p53 pathway targeting mechanics, architectures, and therapeutic advancements:

Vikash Lodhi
M.Sc. (ZOOLOGY)
Department Of Zoology
Agra University, Agra, U.P

Abstract- Most, if not all, human cancers are caused by TP53 (p53) gene dysfunction. There are two main mechanisms causing this dysfunction: MDM2/MDM4-mediated wild-type p53 downregulation and mutation. p53 is a very desirable target for the creation of novel anticancer medications due to its nearly ubiquitous inactivation in cancer. While several approaches have been explored to target defective p53 in cancer treatment, only two of these tactics have produced drugs that are now undergoing clinical trial testing. These tactics include finding substances that can activate the p53 mutant back to its wild-type form and substances that can prevent the interaction of MDM2/MDM4 and wild-type p53.Many p53-MDM2/MDM4 antagonists are presently undergoing clinical trials; the most developed one is called idasanutlin, and it is being tested on patients with relapsed or refractory acute myeloid leukemia in a phase III clinical trial. APR-246 and COTI-2 are the two mutant p53-reactivating substances that have advanced to clinical testing. It is yet unknown if MDM2/MDM4 inhibitors and mutant p53-activating drugs have any clinical utility, despite encouraging results from their trials in preclinical models. Given the high frequency of p53 malfunction in human malignancies, a new era in cancer treatment is expected to dawn if any of the drugs presently undergoing clinical trial evaluation prove to be effective.

Index Terms- Deoxyribonucleic Acid (DNA), Mouse Double Minute 2 (MDM2), Topologically Associating Domains (TADs), Ataxia-Telangiectasia Mutated (ATM), Acute Myelogenous Leukemia (AML).

I. INTRODUCTION

The gene that suppresses tumors. The most often altered gene in human tumors is TP53. 1, 2 There is a close correlation between the development of tumors and the malfunctions brought on by TP53 mutations. 3,4 The primary role of the p53 protein is that of a transcription factor, which controls many different processes, including cell cycle arrest, DNA repair, cell apoptosis, autophagy, and metabolism, and establishes whether cells perish under stressful circumstances. Growing amounts of research over the years have demonstrated the intricacy and interconnectivity of the p53 pathway and, consequently, its function in the immunological microenvironment, metabolic homeostasis, stem cell biology, and other areas. Mutant p53, on the other hand, can change DNA-specific binding, interfere with the protein's thermal stability and spatial conformation and impair p53 function. Because of its inherent tumor suppressor role and high frequency of mutations in tumors, TP53 is a very potential target for cancer therapy. Drug research against p53 has been halted for decades due to the specificity of the p53 structure, the smooth surface lacking an optimal drug-binding pocket, and the challenge of restoring p53 activity. Despite this, scientists continue to think that this challenging aim can be reached and have made considerable headway recently. Our goal in writing this review is to give a thorough overview of the p53 signaling system, its biological role, the structural characteristics of the p53 protein, and the developments in p53-targeted treatments.

II. THE P53 PATHWAYS

A transcription factor called p53 is found in both the cytoplasm and the nucleus. It attaches specifically to DNA and controls a wide range of genes. The negative regulators of p53, MDM2 and MDMX, strictly regulate cellular p53 protein levels under normal circumstances by promoting p53 destruction by ubiquitination. P53 ubiquitination is suppressed in response to both internal and external stresses on cells, including DNA damage, hypoxia, food deprivation, and cancer cell danger. This results in a sharp rise in intracellular p53 protein levels. Posttranslational changes, such as phosphorylation, acetylation, and methylation, activate and stabilize accumulated p53. Stabilized p53 attaches to target DNA, forms tetramers in the nucleus, and controls gene transcription, which modifies downstream signaling pathways. The most extensively studied tumor suppressor gene, p53, transcriptionally activates multiple genes involved in apoptosis and the cell cycle in response to cellular stress, thereby terminating cellular processes and preventing the differentiation of cells with damaged or mutated DNA. However, p53 has also been found to regulate a few "non-classical" pathways, such as autophagy, senescence, tumor microenvironment, metabolic balance, ferroptosis, stem cell differentiation, and so on. The role of p53 in cancer biology. p53 regulates a large number of signaling networks. The questions of precisely how, when, and what P53 acts are not easily answered. However, it is evident that p53 responds to environmental disturbances in a very flexible and diverse manner, deciding whether to maintain cellular homeostasis or cause cell death. By acting as a connecting element for several cellular signaling pathways, p53 regulates a wide range of biological processes in a tasteful and harmonious manner through transcriptional control and protein-protein interactions. {fig.1}

