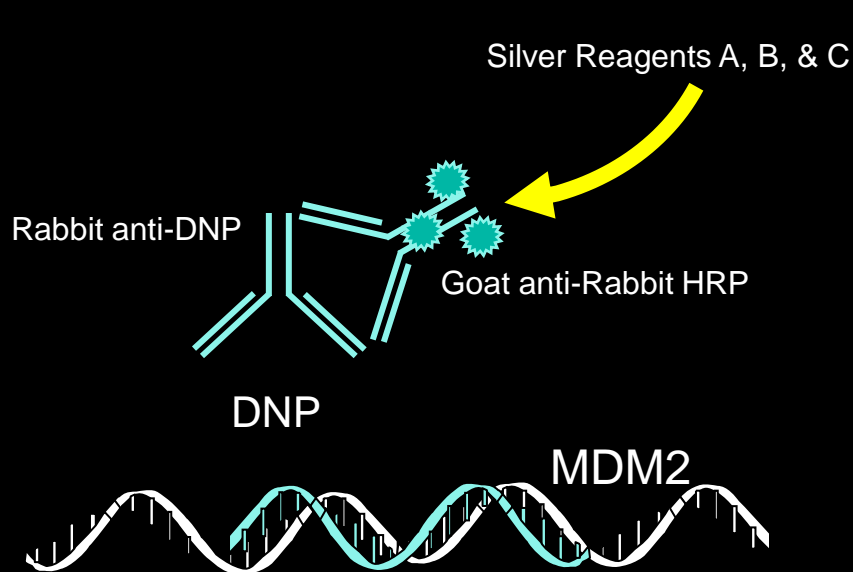
Ventana



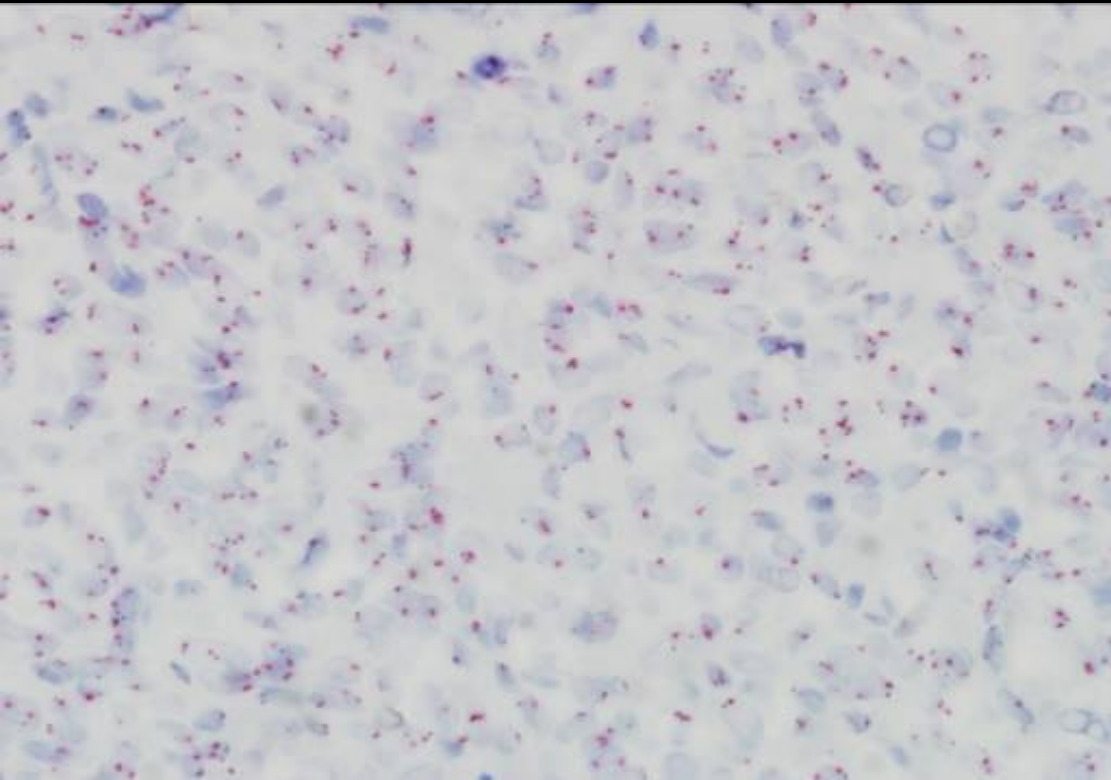
ultraView SISH Detection Kit



