REACTIVIVATION OF P53: TARGET TO TREAT BREAST CANCER

Prasanna Kumar Panda[1], Gitanjali Mishra[2], Ruchi Bhattacharya[3]*, Ritika Singh[3], Renuka Verma[3], Ashish Kumar Netam[3], Jhakeshwar Prasad[3], Trilochan Satapathy[2]

1. University Department of Pharmaceutical Sciences, Utkal University, Odisha
2. School of Pharmaceutical Education and Research, Berhampur University, Odisha
3. Department of Pharmacology, Columbia Institute of Pharmacy, Tekari, Near Vidhansabha, Raipur -493111Dist-Raipur (C.G.) India.

ABSTRACT:

Tumor Protein 53 or Protein 53 (P53) is the single most frequently altered gene in human cancers, with mutations being present in approximately 50% of all invasive tumors. However, in some of the most difficult-to-treat cancers such as high-grade serous ovarian cancers, triple-negative breast cancers, esophageal cancers, Small cell lungs cancers and squamous cell lung cancers, p53 is mutated in at least 80% of samples. Clearly, therefore, mutant p53 protein is an important candidate target against which new anticancer treatments could be developed. Although traditionally regarded as undruggable, several compounds such as p53 reactivation and induction of massive apoptosis-1 (PRIMA-1), a methylated derivative and structural analogue of PRIMA-1, i.e. APR-246, 2-sulfonylpyrimidines (PK11007), pyrazoles such as PK7088, zinc metallochaperone-1 (ZMC1), a third generation thiosemicarbazone developed by Critical Outcome Technologies Inc. (COTI-2) as well as specific peptides have recently been reported to reactive mutant p53 protein by converting it to a form exhibiting wild-type properties. Consistent with the reactivation of mutant p53, these compounds have been shown to exhibit anticancer activity in preclinical models expressing mutant p53.

Keyword: Protein 53, invasive tumors, small cell cancer, anticancer activity, Cancer therapy.

INTRODUCTION:

Breast cancer is the cancer that develops from the cells of the breasts[1] like from the lining of milk ducts and the lobules that supply the ducts with milk[2]. Worldwide, breast cancer is the most common invasive cancer in women. It affects about 12% of women.[3] Breast cancer cells can spread by breaking away from a breast tumor and it can travel through blood vessels or lymph vessels to reach other parts of the body. After spreading, the cancerous cells get attached to other tissues and form new tumors that may damage those tissues. The disease is metastatic breast cancer. Breast cancer is the most common cancer diagnosed in women but can occur in both men and women. Breast Cancer in Men is Less than 1% of all breast cancers occur in men with a rate of 1 in

1,000. Due to the rarity of this condition, it is often found at an advanced stage. These symptoms are having a lump in breast or armpit, bloody nipple discharge, inverted nipple, Orange-peel texture or dimpling of the breast's skin, Breast pain or sore nipple, swollen lymph nodes in the neck or armpit, A change in the size or shape of the breast or nipple.

BREAST CANCER PATHOPHYSIOLOGY:

Breast cancer is a malignant tumor that starts in the cells of the breast. Like other cancers, there are several factors that can raise the risk of getting breast cancer. Damage to the DNA and genetic mutations can lead to breast cancer have been experimentally linked to estrogen exposure. Some individuals inherit defects in the DNA and genes like the BRCA1, BRCA2 and P53 among others. Those people with a family history of ovarian or breast cancer thus is at a high risk of breast cancer. The immune system normally seeks out cancer cells and cells with damaged DNA and destroys them. Breast cancer may be a result of failure of such an effective immune defense and surveillance. These are several signaling systems of growth factors and other mediators that interact between stromal cells and epithelial cells. Disrupting these may lead to breast cancer as well.

Protein 53

Protein 53 **(**P53), also known as Tumor Protein 53 (TP53) is a gene that codes for a protein that regulates the cell cycle and hence functions as a tumor suppression. It is very important for cells in multi cellular organisms to suppress cancer. P53 has been described as "the guardian of the genome", referring to its role in conserving stability by preventing genome mutation (Strachan and Read, 1999). The name is due to its molecular mass: it is in the 53 kilodalton fraction of cell proteins.

