[a].targeting that leads to a variety of cancers

Business, Strategy

A. Targeting negativeregulators of p53: Mdm2 over expressionhas been associated with higher occurrence of breast carcinoma 6. Up regulation of the negativeregulators of p53 results in suppression of wild type p53 expression in cellsthat leads to a variety of cancers 1. An effective approach to activate the wild type p53 in cells is to inhibit itsnegative regulators. The most primary negative regulator of p53 is itsdownstream target, Mdm2 2.

Thehuman Mdm2 gene encodesa 491 amino-acid protein (90 kDa) that contains a p53 binding domain at the N-terminaland a RING domain at the C terminus. 3. Mdm2 hinder p53 either by binding at its N-terminus and blocking the p53 transactivationactivity or it promotes the p53 degradation by acting as an E3 ubiquitin ligase4. Reduction in the coupling ofMdm2-p53 reactivates the wild-type p53 and hence induces cell apoptosis. Thusemploying molecules that disrupt Mdm2-p53 binding can reactivate the p53functions. 1) Mdm2-p53 interactioninhibitors: For smallmolecules to have a great therapeutic potential of Mdm2 inhibitor, it shouldhave the following desirable properties: (a) a high binding affinity and specificityto Mdm2, (b) potent cellular activity in cancer cells with wild-type p53, and(c) a highly desirable pharmacokinetic (PK) profile. Three molecules meet thecriteria of Mdm2 inhibitors: Nutlins, benzodiazepinedione compounds andMI-series analogs 7, 8Nutilins: Nutilins were identified by screening diversechemical library 9.

They are cis-imidazolineanalogs which inhibit the interaction between mdm2 and tumour suppressorprotein p53 10. This series of compoundseffectively inhibits Mdm2-p53 interaction by its high-affinity binding to Mdm24. Nutlin-3, an analog of the serieshas a broad activity against cancer cells with wt p53

both in vitro and invivo. Examples include lung and breast cancer cell lines, haematologicalmalignancies and osteosarcoma cell 11Nutlin canbe used as an alternative to the current cytotoxic chemotherapy, which retain ahigh percentage of p53wild-type status at diagnosis.

Nutlin-3 is used incombination of other anticancer drugs. Nutlin-3 shows a synergistic cytotoxiceffect when used in combination with innovative drugs, such as TRAIL orbortozemib12. RG7112 (2g) , nutlinderivative is the first clinical small-molecule MDM2 inhibitor designed tooccupy the p53-binding pocket of MDM2. In cancer cells expressing wild-typep53, RG7112 stabilizes p53 and activates the p53 pathway, leading to cell cyclearrest, apoptosis, and inhibition or regression of human tumor xenografts13. Nutlin-3, induced cell cycle arrest and apoptosis in 10 randomly selected cancer celllines of seven different tumor types: colon, breast, lung, prostate, melanoma, osteosarcoma, and renal cancer, complete depletion of the S-phase fraction, causing arrest at G1/S and/or G2/M phases in all the cell lines14Benzodiazepinedione: Benzodiazepinedione compound TDP665759, which binds to MDM2 with a IC 50value of 704 nM, inhibits cell proliferation in cells expressing wild-type p5315. It exihibits an excellent cellpermeability that is essential for employing small molecules as anticanceragents. It inhibitis cell proliferationin many cancer cell lines with wild-type p5316MI series: Spiro-oxindole inhibitors MI-63 and MI-219 display excellent specificity for blockingthe MDM2-p53 interaction17.

MI-63and MI-219 interact with MDMX that is closely related homolog of MDM2, and bothproteins interact with p53 at deep hydrophobic binding cleft

with a helicaldomain in their binding partners . MI-219 shows a greater than10, 000-foldselectivity forMDM2 relative to MDMX.. Small molecules have shown antitumoractivity in vitro and in vivo18. MI-219 represent promising therapeutic candidate for drug developmentspecifically targeting the MDM2-p53 interaction and thus reactivation of p53. Compound 5b has been reportedto exhibit significant anti-proliferative activity in nude mice bearing MCF-7xenograft tumor. 19The compound 5bwas found to act via modulation of MDM2 and p53 expression in breast cancercells expressing wild type p53. Compound 5b stimulated p53 activation, causedmodulation of downstream effectors p21, pRb, and cyclin D1 which regulate cellcycle.

