What Is TP53 ?
TP53 Is A Tumor Suppressor Gene

- p53+/+ → 1% at 18 months
- p53+/- → 2% at 9 months
- p53-/- → 75% at 6 months

Donehower et al. 1992
TP53 is Inactivated By Somatic Mutations In Most Human Cancers

![Somatic mutations - Mutation Prevalence](image)
TP53 in the Genomic Era: TP53 is the Most Frequently Mutated Gene in Most Cancers

Exonic mutation frequencies from WES/WGS studies (COSMIC v65)

TP53
TP53 Germline Mutations Are Responsible For Li-Fraumeni Syndrome

The **Li-Fraumeni syndrome** (LFS, OMIM# 151623) is a rare autosomal disorder characterised by a familial clustering of early onset tumors (<45), with a predominance of sarcomas, breast cancers, brain tumors and adrenocortical carcinomas.

In 1990, Malkin et al. found that this syndrome may be caused by a germline mutation in the TP53 gene.
TP53 Encodes The p53 protein, A Transcription Factor

TP53 Encodes The p53 protein, A Transcription Factor

Transactivation (1-42; 43-62)
Proline-rich (63-97)
DNA binding (102-292)
Oligomerisation (323-356)
Regulation (363-393)

Phosphorylation site
Acetylation site

Zn
p53 Is A Stress-activated Emergency Brake For The Control Of Cell Proliferation

Adapted from Soussi (2008) and Geen & Kroemer (2009)
The p53 Pathway

From KEGG database
The p53 pathway: positive and negative feedback loops

From Harris & Levine, 2005
miRNA In The TP53 Pathway (1)
miRNA In The TP53 Pathway (2)

Hermeking, NatRevCancer, 2012
miRNA In The TP53 Pathway (3)

Hermeking, NatRevCancer, 2012
p53 and Ageing

Rufini et al., Oncogene, 2013
p53 and Metabolism:
p53 regulates glucose catabolism
Figure 2. p53 regulates fatty acid metabolism in cancer cells. p53 regulation in fatty acid anabolism and catabolism is partially mediated by the activation of AMPK. p53 upregulates AMPK expression to inactivation of ACC and, thereby, inhibits de novo fatty acid synthesis. In addition, p53 increases fatty acid oxidation by promoting AMPK expression and activation.

Shen, Clin Cancer Res, 2012
Several Post-Transcriptional Modifications On p53 Regulate p53 Activities

The p53-modifications of specific amino-acid residues are indicated in (a) with P (phosphorylation), and in (b) Ac (acetylation), Ub (ubiquitination), M (methylation), N (neddylation) and SU sumoylation). Proteins responsible for the relevant modifications are depicted in boxes in matching shades of grey.

Abbreviations: ATM, ataxia telangectasia mutated; ATR, ataxia telangectasia and Rad3-related protein; AURKA, aurora kinase A; CDK, cyclin-dependent kinase; Chk, checkpoint kinase; CK, casein kinase; CSN, cop-9 signalosome; DNA-PK, DNA-dependent protein kinase; DYRK2, dual-specificity tyrosine phosphorylation-regulated kinase 2; E4F1, Ubiquitin E3 ligase; ERK, extracellular signal-regulated kinase; GSK3b, glycogen synthase kinase 3 b; HIPK2, homeodomain interacting protein kinase2; hMOF, human ortholog of the Drosophila MOF gene (males absent on the first); JNK c-Jun NH2-terminal kinase; MAPKAPK2, mitogen-activated protein kinase-activated protein kinase2; p38, p38 kinase; MDM2, mouse double minute 2 protein; PCAF, p300/CBP associated factor; PKC, protein kinase C; PKR, double stranded RNA-activated kinase; SET9, SET9 ethyltransferase; SMYD2, SET/MYND domain-containing methyltransferase 2; SUMO, small ubiquitin-like modifier 1; TAF1, TATA-binding protein-associated factor 1; Tip60, Tat-interactive protein. y constitutively phosphorylated and dephosphorylated in response to ionizing radiation. * Modification caused by UV-light only. # Overexpression of AURKA causes phosphorylation of p53 at Ser315

