Memorial Sloan Kettering Cancer Center

Molecular Biology of Mesothelioma: Implications for the development of new targeted therapies

Marc Ladanyi, M.D.

Molecular Diagnostics Service
and Human Oncology & Pathogenesis Program
Memorial Sloan-Kettering Cancer Center
New York, NY, USA

Malignant Pleural Mesothelioma

- **Major somatic mutations are in tumor suppressors:**
  - **P16**: 75-80%: homozygous deletion of gene *p16/CDKN2A* (9p21)
    - Testa 1994; Fletcher 1995
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  - **NF2**: 60%: loss of heterozygosity of *NF2* (22q12); inactivating mutations
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  - **BAP1**: 20-25% inactivating mutations; 30% genomic loss
    - 42% cases with either or both
    - Ladanyi 2011; Testa 2011
  - **LATS2:** approx. 5-15% inactivating mutations
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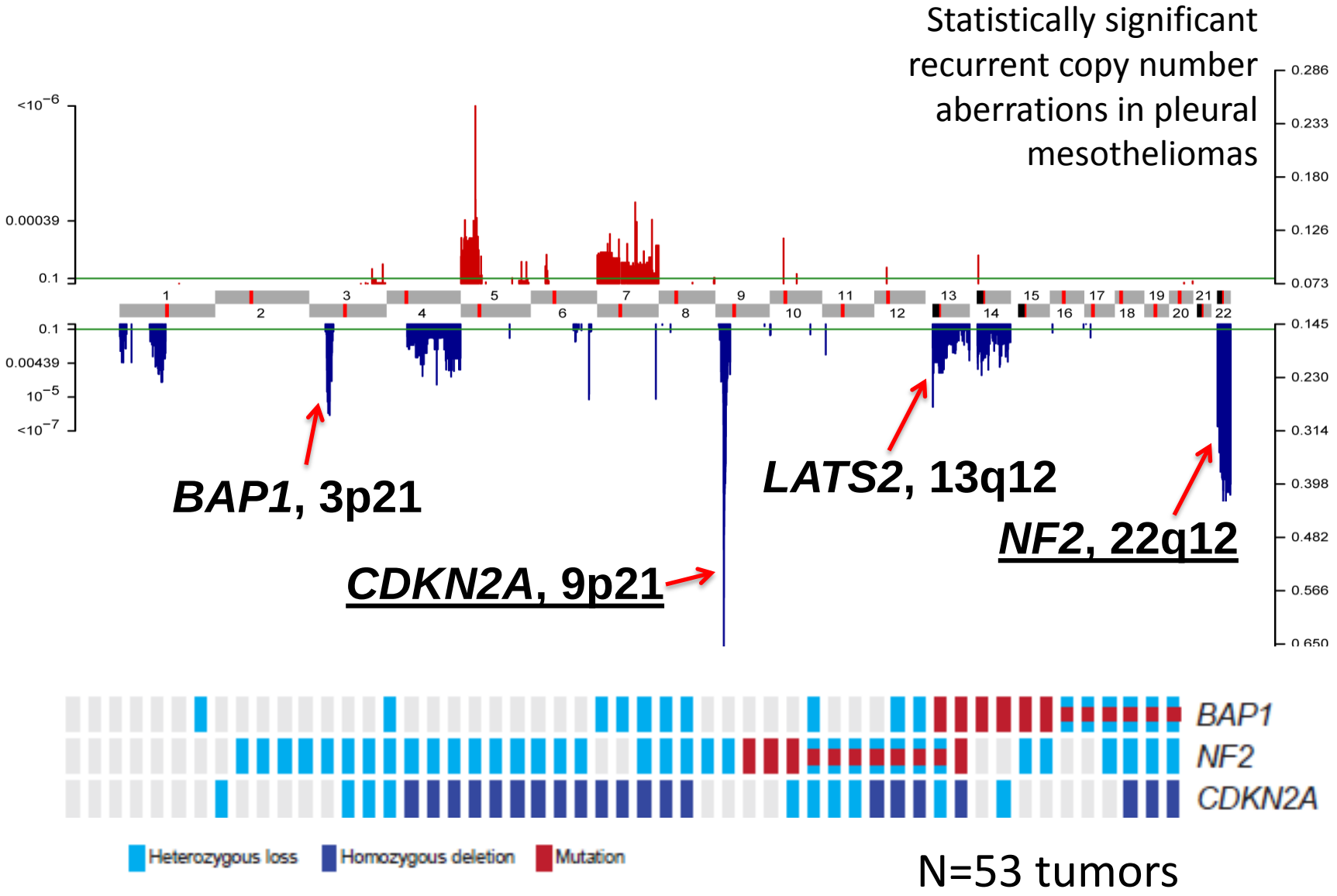
    - Higher in cell lines (up to 35%)

Major mesothelioma tumor suppressors: *P16, NF2, BAP1*



Bott et al. Nature Genetics 43, 668–672, 2011.

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Gene	Histology	Prognosis	Response Prediction	Practical detection methods
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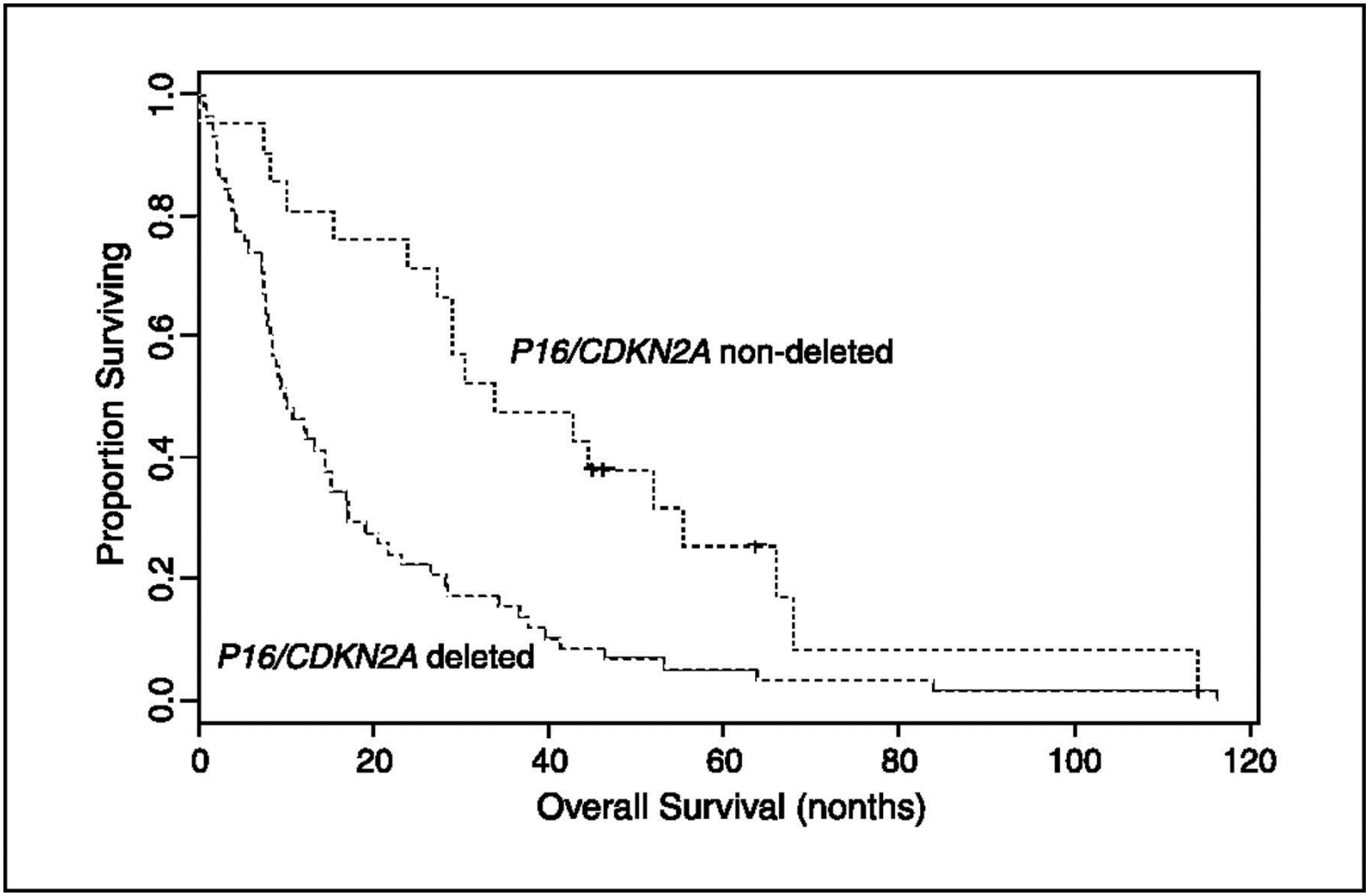
Kaplan-Meier plots of overall survival after surgery according to p16/CDKN2A homozygous deletion status.