Thus, compound triggered G1–S phase cell cycle arrest, which was evidentby flow cytometric analysis of treated breast cancer cells. Thus, compound 5brestores the p53 function, which triggers molecular events consistent with cellcycle arrest at G1/S phase19. Zinc hasbeen reported as an intrinsic factor required for the proper reactivation of p53 byMI-219 20RO5693: RO5693exhibits better cell death activity against MCF-7 cancer cells and other solidtumor cell lines, leading to cell cycle arrest and apoptosis. It depends uponwild type p53 status. This novel class of compounds is advantageous in cancersexpressing high MDMX, including breast cancer21. Most recently, a series of indolylhydantoin compounds RO-2443 and RO-5693 has been demonstrated as potentinhibitors of MDMX by binding to the p53 pocket of MDMX and inducing proteindimerization22. XI-011(NSC146109): It activate wild-type p53 inbreast cancer cells by a mechanism that involved inhibition of MDMX throughtranscriptional repression of the MDMX promoter. These compounds are all stillvery early in

the development process but certainly validate the concept thatMDMX blockade in MDMX over-expressing tumors23.

Tenovin 1 and Tenovin 6: These are the series of compounds thatactivate wild-type p53 through targeting enzymes involved in negativeregulation of p5324. They preventdeacetylation of one of p53 carboxy-terminal lysines (Lys382) which may lead toubiquitination and proteasomal degradation by SirT1. Using a cell based screen, Tenovin 1 was identified to inhibit sirtuins activity 25. A secondary compound Tenovin-6 is seven times more watersoluble and is more cytotoxic. Tenovin 6 has also been proved to decrease tumorgrowth in vivo 26. Targetingmutant p53Smallmolecular weight compounds (SMWC) that restore p53 function and reverse tumor growth : CP-31398, PRIMA-1, P53R, WR-1056 has been given in human breast carcinoma cell lines expressingmutant or wild type (WT) p53 in vitro271) CP-31398(styrylquinazoline): CP-31398was identified through a structure based screening as a compound that canmodify mutant p53 to a wild-type p53 28. CP-31398 promotes the stabilization of the DNA binding domain of p53 andrestore the transactivation function of mutant-p53 proteins in breastcancer(SKBr3) cell lines, SKBr3 causedapoptosis29. It stabilizes theactive conformation of p53 and promotes p53 activity in cancer cell lines withmutant or wild-typep5330.

Thecombination of anticancer drug metformin with p53 reactivating agents, likenutlin-3? and CP/31398, is a promising strategy for improvingmetformin-mediated anti-cancer therapy, especially for tumors with p53mutations31. 2) PRIMA-1 (p53 re-activation and inductionof massive apoptosis): Restorationof

p53 function by converting existingmtp53 to the wild-type p53 (wtp53) conformation promotes apoptosis of tumor cells32. PRIMA-1 is a non-toxic smallmolecule that activates mtp53 and induces cell death in vitro and invivo in estrogen-responsive breast cancer cell lines that express mtp53(BT-474, HCC-1428, and T47-D). PRIMA-1selectively reduces the cell viability of mtp53-expressing breast cancer cells, detected by confirmation specific antiboddies but not in wtp53 containingnormal mammary or endothelial cells33. PRIMA-1 causes covalent modifications i.

e. alkylating the thiol group, ofone or several cysteine residue (cys182, cys229, cys242, cys277) that are exposedon the surface of p53 protein leading to three correctional possibilities. 1)Alkylation might prevents the aggregation caused by thiol groups.