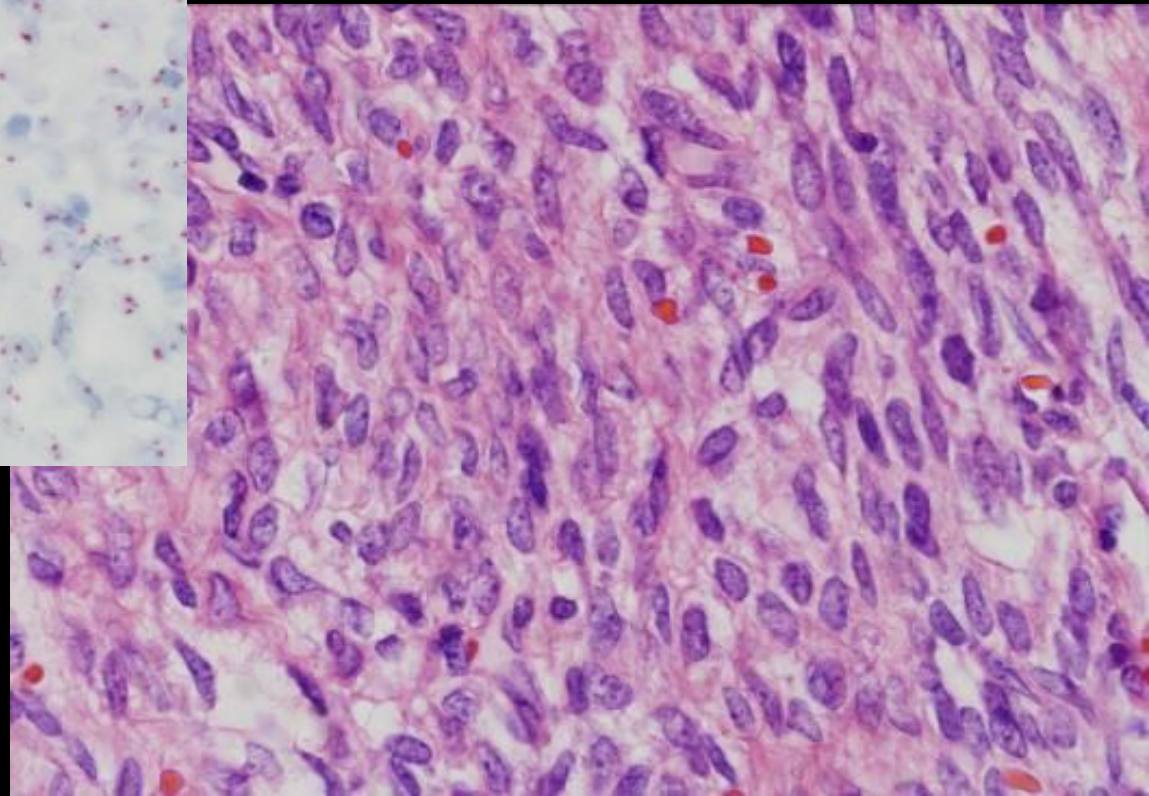
ultraView Red ISH Detection Kit



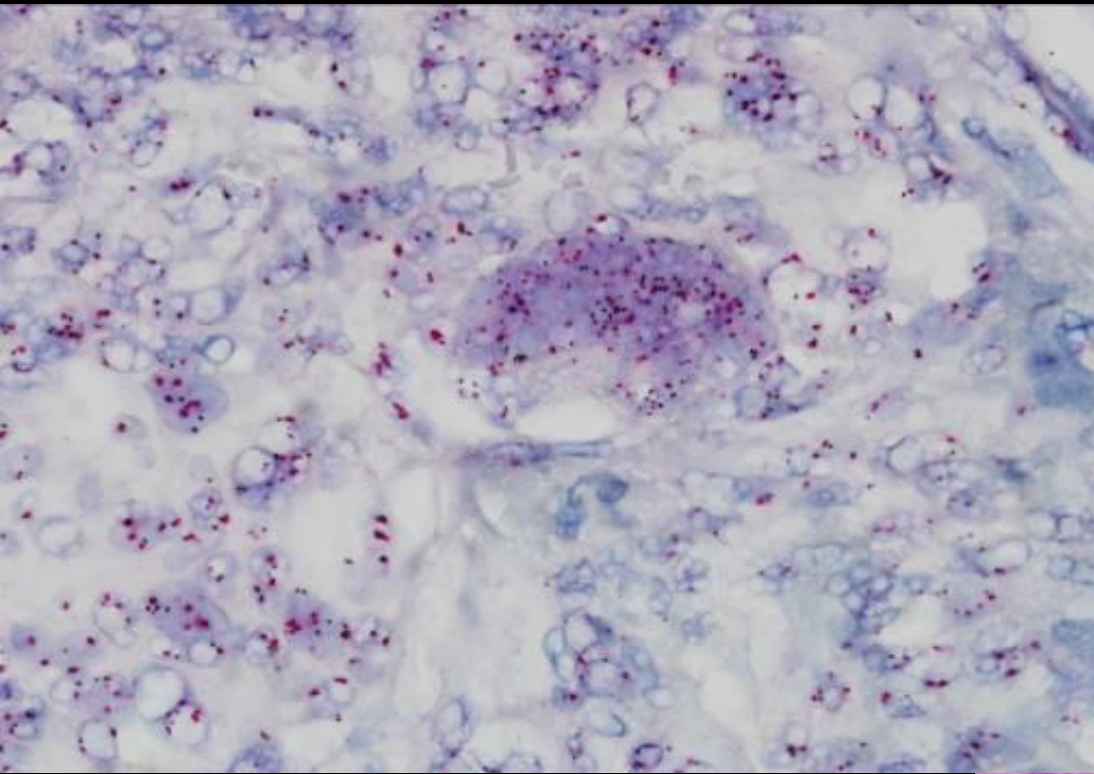