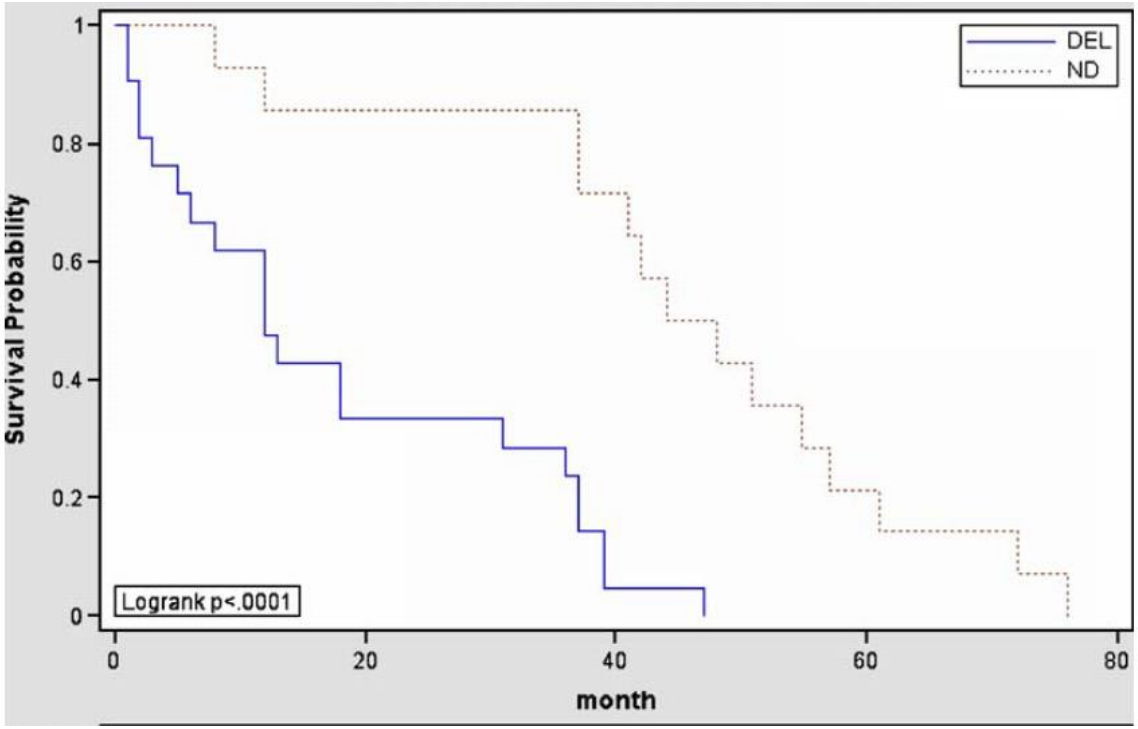
López-Ríos F et al. Cancer Res 2006;66:2970-2979

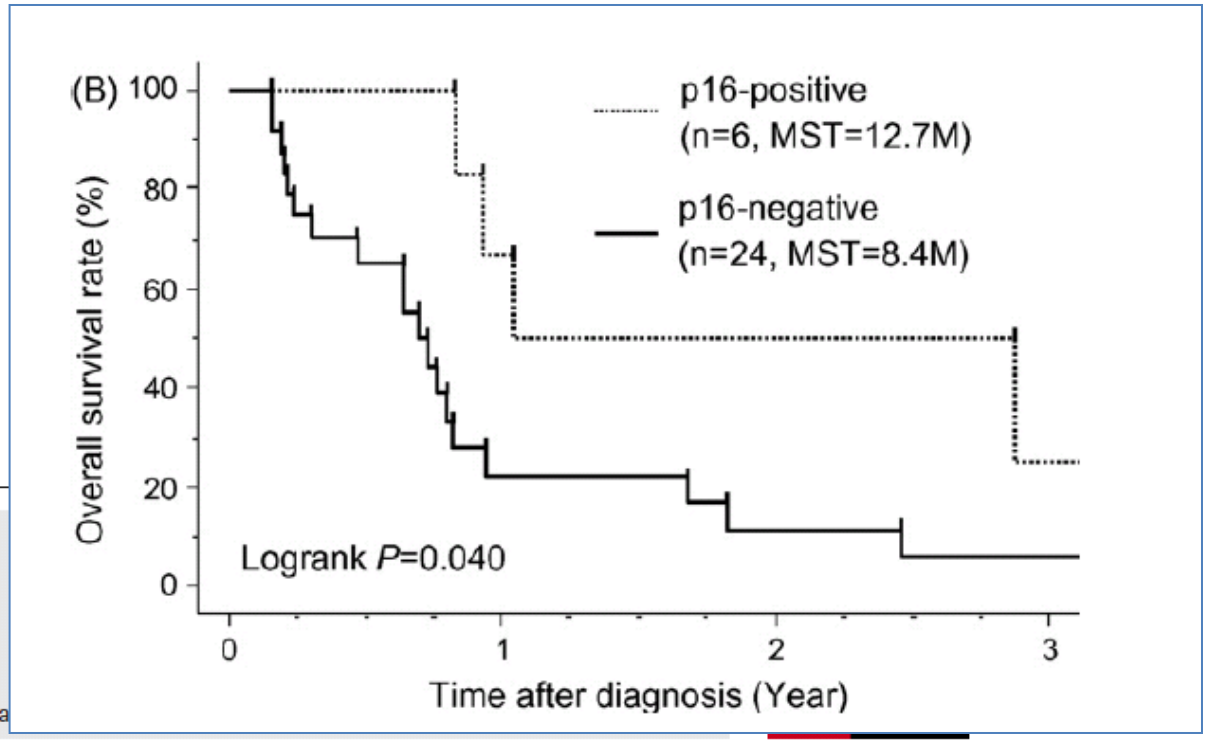
Virchows Arch (2008) 453:627–635
DOI 10.1007/s00428-008-0689-3

ORIGINAL ARTICLE

Prognostic significance of p16/cdkn2a loss in pleural malignant mesotheliomas

Sanja Dacic · Hannelore Kothmaier · Stephanie Land · Yongli Shuai · Iris Halbwedl · Patrizia Morbini · Bruno Murer · Camilla Comin · Françoise Galateau-Salle · Funda Demirag · Handan Zeren · Richard Attanoos · Alan Gibbs · Philip Cagle · Helmut Popper





Lung Cancer (2008) 62, 120–125

ELSEVIER

journa

Frequent p16 inactivation by homozygous deletion or methylation is associated with a poor prognosis in Japanese patients with pleural mesothelioma

Naruyuki Kobayashi[a], Shinichi Toyooka[a,*], Hiroyuki Yanai[b], Junichi Soh[a], Nobukazu Fujimoto[c], Hiromasa Yamamoto[a], Shuji Ichihara[a], Kentaro Kimura[a], Kouichi Ichimura[b], Yoshifumi Sano[a], Takumi Kishimoto[c], Hiroshi Date[d]

P16/CDKN2A correlation with histology

Summary of FISH data on CDKN2A Homozygous deletion in 95 cases of pleural mesothelioma

Histological type

Epithelioid	49/71	69%
Biphasic	16/19	84%
Sarcomatoid	5/5	100%
Total	**70/95**	**74%**

- *Illei PB, et al. Clin Cancer Res 9:2108 , 2003*

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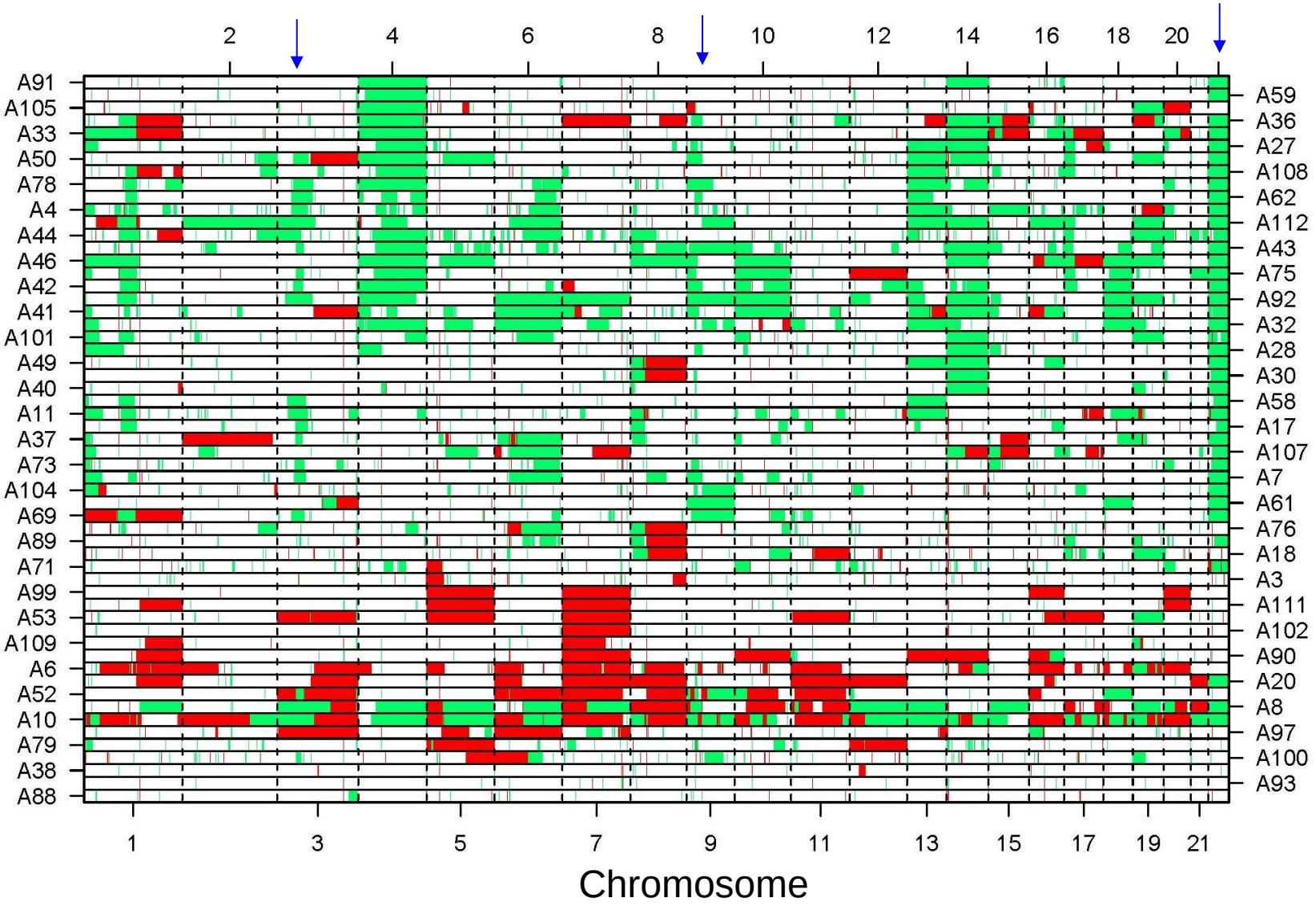
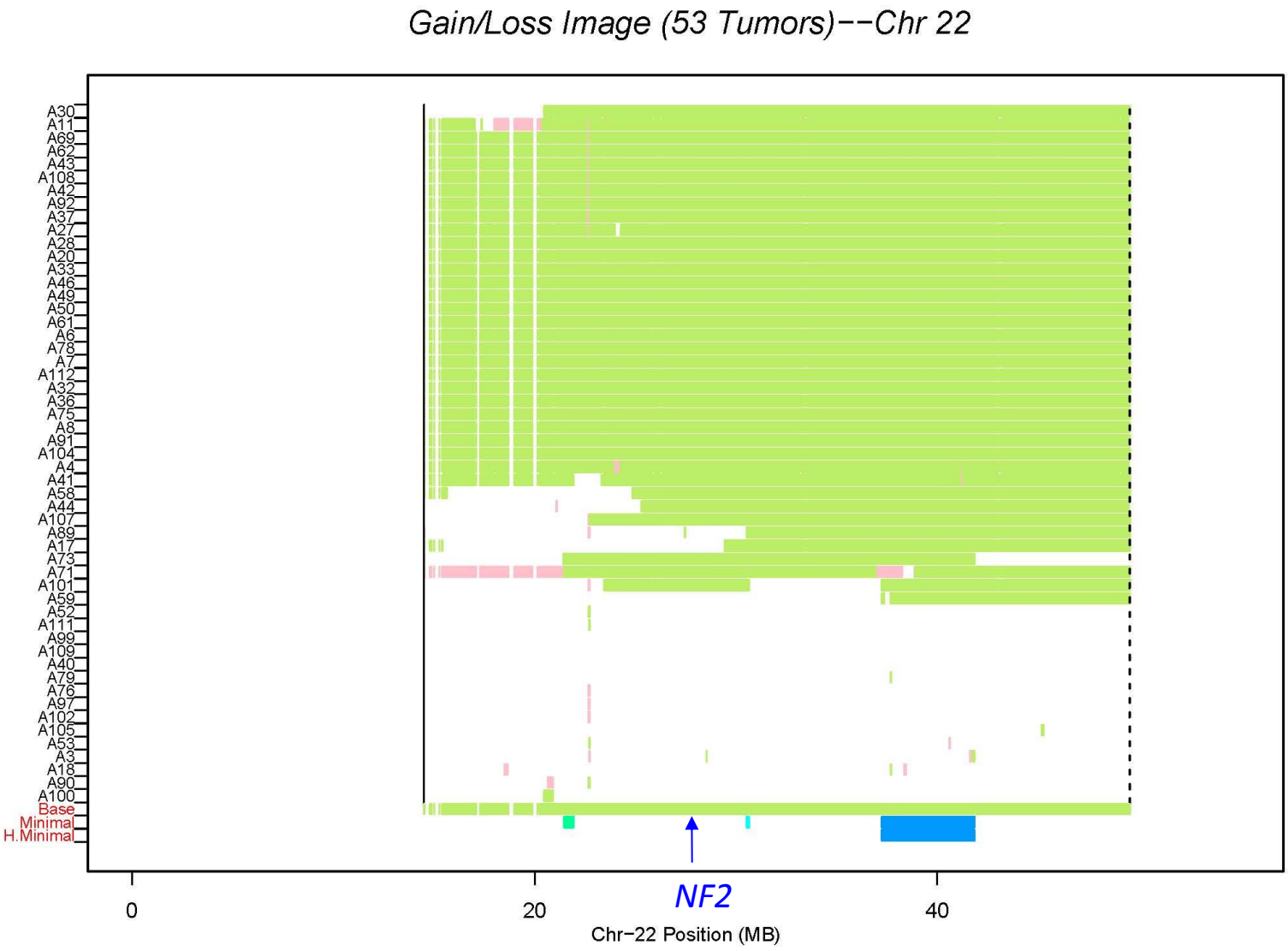
NF2	No correlation	No correlation	MTOR inhibitor CRL inhibitor FAK inhibitor?	FISH, IHC