From Olsson et al., 2007
p53 Activation: Breaking p53-mdm2 Association

Genotoxic stress

ATM/ATR
Chk2
CK2

p53
mdm2

p53 ubiquitination and degradation

Proteasome

Target gene
p53 Regulation by miRNA

Hermeking, NatRevCancer, 2012
TP53 Family Members: Similar Structure

Family of Transcription Factors

Transactivation domain  DNA-binding domain  Oligomerisation & Regulation domain
Sequence Identity Between p53, p63, p73 proteins

From Courtois et al., 2004
p63 & p73 Isoforms Are Generated By Alternative Splicing And Alternative Promoters
p53 Isoforms Described In 2005

p53 Consensus Isoforms In 2010

1st International p53 Isoforms meeting, IARC, Lyon, 2010
Role of p53 Splice Variants in Human Malignancy

Surget et al., OncoTargets and Therapy, 2013
TP53 Family Members: Different Functions

**TP53**
- **17p13(h)**
- 23% female death by exencephaly
- **High frequency of tumor development**

**TP73**
- **1p36(h)**
- Hydrocephalus, chronic infections, inflammation, abnormalities in pheromone sensory pathways

**TP63**
- **3q27-28(h)**
- Defects in the limb, craniofacial and epithelial development.
- **No Skin!**
TP53 Family Members: Mutations In Human Diseases

**TP53**
17p13(h)

- Somatic mutations frequent in almost all cancer types
- Germline mutations cause Li-Fraumeni syndrome

**TP73**
1p36(h)

- LOH or isoforms overexpression in some tumor types, but no somatic mutation
- No disease causing germline mutation

**TP63**
3q27-28(h)

- Isoforms overexpression in some tumor types, but no somatic mutation
- Germline mutations cause developmental disorders but not cancer
**Origin and Evolution of TP53 Family**

*Figure 2.* Scenario of the p53 family gene evolution. The earliest indication of p53 family genes dates back to invertebrates and unicellular choanoflagellae. Aside from independent gene duplications, such as in sea anemone or mosquitos, the earliest indication for mammalian gene splitting dates back to cartilaginous fishes, with tentative orthologs visible in elephant sharks, and all three mammalian paralogs appearing in bony fishes.

Belyi et al., CSHL, 2010
<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1979</td>
<td>discovery</td>
</tr>
<tr>
<td>1983</td>
<td>defined as an oncogene</td>
</tr>
<tr>
<td>1985</td>
<td>cloning of the human gene</td>
</tr>
<tr>
<td>1989</td>
<td>the WT form is defined as a tumor suppressor</td>
</tr>
<tr>
<td>1989</td>
<td>LOH and first mutations identified in cancer</td>
</tr>
<tr>
<td>1990</td>
<td>TP53 is constitutively mutated in Li-Fraumeni syndrome</td>
</tr>
<tr>
<td>1990</td>
<td>p53 is a transcription factor</td>
</tr>
<tr>
<td>1991</td>
<td>participation of p53 in the cellular response to DNA damage</td>
</tr>
<tr>
<td>1991</td>
<td>p53 induces apoptosis</td>
</tr>
<tr>
<td>1991</td>
<td>selective G to T mutation of TP53 gene in HCC from Africa</td>
</tr>
<tr>
<td>1992</td>
<td>TP53-/- mice develop tumors spontaneously</td>
</tr>
<tr>
<td>1993</td>
<td>p53 induces G1 arrest via p21Waf1</td>
</tr>
<tr>
<td>1993</td>
<td>TP53 mutation is associated with poor prognosis in breast cancer</td>
</tr>
<tr>
<td>1993</td>
<td>p53 induces G1 arrest via p21Waf1</td>
</tr>
<tr>
<td>1994</td>
<td>TP73, first TP53 related gene discovered</td>
</tr>
<tr>
<td>1996</td>
<td>p53 is induced by hypoxia</td>
</tr>
<tr>
<td>1997</td>
<td>role of mdm2 in the regulation of p53 stability demonstrated in mice</td>
</tr>
<tr>
<td>1999</td>
<td>TP73, first TP53 related gene discovered</td>
</tr>
<tr>
<td>1999</td>
<td>10,000 human mutations described</td>
</tr>
<tr>
<td>1999</td>
<td>p53 plays a role in DNA repair</td>
</tr>
<tr>
<td>2002</td>
<td>constitutive expression of p53 accelerates ageing in mice</td>
</tr>
<tr>
<td>2002</td>
<td>discovery of a N-terminally truncated variant of p53</td>
</tr>
<tr>
<td>2003</td>
<td>p53 plays a role in global chromatin remodelling</td>
</tr>
<tr>
<td>2004</td>
<td>wt p53 and mutant p53 are targeted for cancer therapy</td>
</tr>
<tr>
<td>2005</td>
<td>description of nine p53 protein isoforms</td>
</tr>
<tr>
<td>2006</td>
<td>p53 plays a direct role in cellular metabolism</td>
</tr>
<tr>
<td>2007</td>
<td>p53 regulation of microRNAs, a new layer of complexity in the p53 network</td>
</tr>
<tr>
<td>2008</td>
<td>a dual role for p53 in autophagy is described</td>
</tr>
<tr>
<td>2009</td>
<td>p53 deficiency help cellular reprogramming and stem cells production</td>
</tr>
</tbody>
</table>
An Elephant in the Room!
Elephant cells have 40 copies of *TP53* gene