STRUCTURE:

The p53 protein is a phosphoprotein made of 393 amino acids. It consists of four domains: (1) A domain that activates transcription factors. (2) A domain that recognizes specific DNA sequences (core domain). (3) A domain that is responsible for the tetramerization of the protein. (4) A domain that recognized damaged DNA, such as misaligned base pairs or single-stranded DNA. Wild-type p53 is a labile protein, comprising folded and unstructured regions which function in a synergistic manner [4] p53 protein has been voted molecule of the year.

P53 MUTATION IN BREAST CANCER:

Mutations in p53 occur in a high proportion of individuals with cancer susceptibility syndrome, which increased the risk of breast cancer[5]. This plays an important role for p53 inactivation in mammary carcinogenesis, and the structure and expression of p53 has been widely studied in breast cancer. Loss of heterozygosity (LOH) in the p53 gene was a common event in primary breast carcinomas[6] and this is

accompanied by mutation in some cases. In breast cancer at least 60% of cases with LOH retain a wild-type p53 allele[6]. Nevertheless, numerous studies have identified coding mutations in p53 in breast cancer and this is now recognised as a common, but by no means ubiquitous, somatic genetic change in breast cancer. Indeed, a comprehensive meta-analysis revealed that only approximately 20% of all cases express mutant p53 [7]. Several studies have sought to identify the stage of breast tumour genesis at which p53 mutation occurs. Careful studies of micro dissected tumour material show that low-grade ductal carcinoma *in situ* (DCIS) is essentially devoid of mutations, whereas mutations are more common in high-grade DCIS[8].

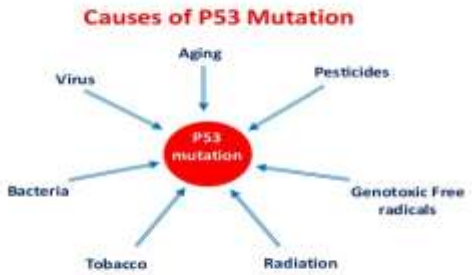

Figure 1: Causes of p53 mutation.

Although the overall frequency of p53 mutation in breast cancer is approximately 20% [7], certain types of the disease are associated with higher frequencies. For example, a number of studies have identified an increased rate of p53 mutations in cancers arising in carriers of germ-line BRCA1 and BRCA2 mutations[9,10]. Moreover, a distinct spectrum of p53 mutations occurs in such carcinomas[11]. Strikingly, in typical modularly breast carcinomas, p53 mutation occur in 100% of cases [12]. This is of particular interest, since it is now well recognised that medullary breast cancers share clinic pathological similarities with BRCA1-associated cases. Indeed, methylation-dependent silencing of BRCA1 expression occurs commonly in medullary breast cancers[13].

P53 PATHWAY:

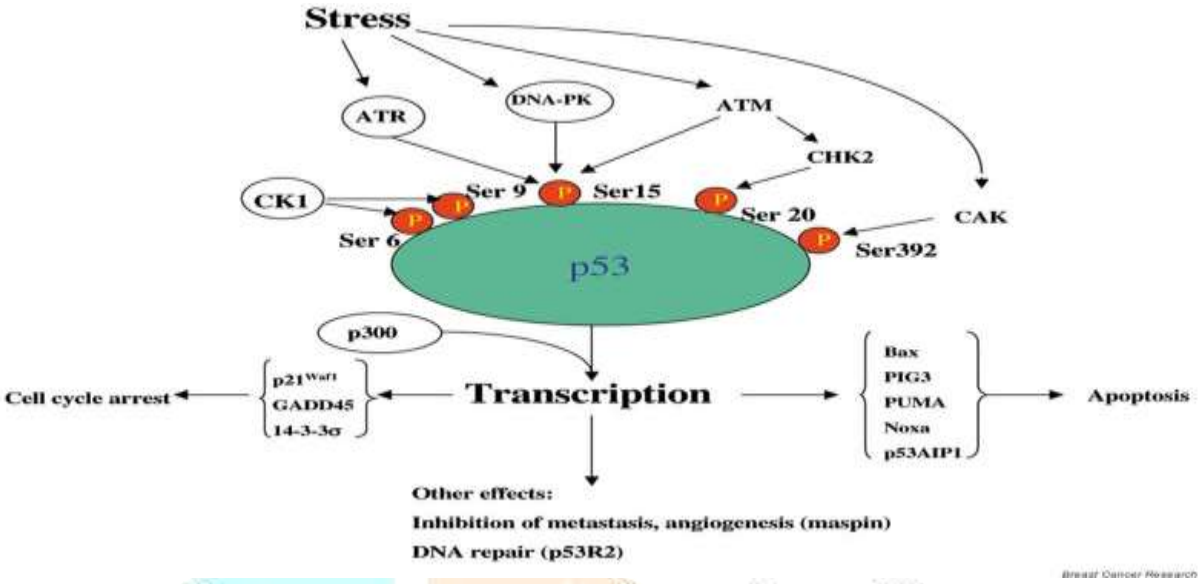

Figure 2: A simplified model of some of the components of p53 signaling.

Under normal conditions, the p53 pathway operates on 'standby' mode. Activation occurs in response to a variety of cellular stresses such as DNA damage and expression of activated Oncogene. Post-translational modifications (such as phosphorylation at the indicated serine residues) activate the protein for DNA binding and Trans activation of downstream 'effector' genes that mediate the tumour suppressor actions of p53. The outcome of activation depends on the nature and magnitude of the stress, its transduction via specific upstream kinases, and the resultant programme of p53-dependent gene expression. Transcriptional co activators like apoptosis stimulating protein of p53 and BRCA1 may further 'fine tune' the response. In some cases, preferentially promote specific cellular responses such as apoptosis. Many of the components of this signalling pathway are targets for genetic and/or epigenetic changes in breast cancer.

P53 REACTIVATION:

Several compounds such as p53 reactivation and induction of massive apoptosis-1 (PRIMA-1) A methylated derivative and structural analogue of PRIMA-1, i.e. APR-246, 2-sulfonylpyrimidines such as PK11007, pyrazoles such as PK7088, zinc metallochaperone-1 (ZMC1), a third generation thiosemicarbazone developed by Critical Outcome Technologies Inc. (COTI-2) as well as specific peptides have recently been reported to reactive mutant p53 protein by converting it to a form exhibiting wild-type properties.

PRIMA-1 and APR-246:

To reactivate mutant p53 and restore its transcription activity [14,15] most widely used are PRIMA-1 and APR-246 (Aprea Therapeutics). Both PRIMA-1 and APR-246 are pro-drugs that must first be converted to methylene quinuclidinone (MQ) in order to bind to p53 [16]. MQ acts by attaching to specific thiol groups in mutant p53, converting it to a Wild-type like conformation[16]. As well as binding to p53, APR-246 has also been shown to inhibit thioredoxin reductase 1 and decrease levels of GSH [17]. Both of these interactions result in increased levels of ROS. Thus, PRIMA-1 and APR- 246 exhibit a dual mechanism of action, i.e., reactivation of mutant p53 and generation of ROS[17]. Both PRIMA-1 and APR-246 have been shown to exhibit anticancer activity in a wide variety of preclinical models[14, 15]. APR-246 was administered as a 2-hour intravenous infusion once per day for 4 consecutive days. APR-246 acted via p53 activation included induction of cell cycle arrest, increased apoptosis and up-regulation of several p53 target genes in leukemic cells recovered from the treated patients, administration of APR-246 at a dose regimen of 67.5 mg/kg, given as a 6 h infusion on four consecutive days was found to be safe and well tolerated[18].