Genomic copy number data in 53 mesotheliomas
Hierarchical clustering



Bott et al. Nature Genetics 43, 668–672, 2011.

Minimal Common Region analysis – Chromosome 22



NF2 mutations in 11/53 tumor samples

Case	cDNA pos	Mutation	Type	Previously described (COSMIC)	Previously mutated in:	Relevance
32	655 G>A	V219M	Missense	Yes	Schwann	In 4.1 domain
99	331 C>T	Q111*	Nonsense	Yes	Schwann	Stop at exon 3
30	1333 G>T	E445*	Nonsense	No		Stop at exon 12
46	172 G>T	E58*	Nonsense	Yes	Meso	Stop at exon 2
97	599 +1		Abnl splice	Yes	Mening	Abnormal splice
102	363 +1		Abnl splice	No		Abnormal splice
43	36-42	S13fs*35	2 bp del	Yes	Schwann	Truncate exon 1
3	271-272	P91fs*32	1 bp del	Yes	Meso	Truncate exon 12
69	1324-1340	S444fs*47	11 bp del	No		Truncate exon 12
7	1641-1642	E547fs*2	1 bp del	No		Truncate exon 15
73	1458-1459	P486fs*8	1 bp insert	No		Truncate exon 14

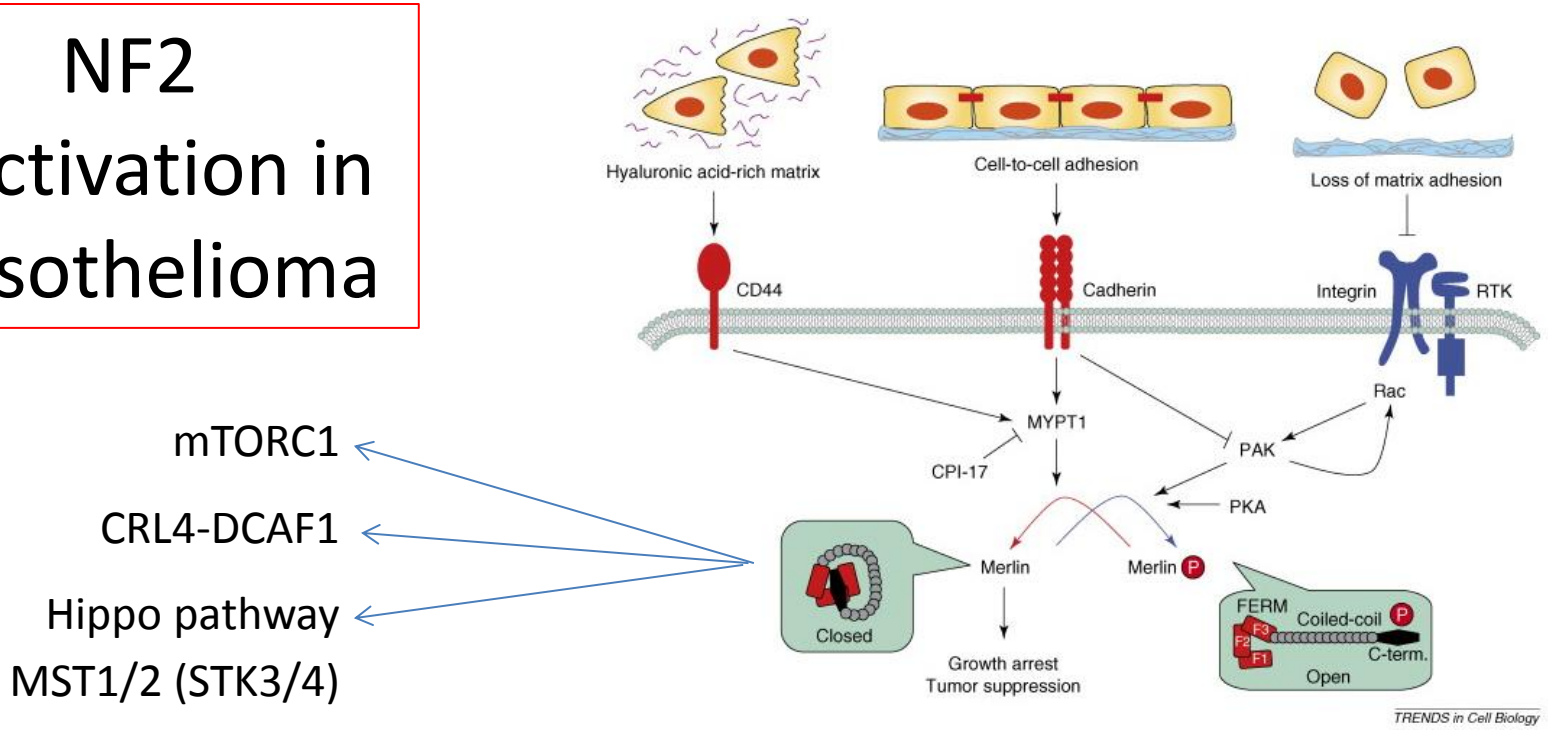
Bott et al. Nature Genetics 43, 668–672, 2011.

NF2 Inactivation in MM: Results

- *NF2* genomic loss in 66% of tumor samples
- Samples with *NF2* loss have 40% decrease in *NF2* mRNA expression (p = 0.005) and lower protein expression by IHC (p=0.007).
- *NF2* genomic loss in 44% of cell lines
- Integrated data in 53 tumors:
  - 7 loss + mutation (13%)
  - 32 either loss or mutation (60%)
  - 14 neither (26%)