Compared with other mammalian species, elephants appeared to have a lower-than-expected rate of cancer.

Compared with human cells, elephant cells demonstrate increased apoptotic response following DNA damage.

Abegglen et al., JAMA, 2015
The IARC TP53 Database
A Locus Specific Database To Study TP53 Gene Variations In Human Cancers

p53.iarc.fr/
Why Study TP53 Mutations?

TP53 somatic mutations are frequent in most types of sporadic human cancers (frequencies vary from 5% to 70% depending on cancer type and stage).

TP53 mutations may also be inherited in families with a predisposition to multiple cancers, as in the Li-Fraumeni syndrome (LFS).

Over 30,000 mutations have been reported in the literature, with more than 2300 different missense mutations.

In several cancers, the nature of TP53 mutations and their distribution along the coding sequence have allowed the identification of tumor-specific mutation spectra, revealing clues on the mechanisms that might have caused the mutation.

The presence of a TP53 mutation may be predictive of the tumor response to treatment and patient survival.

TP53 mutations useful in:
- molecular epidemiology of cancer
- molecular genetics
- molecular pathology of cancer
- structural biology
The IARC Database:
Information System For TP53 Mutations

- Extract TP53 mutation data from publications
- Organize and annotate data into a format that allows easy retrieval and analysis
- Provide a web-based tool to analyse TP53 mutation patterns in cancers
Data Available

- Somatic mutations in human sporadic cancers
- Germline mutations and Li-Fraumeni syndrome
- Polymorphisms in human populations
- TP53 status in cell-lines
- Structure/Function properties of mutant proteins
- Mouse-models with engineered TP53 gene
- Experimentally-induced mutations
Criteria For Inclusion

- **TP53 somatic mutations** associated with human sporadic cancers that have been identified by sequencing and published in peer-reviewed literature. This includes mutations found in normal, pre-neoplastic and neoplastic tissues, including metastases, as well as in cell-lines derived from such tissues.

- Human TP53 **germline mutations** (identified by sequencing and published in peer-reviewed literature) in individuals affected or not by a cancer.

- p53 mutants that have been tested in human cells or yeast assay for **functional activities** such as specific DNA-binding, transcriptional activation, dominant-negative effects on the wild-type protein, gain of function...

- **Mouse-models** with engineered TP53 gene that are included in caMOD database or have been reported in peer-reviewed literature.