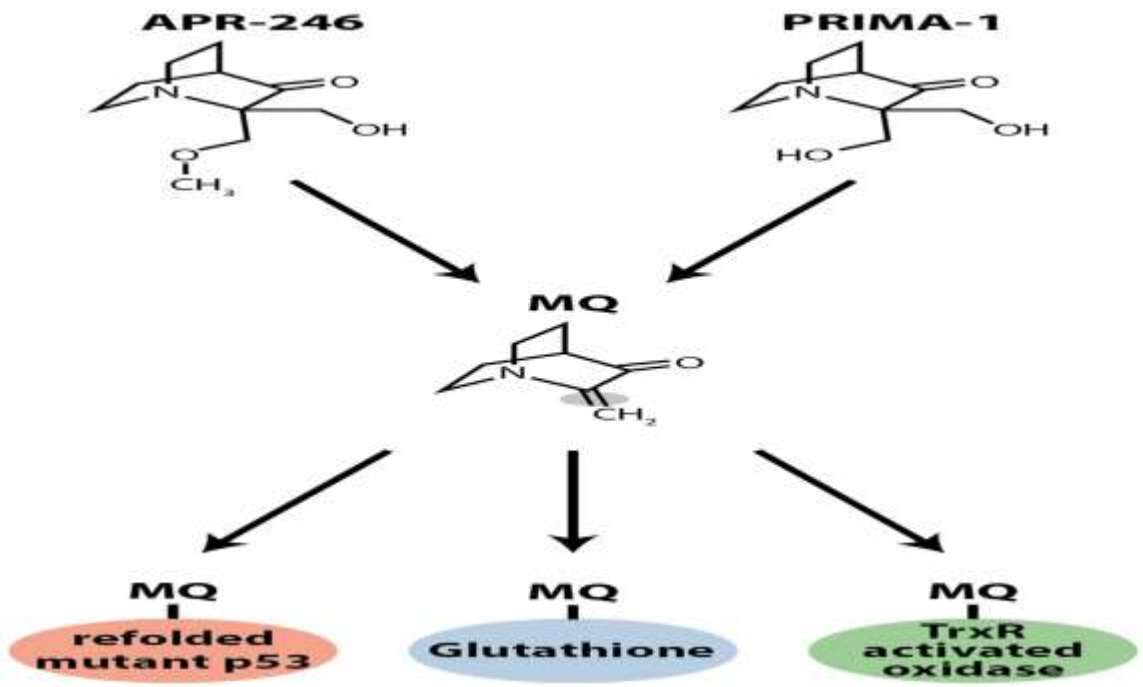

Figure 3. Chemical structure of PRIMA-1and APR-246 (Prima-1Met). Both compounds from the Michael acceptor methylene quinuclidinone (MQ), which is the active moiety.MQ binds covalently to thiol in mutant p53.MQ also targets thioreducxin reductase (TrxR) and glutathione(GSH).MQ binding to TrxR converts the enzyme to an active oxidase, ,which generate ROS, and MQ binding to glutathione depletes intracellular free glutathione, which also induce ROS.

PK11007

Two (2) - sulfonylpyrimidines, including one named PK11007, to be mild thiol alkylators with anticancer activity in several cell lines, especially those with mutationally compromised p53. PK11007 acted by two routes: p53 dependent and p53 independent. PK11007 stabilized p53 *in vitro* via selective alkylation of two surface-exposed cysteines without compromising its DNA binding activity. Unstable p53 was reactivated by PK11007 in some cancer cell lines, leading to up-regulation of p53 target genes such as p21 and PUMA. Generally, there was cell death that was independent of p53 but dependent on glutathione depletion and associated with highly elevated levels of reactive oxygen species and induction of endoplasmic reticulum (ER) stress, as also found for the anticancer agent PRIMA-1MET (APR-246). PK11007 may be a lead for anticancer drugs that target cells with non-functional p53 or impaired reactive oxygen species (ROS) detoxification in a wide variety of mutant p53 cells.

PK7088

PK7088 induces p21- and p53-dependent G_2/M arrest in p53-Y220C cells but not p53 wild-type cells, suggesting restoration of wild-type p53 function of the Y220C mutant in the HUH-7 cells. Interestingly, PK7088 acted synergistically with Nutlin-3. Nutlin-3 mimics the transactivation domain of p53 and competes with binding of the latter to the N-terminal domain of MDM2, thereby inhibiting MDM2-mediated degradation of p53[19]. Nutlin-3-induced effects, such as cell-cycle arrest and induction of apoptosis, are therefore generally only observed in cancer cell lines with wild-type p53, but not in cells that carry inactive mutant p53 [20,21]. As expected, Nutlin-3 had no effect on HUH-7 cells in the absence of PK7088. When combined with PK7088 treatment, however, Nutlin-3 increased p21 expression and G_2/M arrest of HUH-7 cells, indicating levels of active p53 that are further up-regulated through inhibition of one of its degradation pathways.