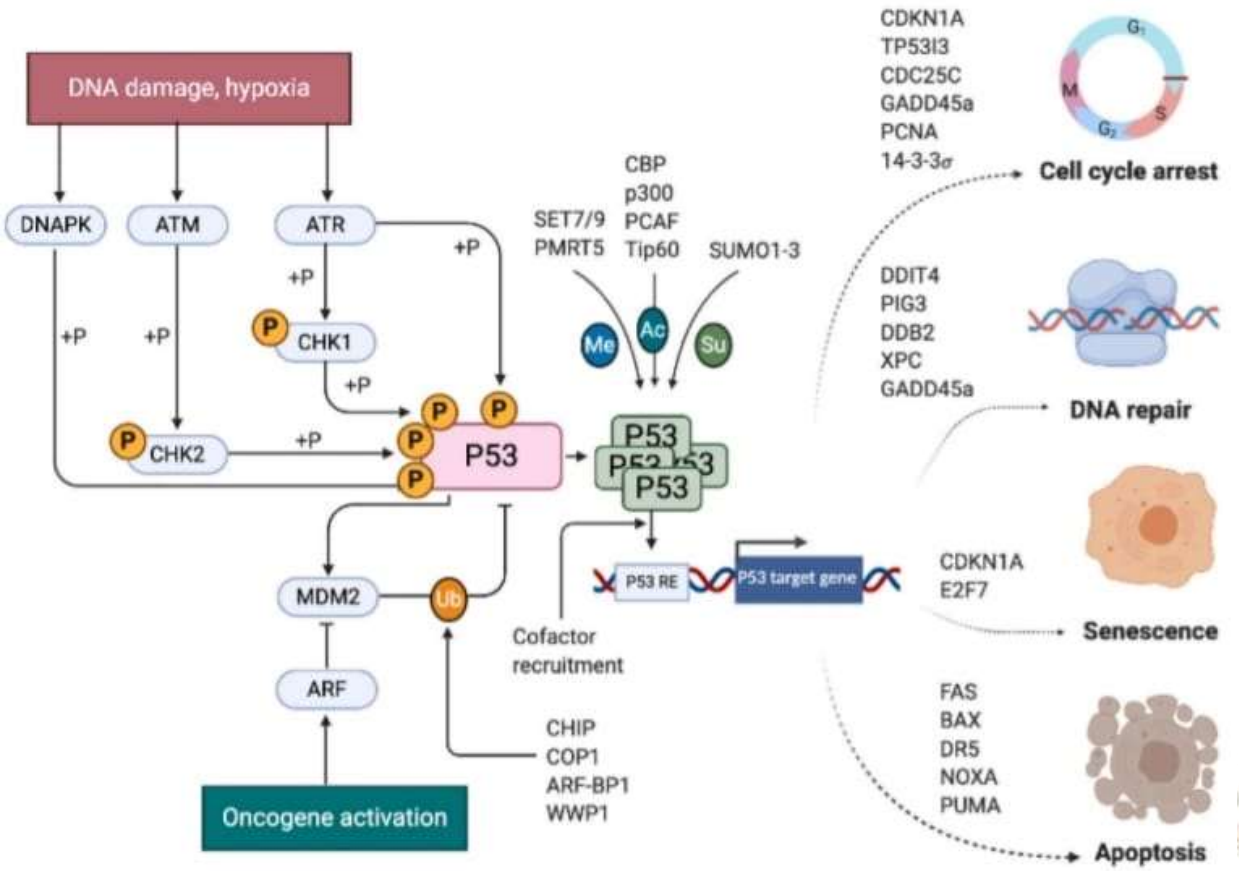

Fig.1. An overview of the output of transcriptional cellular responses and p53 activation and regulation. DNA damage, oncogene activation, hypoxia, and replication/translation stress are examples of cellular stressors that activate the sensor proteins ATM, ATR, Chk1, Chk2, DNA-PK, and p14ARF. These kinases phosphorylate p53, which causes it to oligomerize, stabilize, and bind to the p53RE. MDM2, a p53 target, primarily controls p53 stability, creating a negative feedback loop. Additional protein modifiers and cofactors that attach to the p53 protein control the target genes' transcriptional activity. The multi-step p53 activation pathway ultimately controls the stress input to produce the desired biological response.