Synovial Sarcoma AST01052



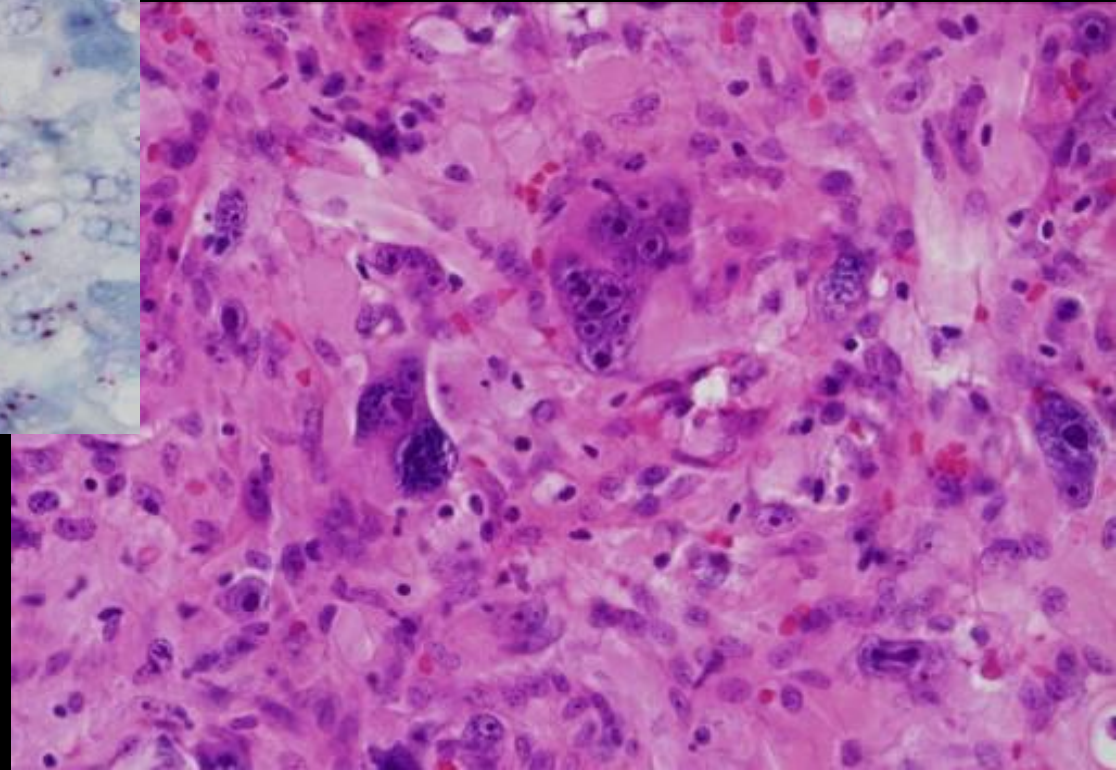
Not amplified for MDM2



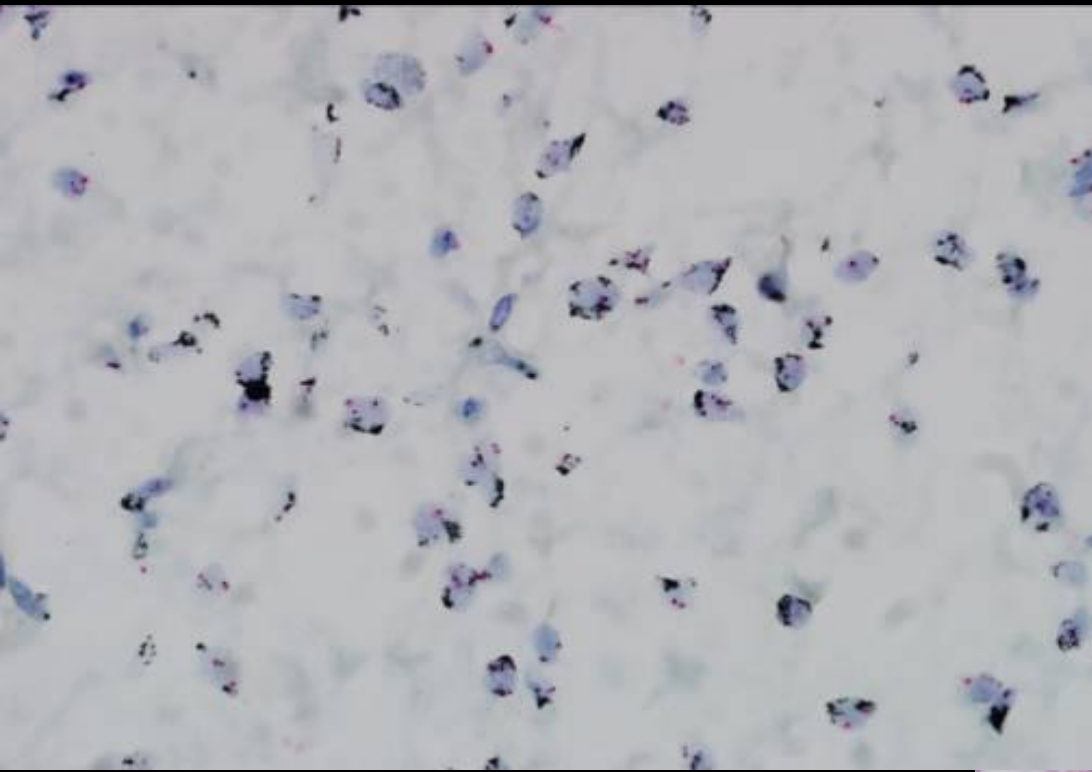