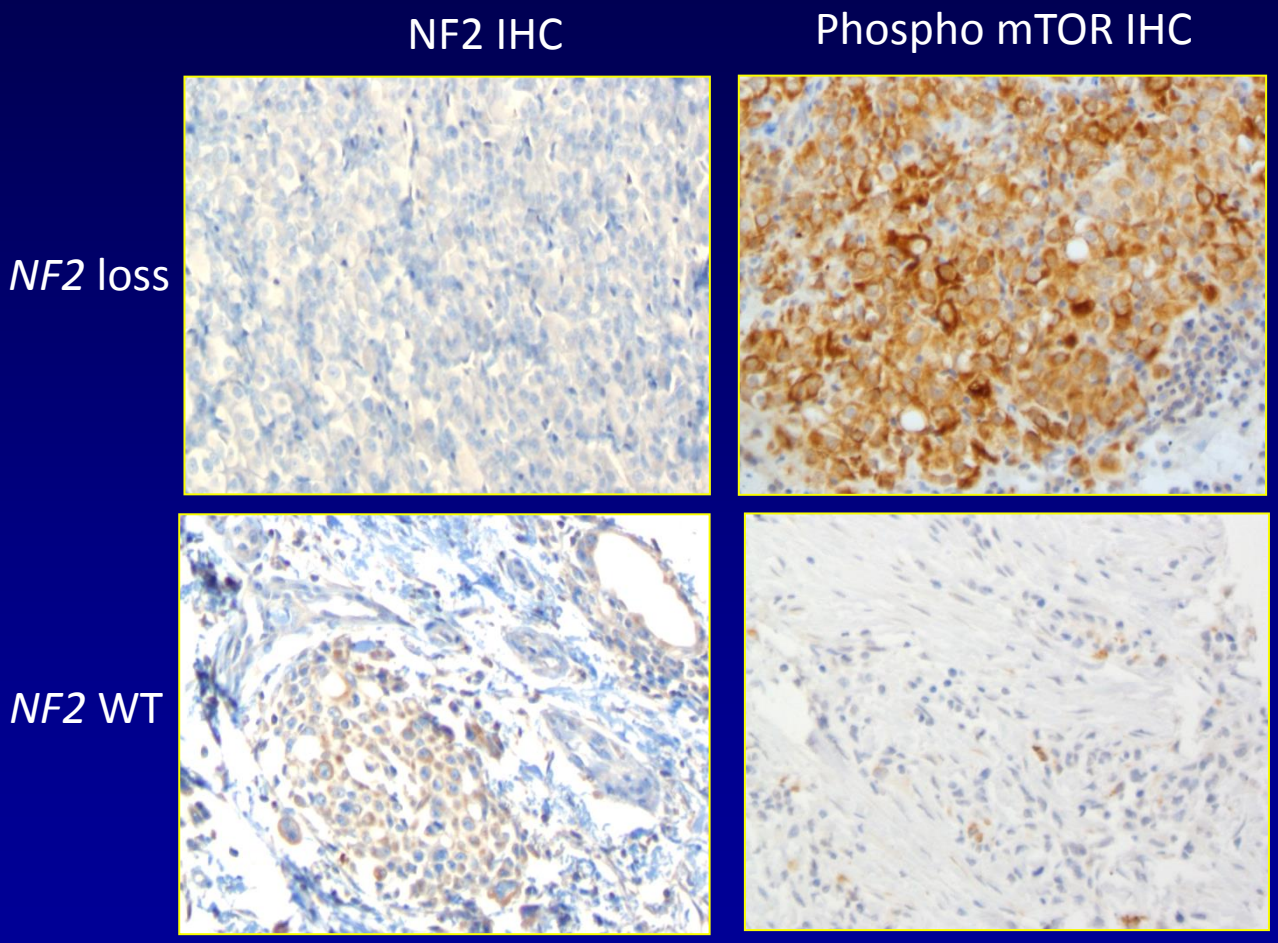
Bott et al. Nature Genetics 43, 668–672, 2011.

NF2 Inactivation in Mesothelioma



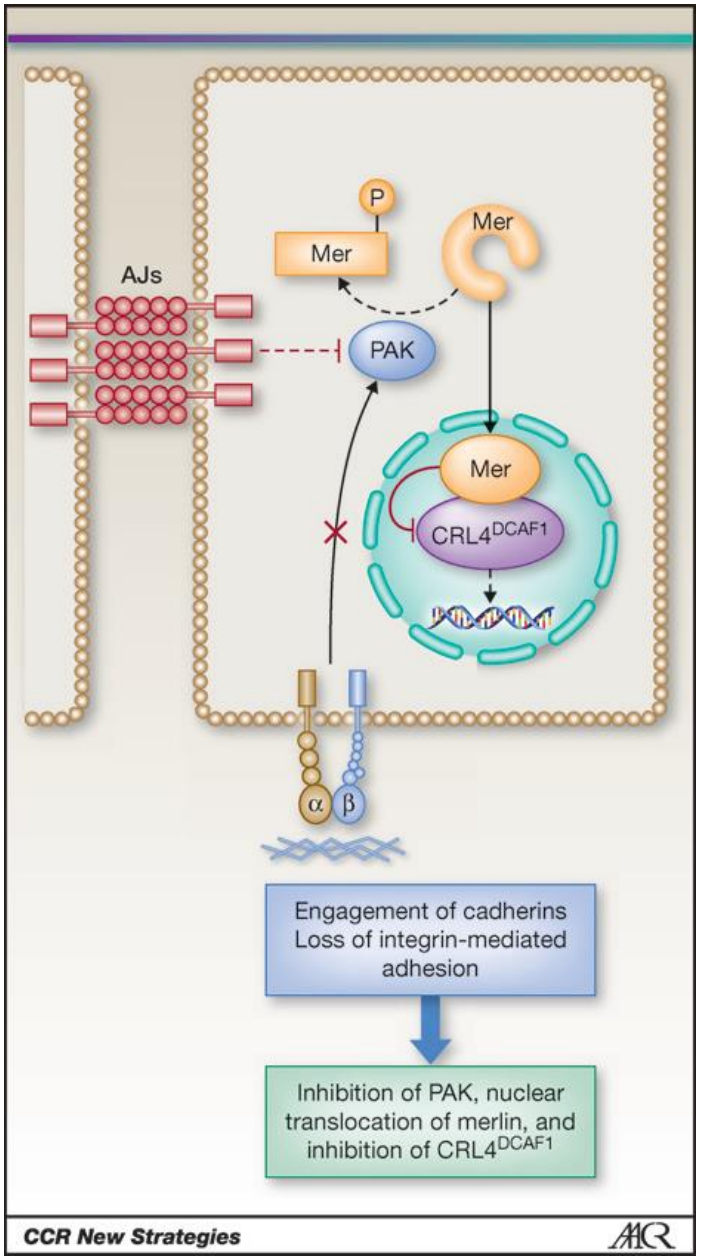
- Loss of NF2 correlates with activation of mTORC1 signaling and sensitivity to mTOR inhibition in MPM cell lines
  - Lopez-Lago MA et al. 2009. Loss of tumor suppressor gene NF2, encoding merlin, constitutively activates integrin-dependent mTORC1 Signaling. Mol Cell Biol 29:4235-4249.
- A phase I study of the dual PI3K/mTOR inhibitor GDC0980 showed a 15% partial response rate in MPM.
- A similar phase I study with the dual PI3K/mTOR inhibitor LY3023414 in MPM is ongoing at MSKCC.

NF2 inactivation increases phospho-mTOR



NF2 loss is associated with increased p-mTOR expression (p=0.002)

→ IHC for NF2 and phospho-mTOR could be useful in selecting MM patients for trials targeting mTOR pathway activation due to NF2 loss



Mechanisms of NF2(Merlin)-mediated inhibition of proliferation

- NF2/Merlin exists in an open, inactive form and a closed, active form. Matrix adhesion and activation of integrin RTK signaling activate the PAK kinase. PAK in turn phosphorylates the C-terminus of NF2/Merlin, disrupting the intramolecular association that maintains the protein in a closed conformation. The resulting inactivation of NF2/Merlin removes a block to cell-cycle progression in normal cells.
- When not inhibited by NF2/Merlin, CRL4/DCAF1 positively regulates a broad oncogenic program of gene expression, which includes mitogenic signaling components, antiapoptotic proteins, and Hippo pathway target genes.

Ladanyi M, et. al. New Strategies in Pleural Mesothelioma: BAP1 and NF2 as Novel Targets for Therapeutic Development and Risk Assessment. Clin Cancer Res. 18:4485-90, 2012.

Rationale for CRL inhibitors in MPM

- The closed, active form of Merlin accumulates in the nucleus and interacts with DCAF1, the receptor component of the E3 ubiquitin ligase CRL4-DCAF1, inhibiting ubiquitination of nuclear proteins.
  - Li W, et al. 2010. Merlin/NF2 suppresses tumorigenesis by inhibiting the E3 ubiquitin ligase CRL4(DCAF1) in the nucleus. Cell 140:477-90.
- De-repressed $CRL4^{DCAF1}$ functions in the nucleus to suppress the output of Hippo signaling. The mechanism of this suppression of Hippo signaling by $CRL4^{DCAF1}$ has recently been elucidated.
  - F. Giancotti lab, Cancer Cell, in press
- This provides a rationale to assess the pharmacological targeting of CRL4 for the treatment for MPM and other NF2 mutant tumors.
- The potential efficacy of the CRL inhibitor MLN4924 **(Millenium-Takeda)** in NF2 mutant MPM will be assessed in patients with previously treated MPM with NF2 loss in a single institution phase II study at MSKCC.

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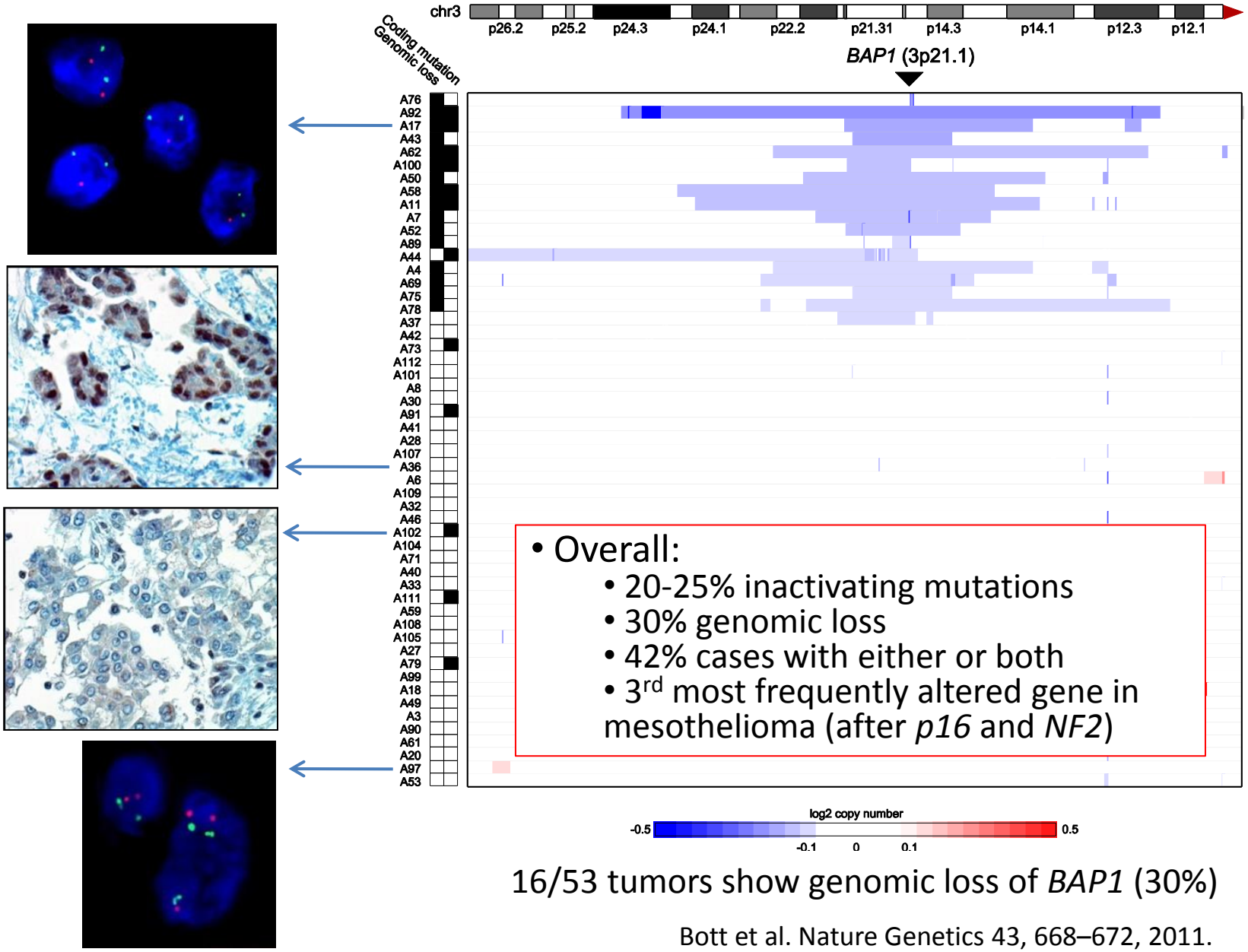
BAP1	No? some data for association with epithelial component	No correlation	? PARP inhibitor ? DNA-PK inhib ? EZH2 inhibitor ? HDAC inhibitor	IHC, FISH, sequencing