III. GENOMIC STABILITY

The p53 gene is thought to protect the genome. It contributes significantly to the preservation of genomic stability. The genome is shielded from damage by p53, which orchestrates several DNA damage response systems.51 The DNA repair proteins XPC and DDB2 are expressed in response to activation by the p53 protein.52 These proteins' interactions with effector proteins can cause apoptosis, senescence, or cancer, among other cell fates. First discovered as a p53 transcriptional target is the p21-encoding gene (CDKN1A). By strongly suppressing cell cycle proteins and the phosphorylation of Rb by the cyclin D1-CDK4, cyclin D2-CDK4, and cyclin E-CDK2 complexes, 55 p21 binds to cyclin-dependent kinases (CDKs). Hypo phosphorylated Rb combines with the E2F transcription factor to produce a complex that impedes E2F's transcriptional activity and blocks the G1 phase. The phosphatase of regenerating liver-3 and PTPRV, which encodes a transmembrane tyrosine phosphatase, are both genes implicated in p53-induced G1 phase block. Moreover, CDKs and cyclin B, which are necessary for mitotic entry and involved in the G2/M phase block, are repressed by p53.The transcription of 14-3-3 sigma is induced by p53, and it also represses the cell cycle protein-dependent kinase Cdc2. The growth halt and DNA damage gene family includes Gadd45. Gadd45 transcription is regulated by p53, which also breaks the cyclin B1/Cdc2 complex and further prevents the G2 phase. Reprime also plays a role in the G2 phase cell cycle arrest caused by p53. These p53 modifications stop oncogene activity and lower the chance of gene mutation. Through reverse transcription of RNA intermediates, retrotransposons are able to multiply and introduce themselves into new genomic locations, resulting in an increase in the number of copies or gene alterations in the host genome. It has been found that disinhibition of retrotransposons is intimately linked to the development of human tumors. Transposon sequence expression is inhibited when p53 attaches to the promoter region of the retrotransposon element LINE1. Cells overexpressing synthetic retrotransposon genes are able to evade apoptosis when p53 is missing. Mutations or deletions of p53 can lead to genomic instability, which can accumulate additional oncogenes and facilitate treatment resistance, metastasis, carcinogenesis, and proliferation. In addition to aiding in genomic mutation and copy number expansion, functional p53 inactivation preserves the viability of cells harboring defective genetic information.

IV. THE SENESCENCE

Senescence is an irreversible stop in the cell cycle. Its tumor suppressive properties are tightly linked to p53 mediated senescence. Senescence is caused by damage to DNA; this process is commonly known as stress-induced premature senescence. The p53 and/or p16INK4A pathways are activated by the DNA damage response pathway, which is triggered by a variety of internal or external stresses. p16INK4A causes cell cycle arrest or senescence by inactivating CDK4/6, phosphorylating Rb accumulation, and inhibiting E2F transcription. On the other hand, ATM/ATR triggers Chk1/Chk2 kinase in response to UV induced DNA damage, which in turn triggers p53 and p21CIP1, resulting in G1 arrest or senescence. Furthermore, p21CIP1 protein levels may cause CDK4/6 activity to be inhibited, which would then cause G1 arrest or senescence. Furthermore, by maintaining fibrinogen activator inhibitor 1, a hallmark of senescent cells, p53 directly causes senescence. Additionally, p53 triggers the transcription of genes that produce the promyelocytic leukemia protein, which results in the senescence of cells.