Histiocytoma AST00432



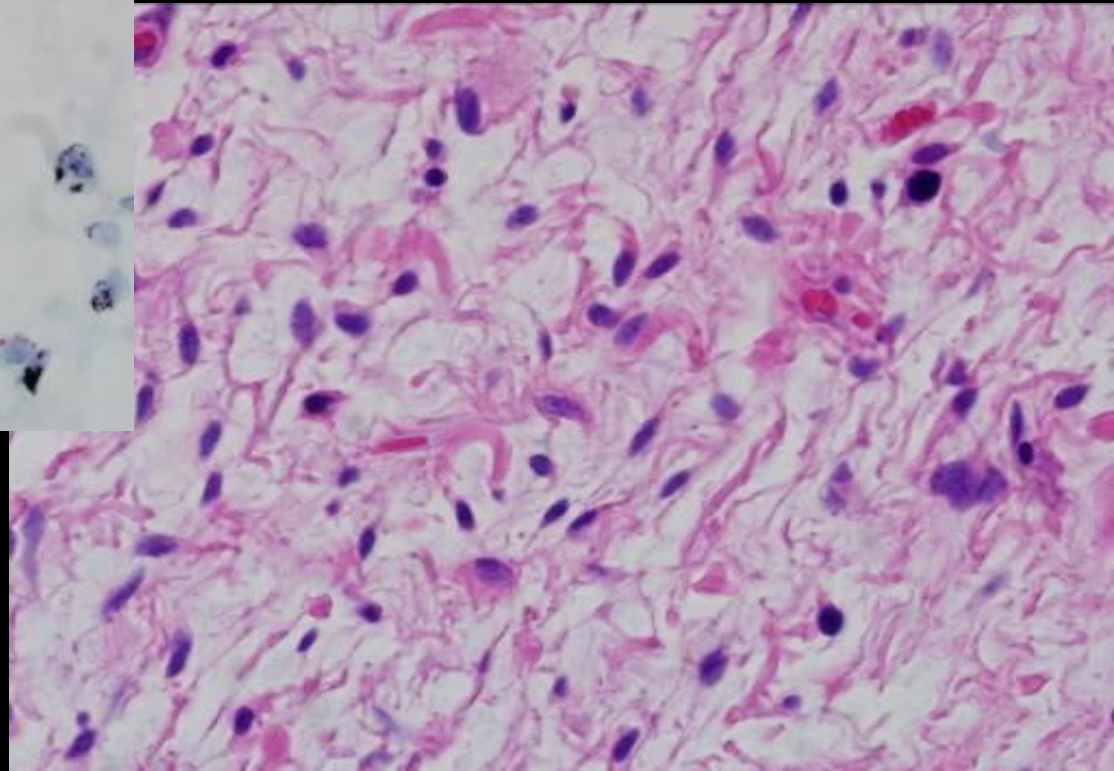
**High polysomy (10-15 copies)
in anaplastic cells**



Liposarcoma AST00442



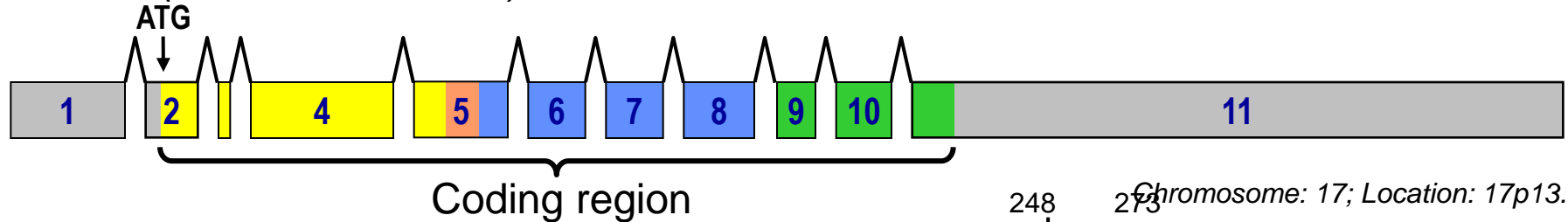
Amplified for MDM2 (>20 copies)