3p21 deletions in mesothelioma centered on *BAP1*



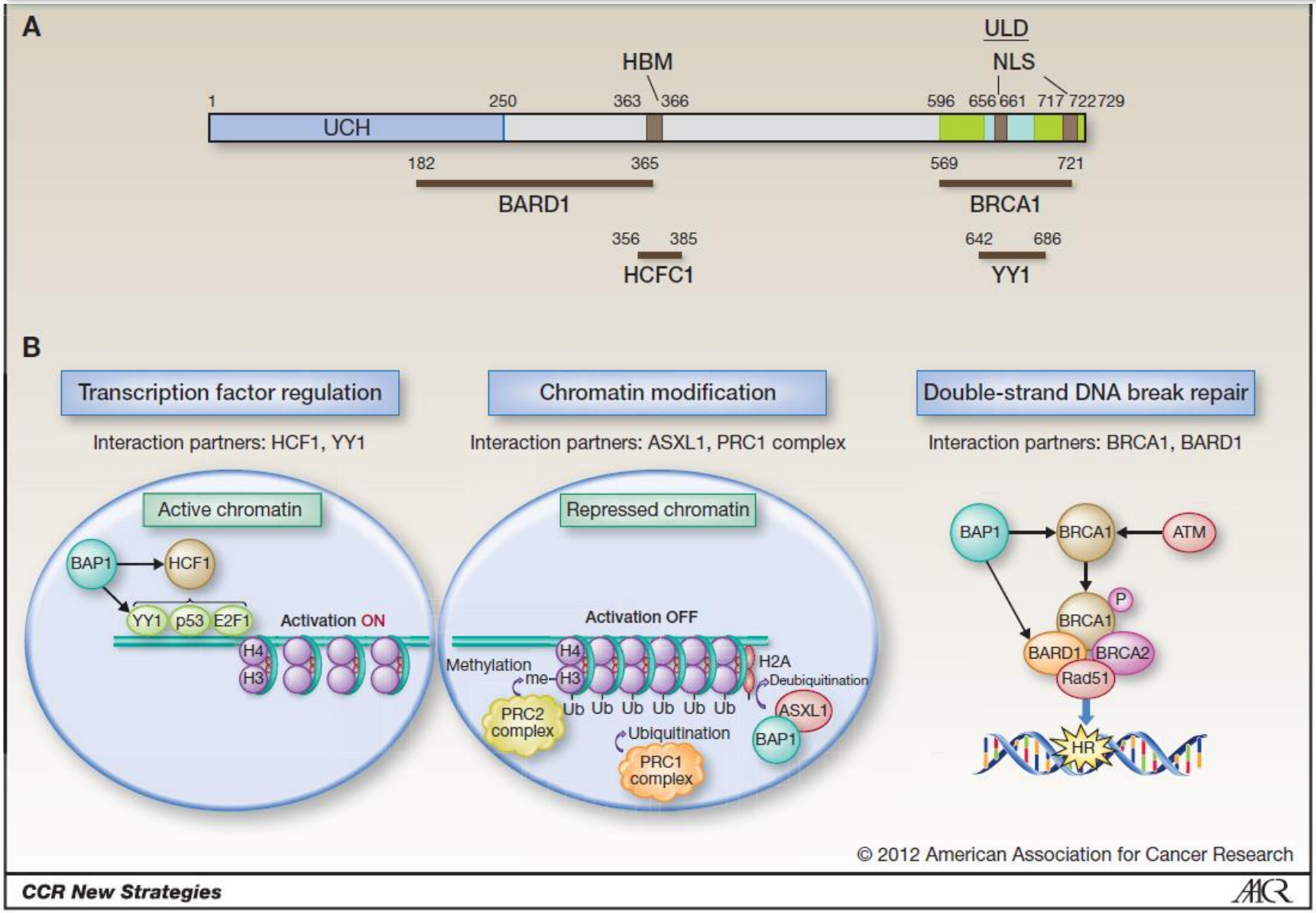
16/53 tumors show genomic loss of *BAP1* (30%)

Bott et al. Nature Genetics 43, 668–672, 2011.

Neoplasms with frequent somatic mutations of BAP1

- Mesothelioma
- Uveal Melanoma
- Clear cell renal carcinoma
- Intrahepatic cholangiocarcinoma
- Special benign cutaneous melanocytic lesions
  - “melanocytic BAP1-mutated atypical intradermal tumors” (MBAITs) / epithelioid atypical Spitz tumours / “Wiesner nevus”

BAP1 protein function: which one is relevant to MPM?



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Clinical features of patients with *BAP1* mutant versus *BAP1* normal mesothelioma

→ patients whose tumors contained *BAP1* mutations were more likely to have smoked.

	BAP1 mutant % (N=24)	**BAP1 normal % (N=97)**	**p-value**
Sex (M/F)	79/21	68/32	0.33
Median age	65	63	0.31
Asbestos exposure	54	46	1
Former and current smoking	75	42	**0.006**
Family history of mesothelioma	4	2	1
Family history of cancer	38	47	0.49
Personal history of cancer	8	8	0.46

Zauderer et al., J Thor Oncol 2013

Clinical Characteristics of Patients with BAP1-mutated mesothelioma

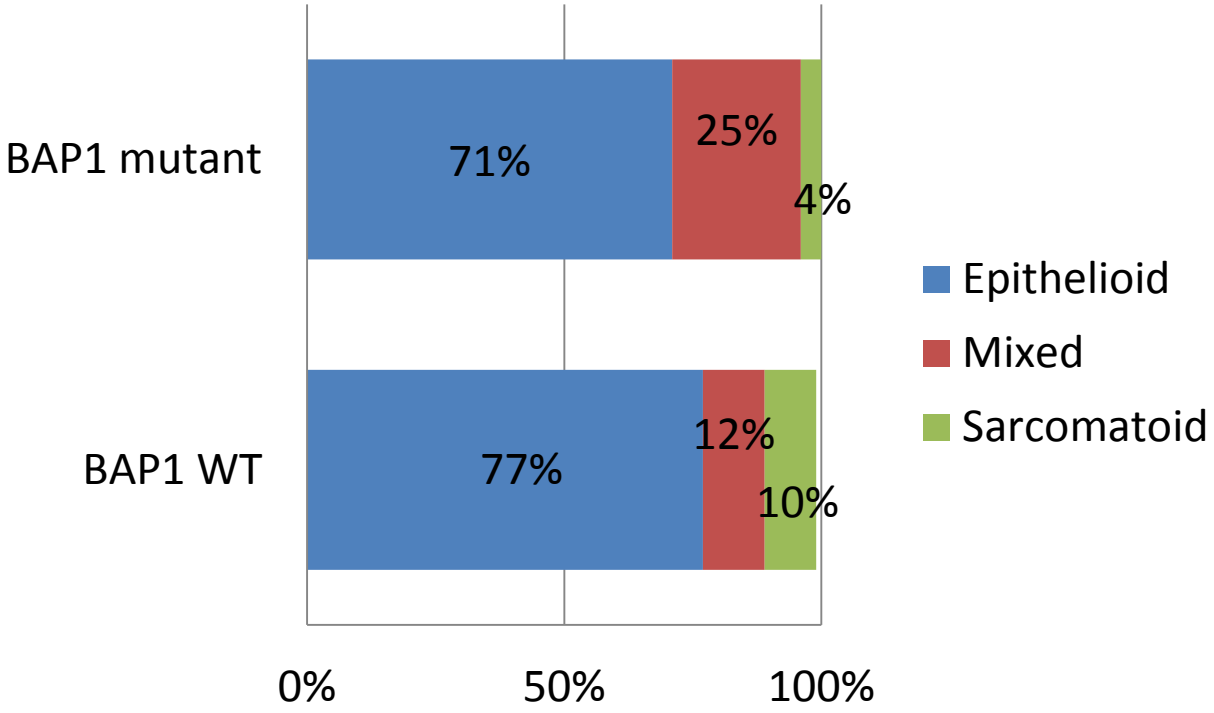
- Age Analysis:

Group	Mean/Median (Range) Age at Diagnosis
+ BAP1 Mutations, Bott et al. Population (n=24)	65 (42-74)
No BAP1 Mutation, Bott et al. Population (n=97)	63 (33-81)
+ Germline BAP1 Mutation (n=22), Carbone et al.	55 (37-85)
National Median Age at Diagnosis	74