V. THE STRUCTURE OF P53 GENE

The p53 protein is encoded by the TP53 gene, which is found on chromosome 17p13.1. Eleven of its thirteen exons are involved in coding for the whole p53 protein. The complete p53 protein, like other transcriptional factors, is made up of several different functional domains. TAD 1, AA residues 1–40, and TAD 2, AA residues, two connected transactivation domains (TAD) that mediate p53's transcriptional activity, are located in the N-terminus. TAD1 and TAD2 can work independently of one another even though they can control the transcription of some shared genes because they can also facilitate the transactivation of distinct genes. The binding location for MDM2, a ubiquitin ligase that targets p53 for proteasomal destruction, is also located in the N-terminus. A proline-rich domain (AA, 62–94) that follows the TAD domains has several repetitions of the sequence PXXP (P is proline; X can be any amino acid). Growth suppression and p53-mediated cell death have been linked to this domain. The DNA binding domain (DBD), located on the C-terminal side of the PXXP region, permits sequence-specific binding to DNA (AA, 95–292). The DBD has two big loops, a loop-sheet-helix structure, and a core β-sandwich scaffold that resembles immunoglobulin. Approximately 80% of TP53 mutations are found in this area, which has been highly conserved throughout evolution. The oligomerization domain (OD) (AA, 326–356) comes after the DBD, followed by a linker region (AA, 291-325). When p53 binds to DNA, it forms a tetramer, which requires the OD region. Furthermore, there is a nuclear export signal sequence in the OD region. The highly unstructured carboxyl terminal domain (AA, 357-993), which experiences significant post-translational changes, has been linked to a number of functions, including subcellular modulation of p53 localization, co-factor recruitment, DNA binding, and protein stability.

(i) *n-terminal region*

A proline-rich area and a highly acidic, intrinsically disordered TAD are found in the N-terminal region of p53.263 TADs, which include TAD1 (residues 1–40) and TAD2 (residues 40–61), can interact with negative regulators to prevent transcriptional activation and bind to transcriptional machinery components and transcriptional coactivators to enhance transcriptional initiation. When TAD binds to partner proteins, it can adopt an amphipathic α-helical conformation. This appears to be an essential binding mode, as evidenced by the structures of TAD bound to replication protein A,271 high-mobility group B1, transcription coactivator CBP267,268/p300269,270 , replication protein A,271 Tfb1 subunits of yeast TFIIH273, and metastasis-associated protein S100A4.Notably, when linked to the human TFIIH275 subunit p62, phosphorylated TAD2 displays an acidic string-like structure . It has been demonstrated that phosphorylation of TAD functions as a switch to quickly turn on and off p53 function as well as a mechanism for a graded p53 response.276,277 All things considered, posttranslational changes along with the TAD's conformational plasticity make p53 an extremely effective transcription factor. It takes the proline-rich region connecting the TAD and DBD for p53 to trigger apoptosis and cell cycle arrest. This area binds to arc - homology 3 domains, which helps it function in signal transduction. Additionally, the PRD interacts with the corepressor protein mSin3a, focal adhesion kinase (FAK), peptidase D, ASPP family members, and five PXXP motifs that can be found in the PRD; some of these motifs may take on the structure of a polyproline helix. It is mainly unknown what PRD's precise structure and interaction mechanism are.

(ii) DNA-binding domain

A tetrahedrally coordinated zinc ion stabilizes two big loops (L2 and L3), a loop-sheet-helix motif (loops L1, S2, and S2', sections of the extended S10 and H2), and an immunoglobulin-like β-sandwich serve as the basic scaffold for the structured DBD. The DBD plays a crucial role in the biological activity of p53 as a transcription factor by binding sequence-specific target DNA. Furthermore, DBD has the ability to interact with a variety of proteins.

(iii) DNA recognition

A large number of distinct genes involved in diverse pathways have their transcription regulated by p53 in response to a wide range of stress signals. Additionally, p53 functions as a pioneer transcription factor by attaching itself to particular nucleosome DNA sequences to encourage chromatin transcriptional activation. Both the DNA helix and the DBD change structurally when they attach to DNA. The spacer region's partially disordered DNA deforms when p53-DBD binds to the BAX response element. This causes the area to unwind and compress, enabling protein-protein interactions. In certain p53/DNA configurations, normal Watson-Crick base pairs are replaced by noncanonical Hogsten base-pairing geometries in the middle A-T doublet of each half site. According to some p53/DNA configurations, loop L1 of two p53 subunits switches conformation, moving from an extended conformation in which Lys120 interacts with DNA directly to a recessed conformation in which Lys120 does not bind with DNA directly.301 The conformational shift and p53's selectivity for binding DNA are connected. Acetylation of the residue in loop L1 In the DNA-bound state, Lys120 increases the conformational space of loop L1 and encourages p53's sequence-dependent DNA-binding modes.