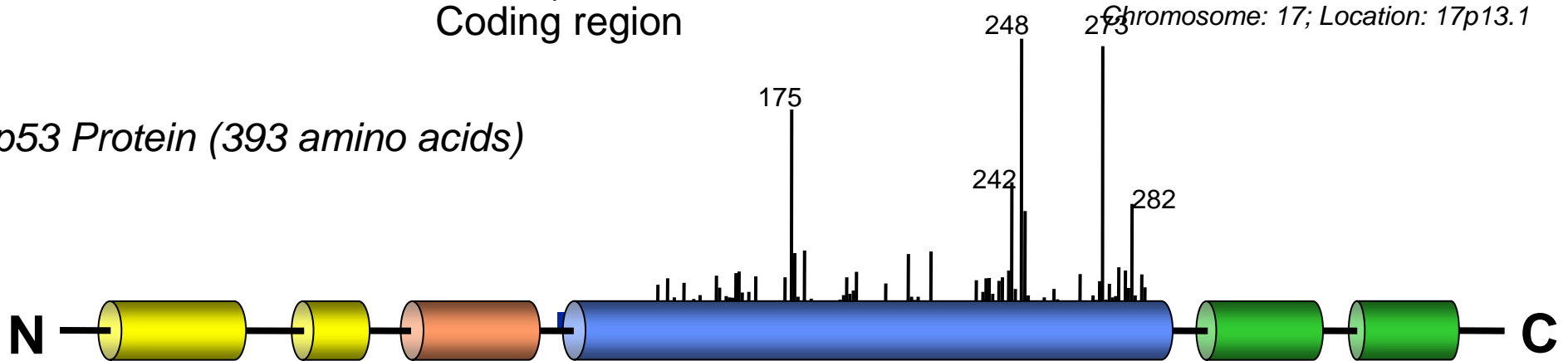
p53 Tumor Suppressor

Most p53 Mutations Are Missense Mutations

p53 Gene (Exon 2-11, ~ 7 Kb)



p53 Protein (393 amino acids)



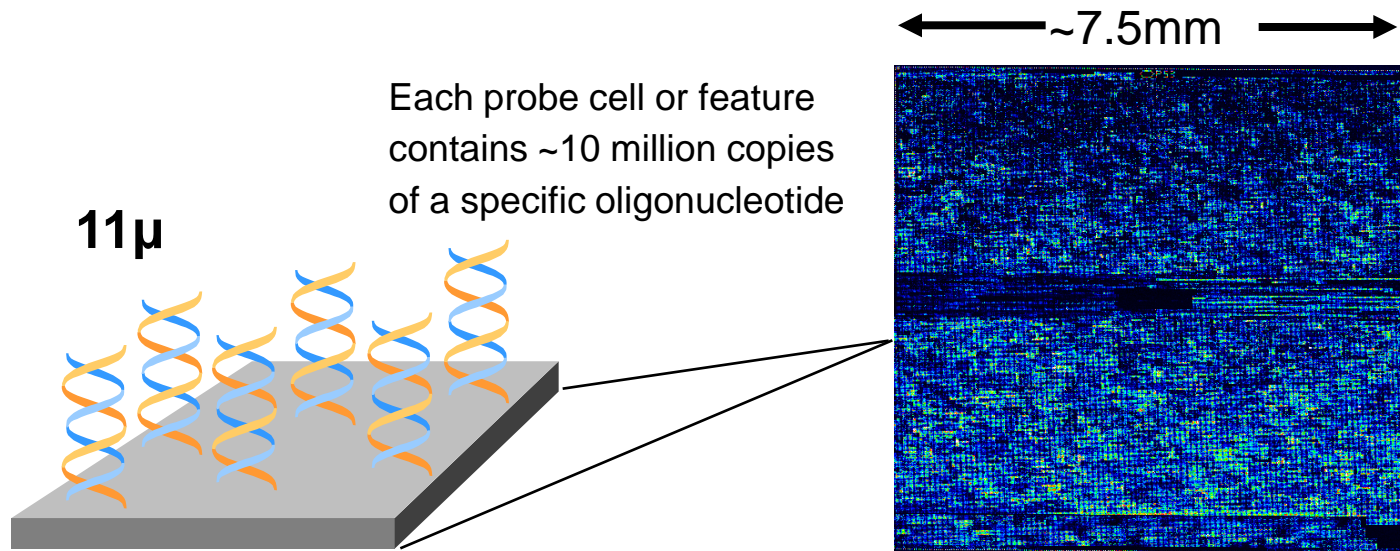
	Transactivation	Proline-rich	DNA binding	Oligomerisation	Regulation
Mutation	1 %	2.3 %	80 %	3.4 %	0.3 %
Missense	50.8 %	45.4 %	82.1 %	36.4 %	72.7 %