- BAP1 mutations were associated with Tobacco Use:
  - 75% of the BAP1 Mutation Positive patients were Current or Former smokers, compared to 42% of the BAP1 WT Population (chi^2 p-value=0.006)
  - 2/24 mutations are point mutations classically associated with smoking
  - Possible mechanism: BAP1 mutation occurs independent of smoking, and then increases sensitivity to carcinogenic effects of cigarette smoke

Zauderer M et al., J Thor Oncol 2013

Distribution of mesothelioma histologic subtype by somatic *BAP1* mutation status



Zauderer M et al., J Thor Oncol 2013

Cancer Science
The official journal of the Japanese Cancer Association

Frequent inactivation of the *BAP1* gene in epithelioid-type malignant mesothelioma

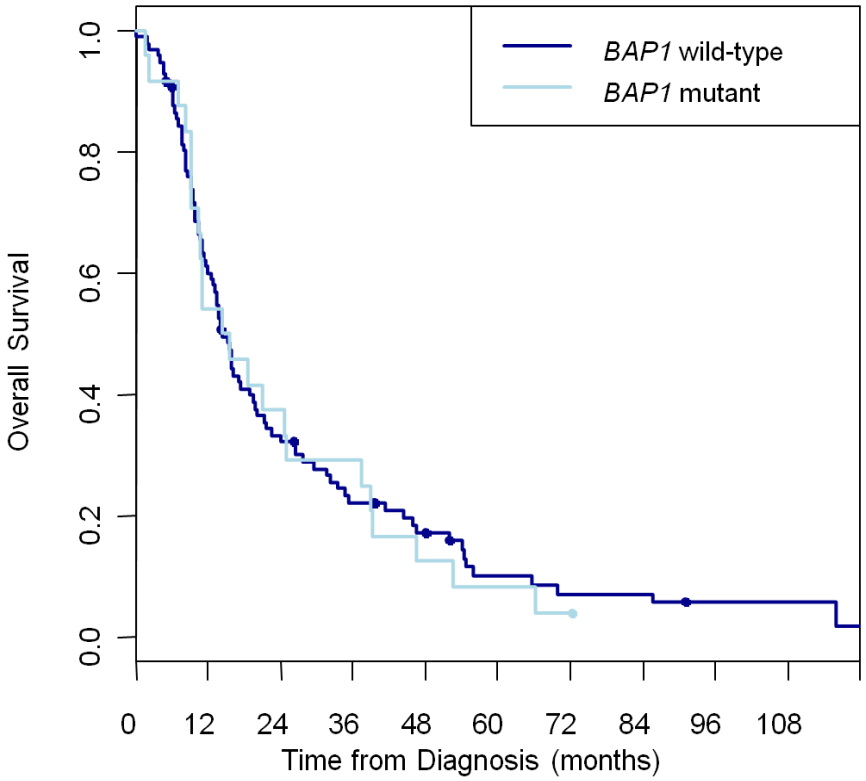
Yoshie Yoshikawa,[1,6] Ayuko Sato,[2] Tohru Tsujimura,[2] Mitsuru Emi,[1,3] Tomonori Morinaga,[1] Kazuya Fukuoka,[4] Shusai Yamada,[4] Aki Murakami,[4] Nobuyuki Kondo,[5] Seiji Matsumoto,[5] Yoshitomo Okumura,[5,7] Fumihiro Tanaka,[5] Seiki Hasegawa,[5] Takashi Nakano[4] and Tomoko Hashimoto-Tamaoki[1]

Cancer Sci | May 2012 | vol. 103 | no. 5 | 868–874

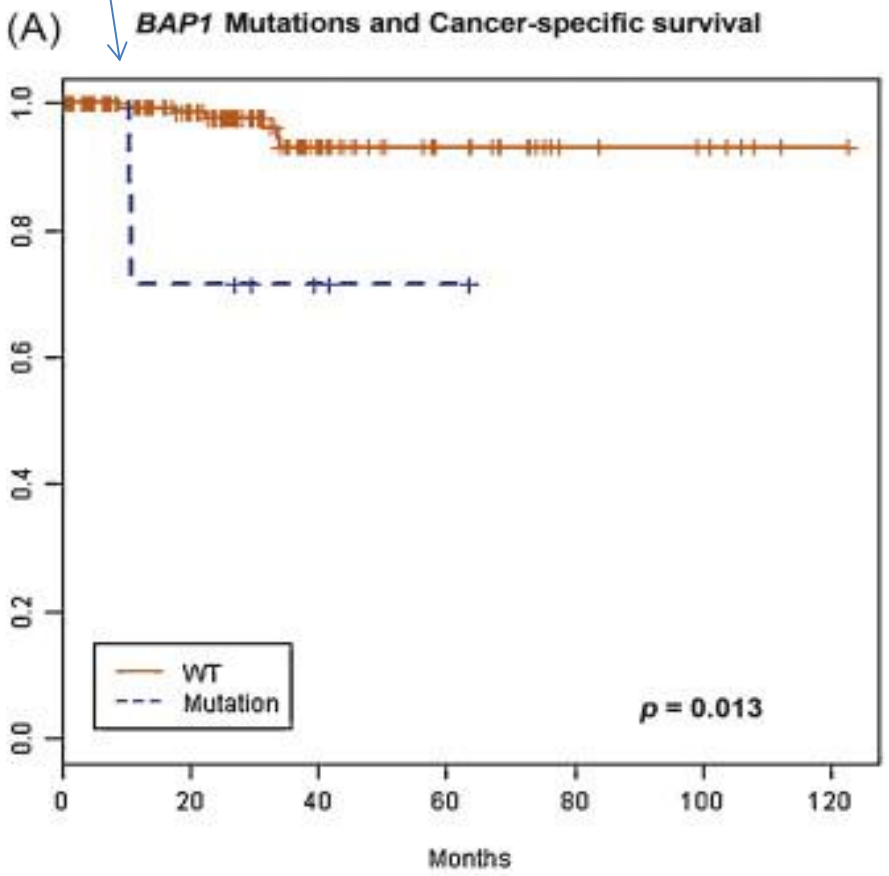
- BAP1 gene mutations found in:
  - 13/16 epithelioid vs 1/7 biphasic or sarcomatous
  - P = 0.005

Impact of *BAP1* mutation status on survival?

→ No in mesothelioma vs Yes in other cancers



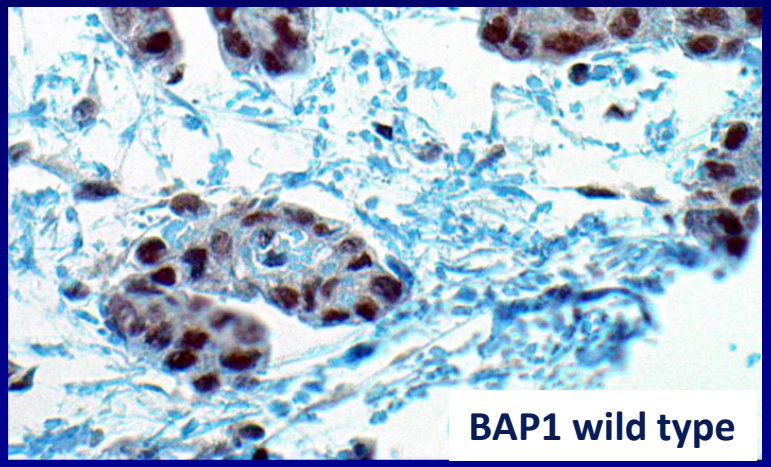
MSKCC Pleural Mesothelioma patients
Zauderer et al., J Thor Oncol 2013

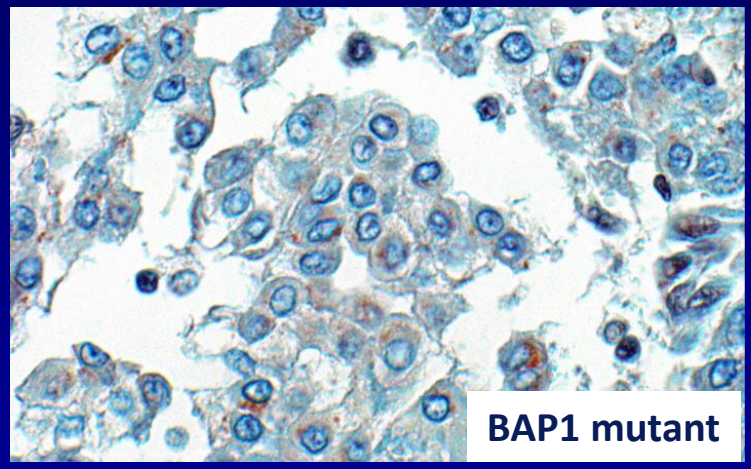


MSKCC Clear Cell Renal Carcinoma patients
Hakimi et al. , European Urology 2012

BAP1 IHC in mesothelioma

- Correlation with *BAP1* expression:
  - mRNA: Tumors with BAP1 genomic losses have lower *BAP1* mRNA expression than those without ($p < 0.0001$) in Affy expression data
  - protein: Tumors with loss and/or mutation also show loss of BAP1 staining in tumor cells by IHC ($p = 0.002$)





  - ..but some cases can have point mutations in ubiquitin hydrolase domain of BAP1 and express non-functional protein
  - ? use BAP1 IHC to select screen mesothelioma patients for germline BAP1 testing