- **Experimentally-induced mutations** obtained in the Hupki mouse models or a yeast assay of mutagenesis.
Database Structure And Contents


- **SOMATIC**
  - Tumor pathology
  - Molecular alterations
  - Patient demographics
  - Patients' life-style
  - Mutation prevalence
  - Prognostic value

- **CELL LINES**
  - Tumor pathology
  - Patient demographics
  - ATCC link
  - COSMIC link

- **GERMLINE**
  - Tumor pathology
  - Individual demographics
  - Family structure
  - Mutation prevalence*

- **EXPERIMENTALLY INDUCED**
  - Cell model
  - Mutagen tested

- **TP53 GENE VARIATIONS**
  - Genomic, coding and protein description
  - Effect

- **POLYMORPHISMS**
  - Validated SNP
  - Link to dbSNP

- **MOUSE MODELS**
  - Affected tissues
  - CaMOD link
  - PubMed link

- **STRUCTURE/FUNCTION**
  - Structural properties
  - Functional assessment
  - Predicted impact

* New in R17
Web Analysis Tools
Database Figures And Facts

- **Database contents:**
  - >29,000 somatic mutations
  - >880 germline mutations
  - >2,300 mutants with functional properties
  - >180 studies on TP53 mutation and clinical outcome
  - > Mouse models with engineered p53
  - > 900 experimentally-induced mutations linked to human exposures

- **Database usage**
  - > 8000 visits per month
  - > 600 downloads per month
  - > 4500 citations in the scientific literature
  - Links with COSMIC, SwissProt, ATCC, HGVS, …
The IARC TP53 Database & Genomic Data Repositories

~ 29,000 somatic mutations in IARC TP53 Database

~ 29,000 TP53 somatic mutations in COSMIC (includes >65% of IARC data)

~ 7,100 TP53 somatic mutations in cBioportal

~ 1,000 TP53 somatic mutations in ICGC data portal

➔ IARC Database has more data on somatic mutations and a broader scope of data (germline mutations, functionnal impact of mutations, exposure-induced mutations,...)
IARC TP53 DATABASE: A Resource For Various Disciplines

- Molecular Epidemiology
- Molecular Pathology
- Medical Genetics
- Structure/Function Research

TP53 MUTATION DATABASE
- Molecular data
- Functional data
- Epi data
- Clinical data
- Recommendations and Guidelines
TP53 Mutations In Human Cancers
Types Of Genetic Alterations In Cancer

Somatic: Acquired during development and present only in cells undergoing clonal expansion

Inherited: present in the germline and detectable in both healthy and cancer cells

- Loss of parts or whole chromosomes
- Duplication of chromosomes
- Chromosome translocations
- Amplifications of chromosome fragments

- Intragenic deletions or insertions
- Recombination between adjacent genes
- Nonsense (Stop) mutations
- Missense mutations (substitutions)
- Methylation
TP53 Somatic Mutations are Frequent in Human Cancers

![Bar chart showing mutation prevalence for various tumor sites](chart.png)
TP53 Germline Mutations Predispose To Several Types of Cancers

Tumors Associated with TP53 germline mutations (N = 1485)
© IARC TP53 Database, R17, November 2013

- Breast: 27.81% (413)
- Soft Tissues: 13.8% (205)
- Brain: 12.93% (192)
- Adrenal Gland: 10.91% (162)
- Other: 8.69% (129)
- Bones: 8.69% (129)
- Hematological: 3.77% (56)
- Colorectum: 2.9% (43)
- Skin: 2.69% (40)
- Lung: 2.29% (34)
- Ovary: 1.75% (26)
- Stomach: 1.28% (19)
- Kidney: 1.08% (16)
- Testis: 0.47% (7)
- Prostate: 0.27% (4)
- Liver: 0.27% (4)
- Larynx: 0.2% (3)
- Head & Neck: 0.13% (2)
- Bladder: 0.07% (1)
p53 Protein is Targeted by Viruses

Polyomaviruses

- pRb
- p53
- Large T (708 aa)

Adenoviruses

- pRb
- E1A (283 aa)
- p53
- E1B (390 aa)

Papillomaviruses

- pRb
- E7 (98 aa)
- E6-AP
- p53
- E6 (158 aa)
In specific types of cancers where TP53 mutations are unfrequent, p53 protein is inactivated by protein interactions.