Thuspotentially increasing the fraction of the protein that binds to DNA andregulate gene target transcriptiopn. 2) Formation of adducts in the core domainmight allow more efficient DNA binding and thus transactivation. 3)PRIMA-1adducts might promote correct folding of the core domain by creating additionalcontacts with amino acids in the core via hydrogen bonding and hydrophobicinteractions34. P53R: p53R3 is a recently identified small molecule that re-stores thesequence-specific DNA binding of p53 mutants (R175H and R273H) after screeninga small library of compounds using an in vitro gel shift assay line LN-308. P53R3 induces p53-dependent antiproliferative effects with much higherspecificity35. The compound wasfound to enhance the recruitment of both wt and mutant p53 to target promotersand to induce the expression of a number of p53 target genes.

4WR-1065: WR1065 is an aminothiol, shown to induce wild-type p53 accumulation through escape from proteasome-dependentdegradation and activation in cultured cells. 36.

p53 accumulation by WR1065 in MCF-7 cells did not resultfrom the formation of DNA-damage as measured by DNA fragmentation and Cometassay, nor from oxidative stress as detected by measurement of glutathionelevels, lipid peroxidation and reactive oxygen species production. p53activation by WR1065 was not prevented by inhibition of PI-3 kinases, phosphatidylinositol 3-ki-nases and is notaccompanied by phosphorylation of Ser-15, -20, or -37, which are common targetsof the ki-nases activated in response to DNA damage. WR1065 induces p53by a pathway different than the one elicited by DNA-damage. Direct reduction byWR1065 of key cysteines in p53 may play an important role in this alternativepathway, as shown by the fact that WR1065 activated the redox-dependent, DNA-binding activity of p53 in vitro37. Further studies reported, WR1065 activates the JNK (c-Jun N-terminalki-nase), decreases complex formation between p53 and inactive JNK, andphosphorylates p53 at Thr-81, a known site of phosphorylation by JNK. Adominant neg-ative form of JNK (JNK-APF) reduces by 50% the activa-tion of p53by WR1065. WR1065 activates p53 through a JNK-dependent signaling pathway.

Thispath-way proves useful for pharmacological modulation of p53 activity throughnongenotoxic mechanisms38 DAPK1: Identificationof targeted therapies for the treatment of ER-negative breast cancers, particularly TNBCs, DAPK1 has been reported to have an unexpected role in altering the

growth of breastcancer cells in a p53-dependent context. In p53-WT cells, confirmed that DAPK1 increases apoptosis in ap53-dependent manner. However, in p53-mutant breast cancer cells whereDAPK1-induced apoptosis is compromised, DAPK1 increases growth of tumor cellsby redirecting its activity through the mTOR/S6 pathway. 39. There is a linkbetween p53-mutation status and cellular sensitivity to DAPK1 inhibition. Pharmacologic inhibition of DAPK1 in p53-mutant, but not WT, cancer cellsdecreases their growth in mouse xenografts, and DAPK1/p53-mutational status isan independent prognostic indicator in breast cancer patients, suggesting thatDAPK1 should be explored as a target for the treatment of p53-mutant cancers, including TNBCs. DAPK1 is reported to both directly transactivatep53 40 and to be a transcriptional target of p5341.

In p53-WT cells, DAPK1may have a dual role in breast cancer cells, regulating both growth and apoptosis, dependin upon whichupstream stimuli are activated and the mutational status of TP5342. YK-3-237: Smallmolecule compound YK-3-237 that reduces acetylation of mtp53 and exhibitsanti-proliferative effects toward triple-negative breast cancer (TNBC) cellscarrying mtp53. YK-3-237 activates SIRT1 enzyme activities in vitro anddeacetylation of both mtp53 and wild type p53 (WTp53) in a SIRT1-dependentmanner.

Deacetylation of mtp53 resulted in depletion of mtp53 protein level andup-regulated the expression of WTp53-target genes, PUMA and NOXA. YK-3-237 alsoinduces PARP-dependent apoptotic cell death and arrests the cell cycle at G2/Mphase in mtp53 TNBC cells 43. Targetingwild-type p53RITA2, 5-bis(5-hydroxymethyl-2-thienyl)Furan: It was the first compound shown to bind the

p53 N-terminus (residues 1-63)without hindering the transcriptional function of p53.