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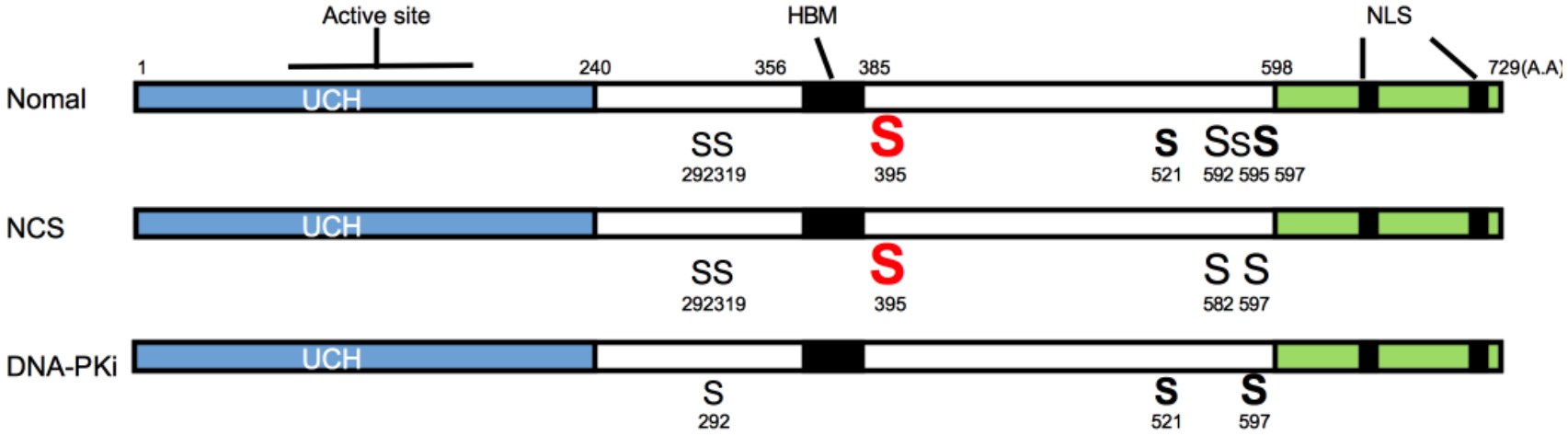
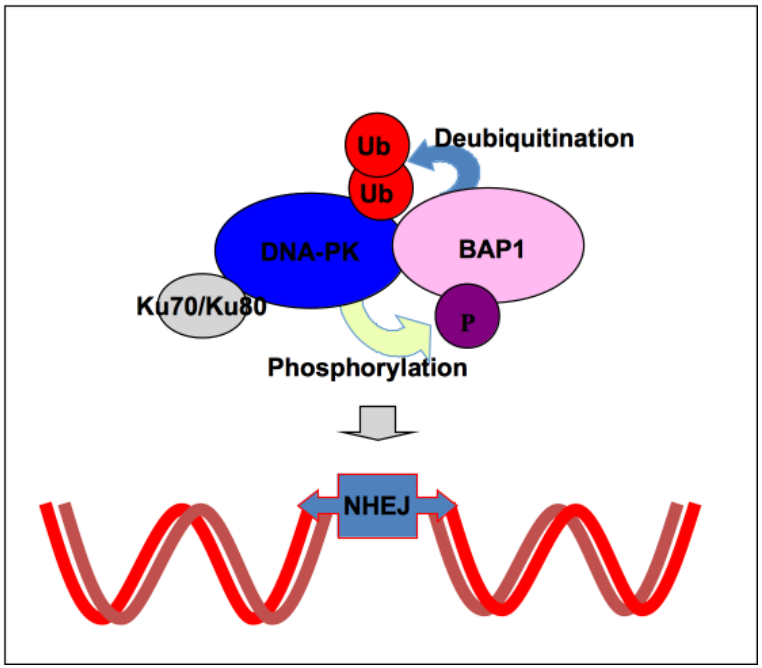
Phosphorylation of BAP1 after DNA damage





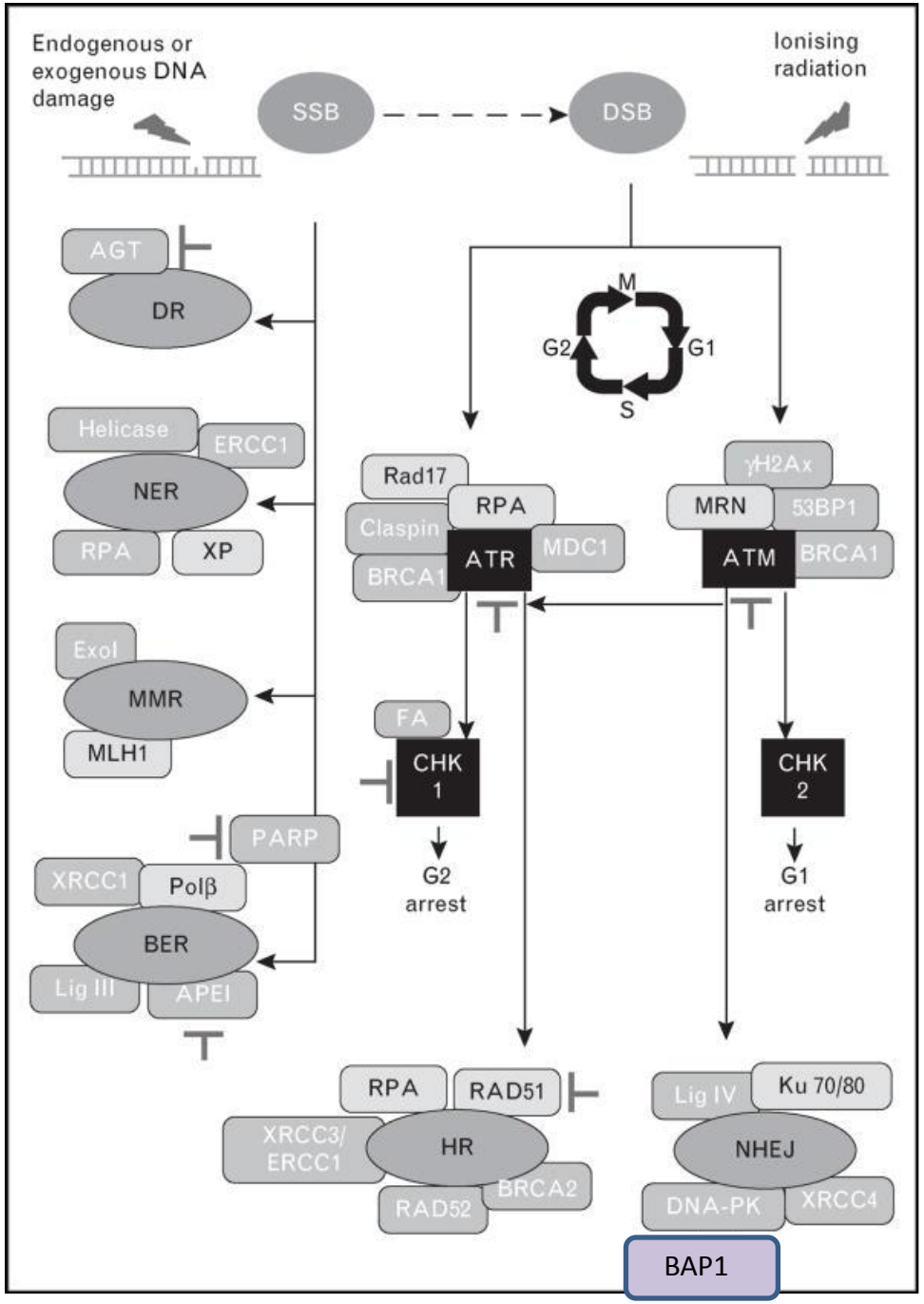
- BAP1 is involved in the non-homologous end joining (NHEJ) pathway of double strand DNA repair through interaction with DNA-PK

- Proposed model: phosphorylation of BAP1 by DNA-PKcs upon DNA damage increases BAP1-mediated de-ubiquitination of DNA-PKcs, stabilizing it at foci of NHEJ DNA repair.

- These data suggest that synthetic lethal targets may exist in DNA repair pathways in BAP1-deficient cells.

Tatsuo Ito

Does BAP1 inactivation in MPM lead to new drug sensitivities based on its role in DNA repair?



Targeting the DNA damage response in oncology: past, present and future perspectives.
Basu, Bristi; Yap, Timothy; Molife, L; de Bono, Johann
Current Opinion in Oncology. 24(3):316-324, 2012.