<table>
<thead>
<tr>
<th>Cancer</th>
<th>TP53 mutation frequency</th>
<th>Inactivating protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroblastoma</td>
<td>&lt; 2%</td>
<td>Twist</td>
</tr>
<tr>
<td>Sarcomas</td>
<td>&lt; 20%</td>
<td>Mdm2</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>&lt; 1%</td>
<td>Mdm4</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>&lt; 10%</td>
<td>E6 (HPV)</td>
</tr>
</tbody>
</table>
The Majority Of TP53 Mutations Are Missense Mutations

- **Germline**
  - missense: 6%
  - frameshift: 7%
  - nonsense: 8%
  - silent: 7%
  - other: 7%
  - splice: 8%
  - Total: 72%

- **Somatic**
  - missense: 5%
  - frameshift: 4%
  - nonsense: 2%
  - silent: 7%
  - other: 9%
  - splice: 7%
  - Total: 73%

p53.iarc.fr
Missense Mutations are Clustered in the DNA-binding Domain

<table>
<thead>
<tr>
<th>Domain</th>
<th>Mut. frequency</th>
<th>Missense mut.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transactivation (1-42; 43-62)</td>
<td>1 %</td>
<td>50.8 %</td>
</tr>
<tr>
<td>Proline-rich (65-97)</td>
<td>2.3 %</td>
<td>45.4 %</td>
</tr>
<tr>
<td>DNA binding (102-292)</td>
<td>80 %</td>
<td>82.1 %</td>
</tr>
<tr>
<td>Oligomerisation (323-356)</td>
<td>3.4 %</td>
<td>36.4 %</td>
</tr>
<tr>
<td>Regulation (363-393)</td>
<td>0.3 %</td>
<td>72.7 %</td>
</tr>
</tbody>
</table>
Post-Translational Modification Sites Are Rarely Mutated In Cancer

Number of missense mutations reported in sporadic cancers (IARC TP53 database, R12).
Most Frequent Mutations Are In The Loops That Make Contact With DNA

Codon: 175 > 248 > 273 > 282 > 249 > 245 > 220 > 176
Effects Of The Most Frequent TP53 Mutations

<table>
<thead>
<tr>
<th>Codon</th>
<th>Residue</th>
<th>Mutant</th>
<th>Effects on protein structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>175</td>
<td>Arg</td>
<td>His</td>
<td>Breaks crucial H-bond bridging loops L2 and L3</td>
</tr>
<tr>
<td>248</td>
<td>Arg</td>
<td>Gln</td>
<td>Breaks main contact with DNA in minor groove</td>
</tr>
<tr>
<td>273</td>
<td>Arg</td>
<td>His</td>
<td>Breaks main contact with DNA in major groove</td>
</tr>
<tr>
<td>248</td>
<td>Arg</td>
<td>Trp</td>
<td>Breaks main contact with DNA in minor groove</td>
</tr>
<tr>
<td>273</td>
<td>Arg</td>
<td>Cys</td>
<td>Breaks main contact with DNA in major groove</td>
</tr>
<tr>
<td>282</td>
<td>Arg</td>
<td>Trp</td>
<td>Destabilizes H2 helix and DNA binding in the major groove and breaks contacts on the β- hairpin</td>
</tr>
</tbody>
</table>
Biological Activities Of p53 Mutant Proteins

WT p53

- Dominant Negative
  - Loss Of Function
  - Retained Function
  - DNA-Binding, Transactivation, Repression

Growth suppression

Mutant p53

- Gain Of Function
  - Protein interactions Transactivation, Repression, ....

Growth promotion

Temperature Sensitivity
Models of Mechanisms Through Which p53 Mutants Function

1. Mutant p53 interacts with DNA directly using mutant p53 binding elements or other regions on the DNA, including MARs, to regulate transcription. Transcriptional cofactors and other proteins can be involved.

   - Examples: PML, EGR1, TOP1
   - p300

2a. Mutant p53 enhances transcription by forming a complex with TFs that can include transcriptional cofactors and other proteins.