(iv) DBD mediated protein- protein interaction

DBD mediates protein-protein interactions with several proteins in addition to DNA recognition. p53's different functions are influenced by interactions with these proteins. A growing number of DBD/protein complex structures have been identified, offering new insights into the processes underlying p53-DBD binding and functional diversity.53BP1 and 53BP2, or the C terminal segment of ASPP2, were first found to bind to p53.303 using a yeast two-hybrid system. They engage in interactions with p53-DBD via the L2 and L3 loops (304–306). In addition to its several functions in DNA damage and repair, 53BP1 has also been shown to improve ASPP2 transcriptional activation mediated by p53. The apoptosis-stimulating p53 protein (ASPP) family includes the inhibitory member iASPP. These proteins regulate p53's apoptotic activity in different ways. In contrast to ASPP2, iASPP interacts with the DBD via the L1 loop, helix H2, and N-terminal loop 308, and preferentially binds p53-PRD283.After attaching to iASPP, the p53 L1 loop separates from other DNA-binding modules and prevents Lys120 from coming into contact with a particular nucleotide. 308 Some understanding of the opposing regulatory mechanism of p53 can be gained from the several binding contacts with the ASPP family. It has been discovered that by blocking the antiapoptotic proteins BCL-2 and BCL-xL, cytoplasmic p53 regulates the mitochondrial apoptosis pathway. The BCL-xl binding surface of p53-DBD largely overlaps with the DNA-binding surface and includes helix H1 and the Zn2+ coordination site 30. This is according to a structural model of BCL-xl/p53 that was determined using the HADDOCK docking method based on 1:1 stoichiometry. Nevertheless, the crystal structure of the p53/BCL-xl complex was recently established, and it shows that p53 binds BCL-xL with a 2:1 stoichiometry. Tyr107 and His178, respectively, are involved in the dimerization process of two p53-DBD molecules through the N-terminal loop and β9-β10 loop of one p53-DBD and the Zn2+-coordination site of the second p53-DBD. After forming a groove and interacting with one BCL-xl, the ensuing p53-DBD dimer forms a ternary complex. BCL-xL and the DNA-contacting residues Arg248 and Arg273 establish direct hydrogen bonds. Compared to other p53-DBD-binding proteins, the binding mechanism is unique.

(v) Tetramerization domain

Through the TET, full-length p53 reversibly forms tetramers. According to the structural study, monomeric TET has a V-shaped structure made up of an α-helix and a β-strand connected by the hinge residue Gly344313–315 (Fig. 7j). The eight backbone hydrogen bonds of the β-sheet support the primary dimer, which is further stabilized by a salt bridge connecting Arg337 and Asp352 and hydrophobic interactions with Phe328, Leu330, Ile332, Phe338, and Phe341.The main hydrophobic contacts (Met340, Phe341, Leu344, Ala347, Leu348, and Leu350) facilitate the dimer-dimer interactions. 35 R337C/H/P and L344P are implicated in Li-Fraumeni Syndrome and Li-Fraumeni-Like Syndrome and have an impact on the synthesis and transcriptional activity of p53 tetramers.316 At physiological temperature, the G334V mutant aided in a beta-dominated structural shift that resulted in the production of amyloid.317 p53's subcellular distribution requires a highly conserved leucine-rich nuclear export signal (NES) inside the TET.318 Although no complex structure has yet been shown, some proteins have been shown to bind directly to TET.3.

(vi) c-terminal regulatory domain

CTD is an intrinsically disordered area, just like NTD. This feature gives it the ability to regulate nearly every aspect of p53, including protein stabilization, cellular localization, cofactor recruitment, and DNA binding. 320 Numerous positively charged residues found in the CTD are highly conserved in mammals. The CTD undergoes extensive posttranslational changes, such as acetylation, methylation, and phosphorylation, all of which are necessary for its stability and functionality. Depending on the binding partners and post-translational modifications, the CTD takes on many conformations.