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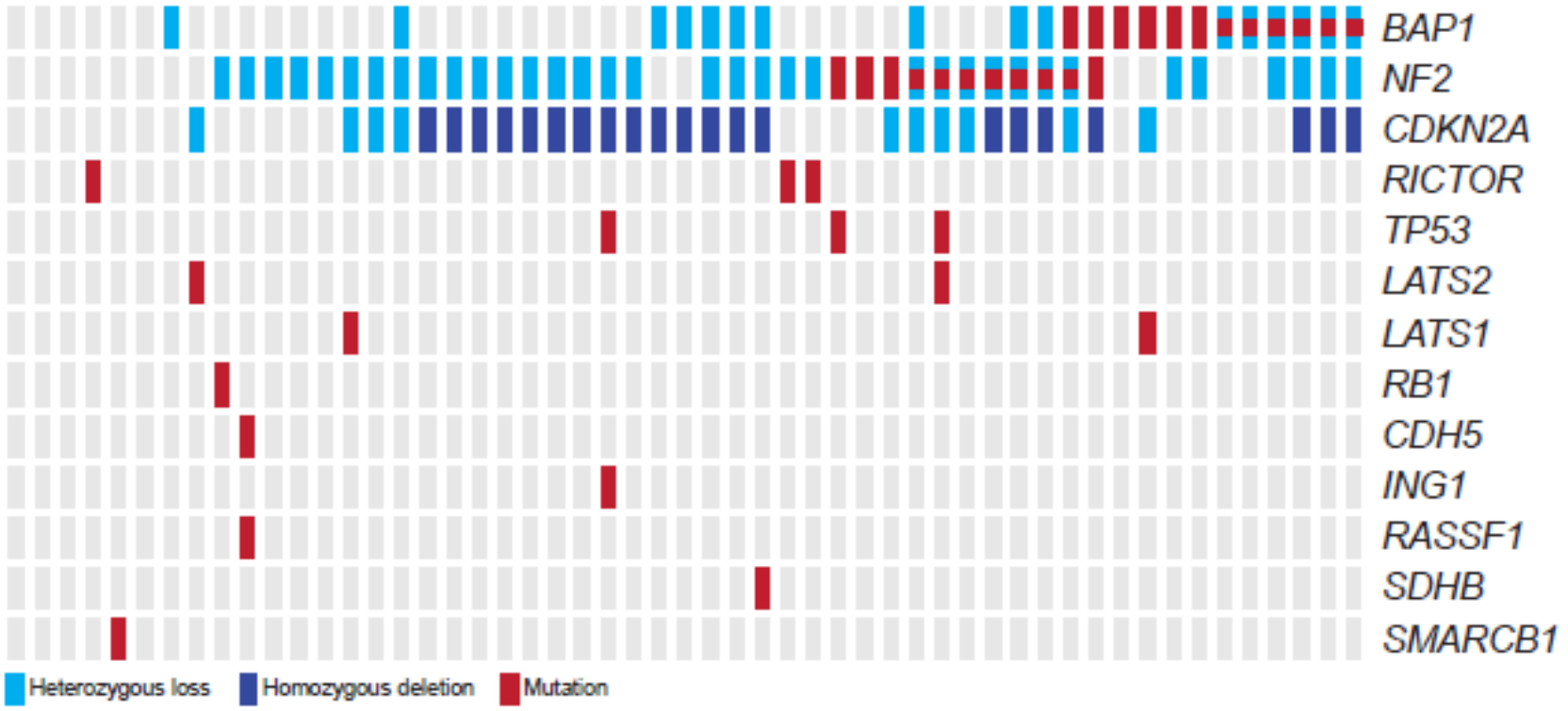
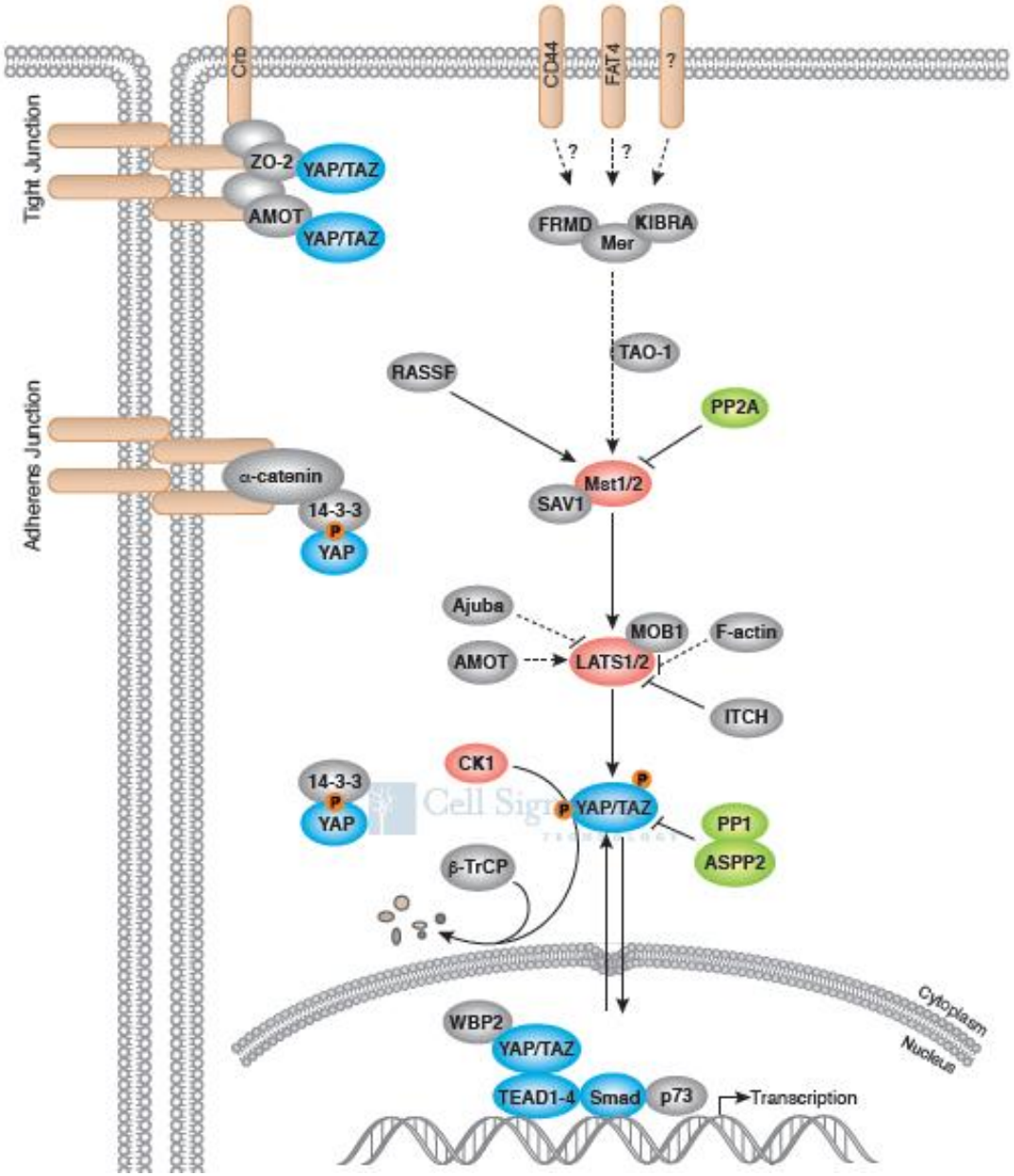
“Oncoprint” map of mesothelioma



N=53 tumors

Bott et al. Nature Genetics 43, 668–672, 2011.

LATS2: Hippo Signaling pathway



Hippo signaling is an evolutionarily conserved pathway that regulates cell proliferation, apoptosis, and stem cell self renewal. The Hippo pathway includes a kinase cascade, wherein Mst1/2 kinases and Sav1 form a complex to phosphorylate and activate LATS1/2. LATS1/2 kinases in turn phosphorylate and inhibit the transcription co-activators YAP and TAZ, two major downstream effectors of the Hippo pathway. When dephosphorylated, YAP/ TAZ translocate into the nucleus and interact with TEAD1-4 and other transcription factors to induce expression of genes that promote cell proliferation and inhibit apoptosis.

Adapted from Cell Signaling Technologies, Inc

Cancer Res 2011;71:873-883.

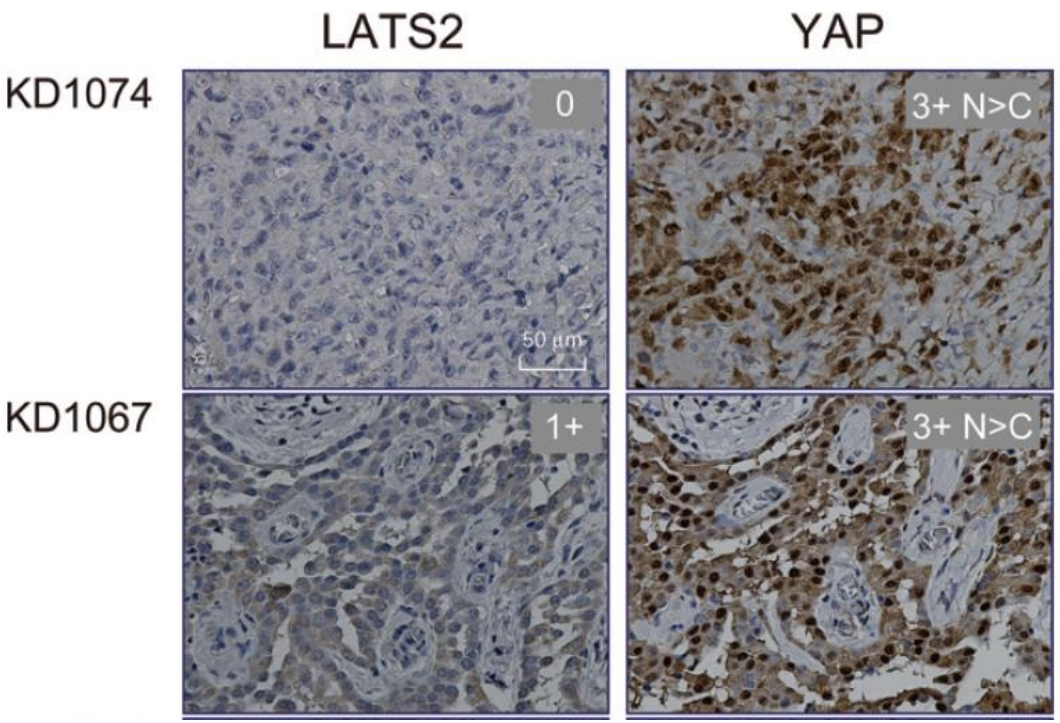
Molecular and Cellular Pathobiology

Cancer Research

LATS2 Is a Tumor Suppressor Gene of Malignant Mesothelioma

Hideki Murakami[1], Tetsuya Mizuno[1,4], Tetsuo Taniguchi[1,4], Makiko Fujii[1], Futoshi Ishiguro[1,4], Takayuki Fukui[2], Shinya Akatsuka[6], Yoshitsugu Horio[3], Toyoaki Hida[3], Yutaka Kondo[1], Shinya Toyokuni[6], Hirotaka Osada[1,5], and Yoshitaka Sekido[1,5]

Cell line	*NF2*[a]	*LATS2*
NCI-H290	HD	+
NCI-H2373	HD	+
ACC-MESO-1	Q389X	+
Y-MESO-9	NM_000268:c.527_528del2	
Y-MESO-12	HD	
Y-MESO-22	HD	
Y-MESO-25	NM_000268:c.532_571del40	
Y-MESO-14	Q196X	
Y-MESO-26B	HD	
NCI-H2052	R341X	
Y-MESO-21	+	
Y-MESO-27	+	
Y-MESO-30	+	
MSTO-211H	+	
Y-MESO-28	–[b]	
Y-MESO-8D	–[b]	
NCI-H28	+	
NCI-H2452	+	
ACC-MESO-4	+	
Y-MESO-29	+	



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    - Higher in cell lines (up to 35%)
  - **TP53**: relatively rare but may define a distinct MPM subset
    - Fletcher 2014
- **Somatic mutations in oncogenes: rare**

Screening mesothelioma samples for oncogene point mutations common in other cancers

- Mass spectrometry-based mutation analysis (Sequenom) of known oncogenic point mutations in 87 tumors:

  KRAS, NRAS, HRAS, EGFR, BRAF, PIK3CA, ERBB2, MEK1, AKT

  – Only 3 mutations identified in 87 MPM samples – confirmed by sequencing

Sample Number	Mutation	Previously described in MM
18	BRAF V600E	No
38	KRAS G12D	No
97	NRAS Q61L	No

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Molecular Biology of Mesothelioma: Implications for the development of new targeted therapies

Marc Ladanyi, M.D.

Molecular Diagnostics Service
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Memorial Sloan-Kettering Cancer Center
New York, NY, USA

THANK YOU