   - Examples: EGR1, TopBP1, PIN1, VDR, ETS1, NF-xB, p63, p73, SP1, SREBP, NF-Y, ETS2, E2F1
   - p300, HDAC, CBP

2b. In response to a stimulus, mutant p53 is recruited to a transcription regulatory complex that can include TFs, transcriptional cofactors and other proteins. This mostly results in activation of target gene expression.

   - Examples: VDR, PLK2, NF-Y, SP1
   - p300
   - stimulus: TPA, vitamin D, DNA damage

3. Mutant p53 decreases transcription by binding TFs and/or transcriptional cofactors and other proteins, sometimes preventing their binding to DNA. This activity can also involve aggregation of mutant p53 with other proteins.

   - Examples: TopBP1, ANKRD11, VDR, SMAD2
   - p63, p73, SP1
   - p300

4. Mutant p53 interacts with other proteins, not directly involved in transcriptional regulation, and enhances or blocks their function.

   - Examples: NRD1, EFEMP2, TOP1, BTG2, MRE11

Muller & Vousden, NatCellBiol, 2012
How to Interpret Mutation Patterns?

- Nature of carcinogen
- Type of mutation
  - Position and sequence context
  - Strand bias/asymmetry
- Metabolization and intracellular processing
- Effects of the mutation on cell behavior
- Cellular strategies to cope with the lesion: DNA repair

ADAPTATION
Sequence Context: CpG Mutations Are The Most Frequent Type Found In Human Cancers

C>T mutations occur frequently at CpG sites by endogenous mechanisms

From Yang et al., 1995
Strand Bias

There is a strand bias (or strand asymmetry) when a mutation event occurs preferentially on one strand of DNA.
Interplay Between Mutagenesis And Biological Selection

Most frequent mutations are those arising from mutation events with high dinucleotide substitution rates and that produce non-functional proteins.

*Substitution rates calculated according to dinucleotide substitution rates derived from human-mouse aligned sequences of chromosomes 21 and 10 (Lunter and Hein 2004) represent an estimate of the expected frequency of mutation events.
The Hupki Mouse And HUF Assay: New Tools To Investigate Human p53 Mutagenesis In Vivo

HUF is an embryonic fibroblast immortalization assay that uses cells from the Hupki mice, which can be used as an in vitro approximation of p53 gene mutagenesis in human cancer development.

Adapted from Hollstein
A Specific Mutation Pattern In Lung Cancer From Smokers

G:C>T:A

G>T mutations are more frequent in lung cancers from smokers than in non-tobacco related cancers.

Adapted from Pfeifer et al. Oncogene (2002)
Mutagenesis And Selection in Lung Cancer

Lung cancers

G>T mutations at hotspot residues

Breast, Brain, CRC

% of all mutations

Adapted from Pfeifer et al. Oncogene (2002)
Tobacco carcinogens

- Cigarettes contain many hazardous substances that damage the lungs when inhaled.
- Enlarged view of air sacs (alveoli)
- Damaged air sacs (alveoli)

- Tobacco carcinogens per cigarette:
  - 4-Aminobiphenyl: 15-40 mg
  - 2-Naphthylamine: 10-23 mg
  - Benzo(a)pyrene: 20-600 mg
  - Acrylamide: 20-50 mg
  - 4-Nitroquinoline N-oxide: 0-200 mg
  - 4-OH-aminobiphenyl: 1.3-16 mg
  - 2-Naphthylamine: 20-70 mg
  - Benzo(a)pyrene: 2.4-4.6 ng
  - 4-Aminobiphenyl: 1.7-22 ng

Source: IARC monograph vol 138
TP53 Mutations Hotspots In Lung Tumors Of Smokers Coincide With Experimentally Induced B(a)P Adducts

Benzo(a)pyrene

G to T Codons 157, 158, 248, 273
Lung cancer: 30%
Other cancers: <10%

From Pfeifer et al.
TP53 Mutations Induced By B(a)P In the HUF assay Are Similar To The One Found In Lung Tumors Of Smokers

From Hollstein et al.