ZMC1:

p53 is a Zn^{2+}-dependent tumor suppressor inactivated in >50% of human cancers. The most common mutation, R175H, inactivates p53 by reducing its affinity for the essential zinc ion, leaving the mutant protein unable to bind the metal in the low [Zn^{2+}] environment of the cell. The drug zinc metallochaperone-1 (ZMC1) was able to reactivate p53 and other Zn^{2+}-binding mutants by binding Zn^{2+} and buffering it to a level such that Zn^{2+} can repopulate the defective binding site, but how it accomplishes this in the context of living cells and organisms is unclear.

COTI-2:

COTI-2 normalizes mutant p53 to wildtypelike conformation to promote apoptosis/cell death. The mechanism is most likely through zinc chelation by COTI-2's thiosemicarbazone core structure. Metal ion chelation has been shown to induce p53 conformational changes. Wild-type p53 binds zinc and requires it for proper function. Mutant p53 is relatively unable to bind zinc. Metal chelation by a thiosemicarbazone is to provide a source of zinc that allows for a p53 mutant conformation change. Thiosemicarbazones induce a wild-type-like conformational change in the p53 mutant protein that restores sequence-specific p53 transcription.

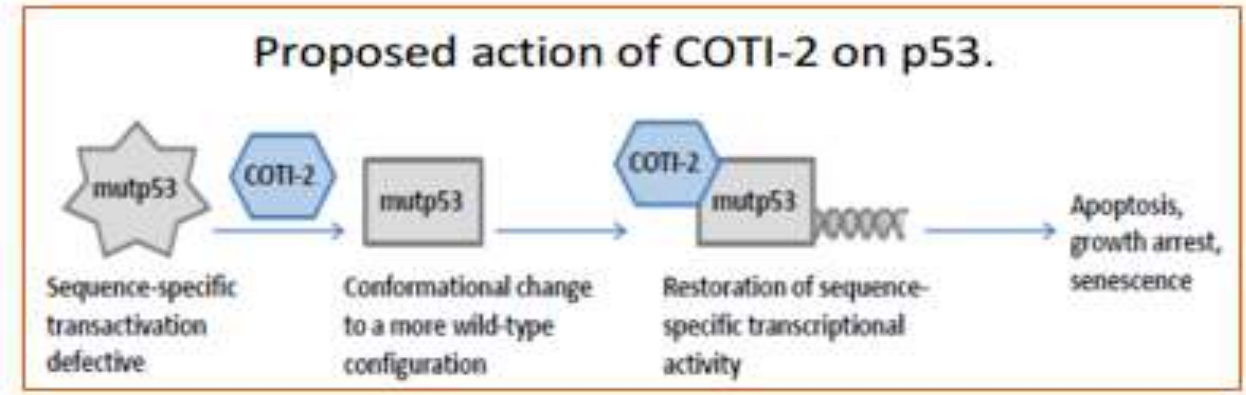

Figure 4: Action of COTI-2 on p53.

Applications of p53-Based Cancer Therapy:

Because most, if not all, human cancers altered p53, the concept of restoration of p53 for cancer therapy is very attractive**.**

p53 Stabilization

MDM2 is an E3 ubiquitin ligase which controls p53 degradation. Many tumors overexpress MDM2[22], even tumors without p53 mutations [23]. Targeting MDM2 for p53 stabilization seems to be promising; so many reports on targeting MDM2 or the MDM2-p53 have been published. For example, the nutlins are cis-imidazoline compounds that act as antagonists of the MDM2-p53 interaction. Analysis of the crystal structure showed that nutlin binds in the pocket of MDM2 to prevent the p53-MDM2 interaction. Nutlin can activate the p53 pathway, thereby inducing cancer cells and xenograft tumors in mice to undergo cell cycle arrest, apoptosis, and growth inhibition[24]. MI-219 is another small molecule that inhibits the MDM2-p53 interaction. MI-219 also activates the p53 pathway in cells with wild-type p53. Apoptosis and cell cycle arrest were observed in xenograft tumors which resulted in tumor regression.