RITA triggers aconformational shift preventing p53’s interaction with its negative regulatorssuch as Mdm244. RITA suppressesthe growth and induces apoptosis in a p53-dependent manner, along with theinduction of p53 target genes, in a variety of mutant p53 carrying cell linesof different origin 45. RITAreactivate wild type p53 by interfering with Mdm2-mediated degradation .

It prevent the docking of the central domain ofMdm2 to p53 DBD, which is required for the efficient ubiquitination of p53 byMdm2. Alternatively, it might blockother facets of Mdm2-mediated inhibition of p5346. It has been also shown that mdm2 released from p53 byRITA promotes degradation of p21 and thep53 cofactor hnRNP K, required for the transcription of p21. MDM2-dependentinhibition of p21 acts as a switch capable of regulating cell fate decisionsupon p53 reactivation. RITA-mediated p53 activation unleashes thetranscriptional repression of anti-apoptotic proteins, Mcl-1, Bcl-2, MAP4, andsurvivin. In addition, it blocks the Akt signaling pathway on various levels bydown regulating c-Myc, cyclin E, and j3-catenin 47.

NSC319726(Zinc mettalochaperone-1): Zn(2+) is a knownregulator of p53 binding to the target genes48. Amino acid substitutions in the p53 Zn(2+)-binding pocket canpresumably exert an influence on Zn(2+) position in the Zn(2+)-p53 complex andthereby affect p53 binding to DNA. Molecular dynamics trajectories demonstratedeffect of the putative changes in the Zn(2+) position in its binding pocket dueto the G245C and G245D

substitutions on the p53 DNA-binding motif. It leads to changesin the conformation of the p53 DNA-binding motif, as compared with thewild-type (WT) p5349. Binding ofZn(2+) to the p53 mutant forms reduced the effect of the substitutions onconformational change. Zn(2+) binding in the normal position compensated theeffect of the mutations on the conformation in comparison to the altered Zn(2+)position49. NSC319726 analogrestores the wild type like conformation of mutant p53 and upregulatesp53downstreaming target genes (p21, PUMA, MDM2) through increasing ROS levels NSC319726 (ZMC1) is a small molecule that reactivatesmutant p53 by restoration of WT structure/function to the most common p53missense mutant (p53-R175H)50.

Twomechanism has been reported by which ZMC1 reactivates p53-R175H and provideevidence that ZMC1: 1) restores WT structure by functioning as azinc-metallochaperone, providing an optimal concentration of zinc to facilitateproper folding; and 2) increases cellular reactive oxygen species thattransactivate the newly conformed p53-R175H (via post-translationalmodifications), inducing an apoptotic program. 51 RPS6KA6 belonging to the ribosomal s6 kinase (RSK)family plays a role in cell growth and proliferation. RPS6KA6 was overexpressedin breast cancer cell lines, supporting its role in breast tumorigenesis. Atpresent, RPS6KA6 has been found to be downregulated in NSC319726-treatedsamples, which may suggest the inhibi-tory action of NSC319726 on theexpression of RPS6KA652. Thepharmacologic delivery of a metal ion to restore proper folding of a mutantprotein is unique to medicinal chemistry and represents a new pathway to drugmutant p5353. Survivinmutant adenovirus: As p53function is

lost in many cancers, it is essential to restore p53 function byreintroducing wt p5399.

Introduction of p53 using viruses is one of the mostpracticed gene therapy in cancer treatment. Replication -deficient adenovirusencoding a nonphosphorylatable Thr34 to Ala mutant of the apoptosis inhibitorsurvivin (pAd-T34A) have been reported to cause spontaneous apoptosis in tumourcell viability in vitro and in vivo. Infection with pAd-T34A caused spontaneousapoptosis breast cancer cell lines 54. Peptidethat binds and stabilizes p53: Designed peptide CDB3 bindsto a site in p53 that partly overlaps with its positively charged binding sitefor DNA. These peptides act as chaperones.

CDB3 binds to p53 and mutants duringbiosynthesis, raises melting temperature to above body temperature so it canfold, and then transfers p53 to its natural binding partners in the cell thatwould take over the stabilizing function. 55