Table 1. $p53$ gene mutations in HUF cell lines derived from BaP-exposed and untreated primary cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Base change*</th>
<th>Amino acid substitution</th>
<th>Lung cancer $p53$ mutation† (rank)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bap-treated³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HUF-BaP-4</td>
<td>GCC to GGC (C to G)</td>
<td>A 138 G</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GTC to GTT (C to T)</td>
<td>V 157 V</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CGC to CCC (G to C)</td>
<td>R 158 P</td>
<td></td>
</tr>
<tr>
<td>HUF-BaP-13</td>
<td>CGT to CAT (G to A)</td>
<td>R 273 H</td>
<td></td>
</tr>
<tr>
<td>HUF-BaP-14</td>
<td>GGG to TGG (G to T)</td>
<td>G 117 W</td>
<td></td>
</tr>
<tr>
<td>HUF-BaP-D14B</td>
<td>GTC to TTC (G to T)</td>
<td>V 157 F</td>
<td></td>
</tr>
<tr>
<td>HUF-BaP-15</td>
<td>g to t (G to T)</td>
<td>(intron 7)†</td>
<td></td>
</tr>
<tr>
<td>HUF-BaP-16</td>
<td>GAG to GAA (G to A)</td>
<td>E 224F†</td>
<td></td>
</tr>
<tr>
<td>HUF-BaP-20</td>
<td>g to c (G to C)</td>
<td>(intron 5)†</td>
<td></td>
</tr>
<tr>
<td>HUF-BaP-28</td>
<td>GTC to GTT (C to T)</td>
<td>V 157 V</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CGC to CTG (G to T)</td>
<td>R 158 L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>g to t (G to T)</td>
<td>(intron 5)†</td>
<td></td>
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<tr>
<td>HUF-BaP-48</td>
<td>AGA to GGA (A to G)</td>
<td>R 280 G</td>
<td></td>
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<tr>
<td>HUF-BaP-106</td>
<td>CCT to TCT (C to T)</td>
<td>P 278 S</td>
<td></td>
</tr>
<tr>
<td>HUF-BaP-116</td>
<td>GGG to TGG (G to T)</td>
<td>G 279 W</td>
<td></td>
</tr>
</tbody>
</table>
TP53 Mutations In Skin Cancer: Effect Of UV Exposure

Skin SCC in XP patients

- CC > TT: 47%
- Insertion/deletion: 6%
- Other: 24%

Sporadic Skin SCC

- CC > TT: 24%
- Insertion/deletion: 27%
- Other: 3%
TP53 Mutations In Breast Cancer: Effect Of Genetic Background

All Breast

24% AT

BRCA1/2 carriers

36% AT

A:T>C:G
A:T>G:C
A:T>T:A
G:C>A:T
G:C>A:T at CpG
G:C>C:G
G:C>T:A
del
ins
other
Mutations As “Carcinogen Fingerprints”

A mutation can be considered as a carcinogen fingerprint if there is:

✓ Evidence that a characteristic mutation pattern is found in exposed compared with non-exposed individuals. This pattern should be distinct by at least one of the following criterion: type of mutations, site of mutations, strand bias.

✓ Evidence that the suspected carcinogen induces similar mutations in experimental model systems.

✓ Evidence that mutations occur early in tumor development.
# Known TP53 Mutation Fingerprints

<table>
<thead>
<tr>
<th>Source</th>
<th>Mutagen</th>
<th>Adduct</th>
<th>TP53 mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV radiations</td>
<td></td>
<td></td>
<td><strong>CC to TT</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Various codons</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Skin cancer: 15%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Other cancers: &lt;1%</td>
</tr>
<tr>
<td>Aflatoxins</td>
<td></td>
<td></td>
<td><strong>G toT</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Codon 249</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver cancer: &gt;30%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Other cancers: &lt;1%</td>
</tr>
<tr>
<td>Tobacco smoke</td>
<td></td>
<td></td>
<td><strong>G toT</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Codons 157, 158, 248, 273</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lung cancer: 30%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Other cancers: &lt;10%</td>
</tr>
<tr>
<td>Aristolochic acids</td>
<td></td>
<td></td>
<td><strong>A toT</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Codon 131</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Urothelial cancer</td>
</tr>
</tbody>
</table>
Clinical Applications Of TP53 Mutations
From Early Detection To Gene Therapy