Other Classes of Drugs for p53 Stabilization:

Tenovin was found by a cell-based drug which was screen to activate p53. Tenovin acts as an inhibitor of the protein-deacetylating activities of SirT1 and SirT2. The intraperitoneal administration of tenovin-6 has been demonstrated to induce a regression of xenograft tumors.RITA induced apoptosis in various cancer cells that retained wild type p53. They also found that the p53 released from MDM2 by RITA promotes p21 and hnRNP K (a p53 cofactor), thus implying that p21 plays a major role in regulating the cancer cell fate after p53 reactivation.

p53 Inhibition for Cancer Therapy:

The inhibition of p53 can protect normal cells during genotoxic chemotherapy or radiation therapy. The side effects of genotoxic therapy for cancer are largely caused by p53-mediated apoptosis. The small molecule pifithrin-alpha can block p53-dependent transcriptional activity and protect mice from the lethal side effects associated with anticancer treatment. If we can avoid dose-limiting genotoxic stress to normal cells during chemotherapy or radiotherapy for cancer, it will thus allow a higher dose to be used for patients who are not sufficiently responsive to conventional chemotherapy.

p53 Gene Therapy:

The first p53-based gene therapy was reported in 1996. A retroviral vector containing the wild-type p53 gene under the control of an actin promoter was injected directly into tumors of nonsmall cell lung cancer patients. After development of a replication-defective recombinant p53 virus (Ad5CMV-p53), many clinical trials have been performed, including one in esophageal cancer patients. A few trials reached phase III, but final approval from the FDA has not yet been granted. Recently, p53-based gene therapy has been developing in China.

p53-Based Immunotherapy:

Tumor-associated antigen-specific cytotoxic T lymphocyte can mediate immune response of host against cancer in vivo. P53 protein, especially targeting missense mutation of p53, can be candidate of tumor antigen . Some cancer patients have antibodies against p53, the frequency and clinical significance are still under debate. Speetjens et al. reported clinical trials of a p53-specific synthetic long peptide (p53-SLP) vaccine for metastatic colorectal cancer patients . Ten patients were vaccinated with p53-SLP in a Phase I and Phase II trial. Toxicity was tolerable, and p53-specific immune response was detected in 9 of 10 patients. P53-specific T-cell reactivity persisted more than 6 month in 6 of 9 patients. Although the trial was Phase I/II, the clinical benefit may be hard to obtain because most patients had T-helper cells that lacked key cytokines. Preclinical phase I/ II trial of INGN-225 (Introgen), a p53-modified adenovirus-induced dendritic cell vaccine for small cell lung cancer

(SCLC) patients, has been reported. INGN-225 was well tolerated and induced p53-specific immune response in 18/43 (41.8%) patients and sensitized SCLC to subsequent chemotherapy.

CONCLUSION:

In this paper, we focused on the functions of p53 and clinical applications targeting p53 for cancer therapy. p53 has been reported to induce apoptosis independent of its transcription of genes . The next generation of p53-based cancer therapeutic greater approaches should therefore be developed to take advantage of this cytosolic function. This may be safer than regulating the transcription modulation of wild-type p53, which can induce both pro-survival and pro-apoptotic effects in tumor cells, as discussed above. Several important issues have been addressed to most efficiently apply p53-reactivating drugs. The search for the most efficient combinations of drugs that can act synergistically when combined with p53 reactivate seems very promising. We believe that the clinical application of p53-based therapies and their combinations with other drugs will help to fight cancer.

REFERENCES:

1. "Breast Cancer". *NCI*. Archived from the original on 25 June 2014. Retrieved 29 June2014.
2. *"Breast Cancer Treatment (PDQ®)". NCI. 23 May 2014. Archived from the original on 5 July 2014. Retrieved 29 June 2014.*
3. McGuire,A;Brown,JA; Malone, C;MCLaughlin, R; Kerin,MJ(22 May 2015). "Effects of age on the detection and management of breast cancer".Cancers.7(2):908-29.
4. Breast J. 2010 May-Jun;16(3):245-51.
5. Malkin D, Li FP, Strong LC, Fraumeni JF, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA, Friend SH: Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science. 1990, 250: 1233-1238.
6. Davidoff AM, Humphrey PA, Iglehart JD, Marks JR: Genetic basis for p53 overexpression in human breast cancer. Proc Natl Acad Sci USA. 1991, 88: 5006-5010.
7. Pharaoh PD, Day NE, Caldas C: Somatic mutations in the p53 gene and prognosis in breast cancer: a meta-analysis. Br J Cancer. 1999, 80: 1968-1973. 10.1038/sj.bjc.6690628.
8. Bartek J, Iggo R, Gannon J, Lane DP: Genetic and immunocytochemical analysis of mutant p53 in human breast cancer. Oncogene. 1990, 5: 893-899.
9. Smith PD, Crossland S, Parker G, Osin P, Brooks L, Waller J, Philp E, Crompton MR, Gusterson BA, Allday MJ, Crook T: Novel p53 mutants selected in BRCA-associated tumours which dissociate