AmpliChip p53 Microarray

Designed by Roche, Manufactured by Affymetrix

Roche

- Re-sequences p53 exons 2-11
- Total 1240 nucleotide positions
- Each position queried by redundant probe sets
 - Single-base substitutions
 - Single-base deletions
- ~220,000 oligonucleotide probes
- 169-array format yields ~155 chips/wafer



Re-sequencing with AmpliChip p53

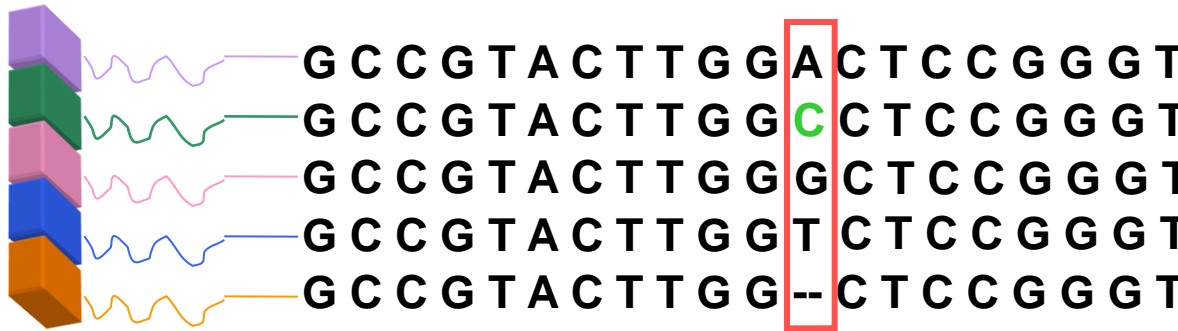
Somatic Mutations Detected in Exons 2-11 of the p53 Gene

Eg., Exon 7, codon 248

Wild-type reference sequence

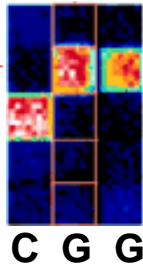
5' T G G G C G G C A T G A A C C G G A G G C C C A T C 3'

Complementary sense probe set: each probe type contained in a probe cell

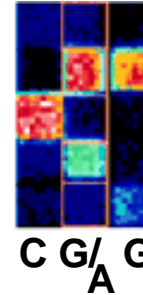


Substitution position

Base cell G: wild-type

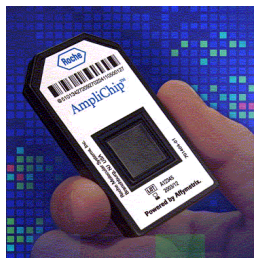


For **every** base position:
 At least 11 Sense Probe sets
 At least 11 Anti-sense Probe sets
 Varying probe lengths of 15-25 base
 Varying substitution position (8-12th)



Base cell A/G: mixture of mutant and wild-type sequences

At least 22 replicates for each position in the same chip



AmpliChip p53 Assay Workflow



RG7112: Clinical Ph I status summary



As of March 2010 :

- Two Ph I studies in late dose escalation
 - SOLID TUMOR STUDY (relapsed/refractory solid tumors)
 - LEUKEMIA STUDY (relapsed/refractory acute leukemias and CLL)
- Acceptable safety to date
- Exposures at efficacious range seen in xenograft studies
- Evidence of clinical benefit
 - **Solid tumor study :**
 - Several patients with long-term stable disease
 - Preliminary activity seen by FLT-PET
 - One liposarcoma patient with a minor response by RECIST.
 - **Leukemia study :**
 - AML patient with complete remission in BM and recovery of blood counts after cycle 2- currently in cycle 8
 - Two CLL patients with marked reduction in lymphocyte counts
 - Several CLL patients in low dose cohorts with stable disease >6 months

Thank You