- Cancer diagnosis
  - Early detection of cancerous lesion
  - Identification of cancer type
  - Marker of clonality

- Cancer aetiology
  - Identification of cause/exposure

- Patient outcome
  - Predict patient survival
  - Surveillance of recurrence

- Cancer treatment
  - Prediction of treatment response, treatment selection
  - Gene therapy
  - Re-activating TP53 in mutated cells
# Prognostic Value Of TP53 Mutations

<table>
<thead>
<tr>
<th>Tumor Site</th>
<th>Related to Bad Prognosis</th>
<th>Related to Good Prognosis</th>
<th>Not Related to Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>4</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Bones</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Brain</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Breast</td>
<td>28</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Colorectum</td>
<td>16</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Esophagus</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Head &amp; Neck</td>
<td>7</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Hematol.</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Larynx</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Liver</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lung</td>
<td>8</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Ovary</td>
<td>7</td>
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<td>2</td>
</tr>
<tr>
<td>Pancreas</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Prostate</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Soft tissues</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stomach</td>
<td>1</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Renal pelvis</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Number of studies reporting that TP53 mutations are:
  * Only studies with > 50 cases were included.

© IARC TP53 Database 2012
Assessing p53 status: Protein Accumulation vs Mutation

- 95% are negative by IHC
- 90% are negative by IHC
- 90% are negative by IHC
- 96% are positive by IHC

- 23% of cases with mutations may stain negative

Studies using IHC to investigate p53 prognostic value have yielded inconsistent results.

IHC alone is not suitable for assessing TP53 mutation status.
TP53 Mutations Are Associated With Shorter Survival in Breast Cancer Independently Of Stage, Grade And Hormone Receptors Status

Tumor grade <3, tumor size <5 cm, node negative and ER or PR positive cases (204 patients)

B

No mutation

40% decrease in survival at 5 years

p<0.0001

# at risk:

<table>
<thead>
<tr>
<th></th>
<th>Mutation</th>
<th>No mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>190</td>
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<td>175</td>
</tr>
<tr>
<td>157</td>
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<tr>
<td>2</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

Olivier et al., Clin Cancer Res, 2006
TP53 Missense Mutations Within The DNA-binding Loops And Non-Missense Mutations Are Associated With The Worst Prognosis In Breast Cancer

No Mutation
Missense in DBM
Other missense
Non-missense mutations

Olivier et al., Clin Cancer Res, 2006
TP53 Mutations Are Unevenly Distributed Across Molecular Subtypes

Frequency of TP53 mutations:
- Luminal A: 8/49 (16%)
- Luminal B: 12/17 (71%)
- Basal: 12/16 (75%)
- ERBB2+: 18/21 (86%)

p<0.001

115 breast tumors stage III/IV

From Langerod et al., Breast Cancer Res, 2007 & Sorlie et al., PNAS, 2002
Gene Expression Signature Of TP53 Status

Langerod et al., Breast Cancer Res, 2007
Strategies for Efficient Cancer Therapy: Virus-based Therapies

- **ONYX-015**
  - Is an attenuated chimeric human group C adenovirus, that has been developed to preferentially replicates in and lyses tumor cells that are p53 negative (McCormick, Cancer Biol Ther. 2003).

- **Advexin (Ad5CMV-p53)**
  - Is a non-replicating, non-integrating adenoviral vector that carries the p53 gene (Gabrilovich, Expert Opin Biol Ther., 2006).
Strategies for Efficient Cancer Therapy: Small Molecules That Target p53

- Inhibition of mdm2/p53 interaction (*Nutlin-3, RITA*)
  - Kill cancer cells with WT p53

- Reactivation of mutant p53 (*PRIMA-1, CP31398, WR1065, MIRA-1, STIMA-1, RETRA*)
  - Kill cancer cells with MUT p53
Further Information

Selected references at:

p53 story at:
http://p53.free.fr/

p53 Knowledgebase at:

TP53 Mutations in Human Cancers: Origins, Consequences, and Clinical Use

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²LIGHT Laboratories, University of Leeds, United Kingdom