transformation suppression from other wild-type p53 functions. Oncogene. 1999, 18: 2451-2459. 10.1038/sj/onc/1202565.

10. Phillips KA, Nichol K, Ozcelik H, Knight J, Done SJ, Goodwin PJ, Andrulis IL: Frequency of p53 mutations in breast carcinomas from Ashkenazi Jewish carriers of BRCA1 mutations. J Natl Cancer Inst. 1999, 91: 469-473. 10.1093/jnci/91.5.469.
11. Greenblatt MS, Chappuis PO, Bond JP, Hamel N, Foulkes WD: TP53 mutations in breast cancer associated with BRCA1 or BRCA2 germ-line mutations: distinctive spectrum and structural distribution. Cancer Res. 2001, 61: 4092-4
12. De Cremoux P, Salomon AV, Liva S, Dendale R, Bouchind'homme B, Martin E, Sastre-Garau X, Magdelenat H, Fourquet A, Soussi T: p53 mutation as a genetic trait of typical medullary breast carcinoma. J Natl Cancer Inst. 1999, 91: 641-643. 10.1093/jnci/91.7.641.
13. Esteller M, Silva JM, Domingue G, Bonilla F, Matias-Guiu X, Lerma E, Bussaglia E, Prat J, Harkes IC, Repasky EA, Gabrielson E, Schutte M, Baylin SB, Herman JG: Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst. 2001, 92: 564-569
14. Duffy MJ, Synnott NC, McGowan PM, et al. p53 as a target for the treatment of cancer. Cancer Treat Rev 2014; 40:1153-60.
15. Parrales A, Iwakuma T. Targeting Oncogenic Mutant p53 for Cancer Therapy. Front Oncol 2015;5:288
16. Lambert JM, Gorzov P, Veprintsev DB, et al. PRIMA-1 reactivates mutant p53 by covalent binding to the core domain. Cancer Cell 2009;15:376-88.
17. Bykov VJ, Zhang Q, Zhang M, et al. Targeting of Mutant p53 and the Cellular Redox Balance by APR-246 as a Strategy for Efficient Cancer Therapy. Front Oncol 2016;6:21.
18. Deneberg S, Cherif H, Lazarevic V, et al. An open-label phase I dose-finding study of APR-246 in hematological malignancies. Blood Cancer J 2016; 6:e447.
19. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science. 2004;303:844–848.
20. Kojima K, Konopleva M, Samudio IJ, Shikami M, Cabreira-Hansen M, McQueen T, Ruvolo V, Tsao T, Zeng Z, Vassilev LT, et al. MDM2 antagonists induce p53-dependent apoptosis in AML: implications for leukemia therapy. Blood. 2005; 106:3150–3159.
21. Endo S, Yamato K, Hirai S, Moriwaki T, Fukuda K, Suzuki H, Abei M, Nakagawa I, Hyodo I. Potent in vitro and in vivo antitumor effects of MDM2 inhibitor nutlin-3 in gastric cancer cells. Cancer Sci. 2011; 102:605–613.
22. Folkman J: Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1: 27-31, 1995.

23. Bergers G and Benjamin LE: Tumorigenesis and the angiogenic switch. Nat Rev Cancer 3: 401-410, 2003.

24. Dohi, T., Okada, K., Xia, F., Wilford, C.E., Samuel, T., Welsh, K. et al, An IAP-IAP complex inhibits apoptosis. Journal of Biological Chemistry, 2004; 279: 34087-34090.