VI. FULL LENGTH P53-

It is challenging to ascertain the high-resolution structure of full-length p53 due to the highly intrinsic unfolded areas present in p53. The DBD targets the upstream DNA-binding site within RNA polymerase II (Pol II), and the subsequent TET is exposed on top of the DBD, according to a 4.6 Å resolution structure of Pol II with full-length p53,322. The jaw of Pol II that makes contact with downstream DNA is bound by the TAD distal to the DBD, which helices. Through this interaction, Pol II undergoes a conformational shift that sheds light on the p53-mediated control of gene expression. There is still much to learn about the interactions between an intact p53 tetramer and proteins and DNA. Small-angle X-ray scattering, electron microscopy, FRET, and NMR methods all work together to show that the free p53 tetramer in solution forms an open, cross-shaped structure with two loosely connected DBD. 323,324 When the oligomers attach to a particular DNA response element, they close to form a compact complex. Additionally, mass spectrometry and chemical cross-linking techniques are used to investigate the structural dynamics of full-length p53.Cryo-electron microscopy of p53-DBD and full-length p53 complexed with a nucleosome has been reported in a recent study. In this process, the DBD attaches to the DNA as a tetramer and removes the DNA from the histone surface. The cryo-EM maps did not show the N- or C-terminal areas; however, a biochemical study indicates that p53's C terminus may have an extra DNA-binding domain. The major task of protein amino acid sequence to structure has been successfully completed by the artificial intelligence system AlphaFold, greatly advancing the study of biology, particularly structural biology.326–328 The full-length p53 protein's predicted structure was published by AlphaFold2 in 2021. The DBD and TET portions of the projected full-length p53 structure are shown as monomers, similar to the known structures of specific structural domains. However, it doesn't go into further detail on how the disordered areas fold or how p53 forms a tetramer. Furthermore, missense mutations that cause p53 to lose or change in function are its most significant characteristic. At this time, AlphaFold2 is unable to accurately forecast how missense mutations would affect structure. 330 Additionally, the P53 protein binds to a variety of ligands, including metal ions, DNA, small molecules, and other proteins. To help with the development of drugs against p53 and the identification of protein complexes, structural knowledge on this interaction is currently lacking. We anticipate that artificial intelligence will produce more useful knowledge in the future as a result of the technological revolution. In any case, structural research on full-length partner proteins, full-length p53 in complex with other DNA targets, and even higher-order complexes is required. Not only can this structural biology knowledge aid in comprehending the function of p53 in cellular processes, but these structures might also be useful in the development of p53-targeting medications.

VII. TARGETING P53 BASED ON STRUCTURE

Analysis based on biochemistry and structure has shed light on the role of p53. There are still a lot of unanswered problems concerning the precise regulation and structure-function of p53. Recent advancements in NMR, protein crystallography, and cryo-electron microscopy techniques have yielded a wealth of structural data regarding p53's ligand-binding details. Structure-based design techniques can be used to optimize the structure and, hence, increase activity and selectivity based on this crucial information. {fig.2}

(i) MDM/MDMX-p53, a central hub for activation

The primary negative regulator of p53 is MDM2/MDMX. The N-terminal of MDM2 binds to p53-TAD and represses the transcriptional activity of p53, while wild-type p53 stimulates the transcription of MDM2. This is the MDM2-p53 negative feedback regulatory loop. Furthermore, E3 ubiquitin ligase, an enzyme present in the C-terminus of MDM2, facilitates nuclear export and p53 degradation. MDMX, also known as MDM4, is a homolog of MDM2 that shares structural similarities with MDM2 but does not have ubiquitin ligase activity. Instead, it can increase MDM2's ubiquitination activity by creating a dimer with MDM2. Many human tumors include overexpression or activation of MDM2/MDM4, which results in p53 inactivation. Several anticancer medications that target the interactions between MDM2/MDMX and p53 have been discovered recently.

(ii) MDM2-p53 inhibitor

The cocrystal structure of p53-TAD with MDM2 shows that Phe19, Trp23, and Leu26 are on the α-helix of p53. penetrate deeply into the hydrophobic cleft of MDM2. with Leu22 providing additional van der Waals forces. These The structural features of the p53/MDM2 complex provide a basis for Finding inhibitors that block the interaction between these two proteins.

(iii) RG7112

Researchers created imidazole-like MDM2 antagonists based on the structural characteristics of p53/MDM2, with RG7112 being the first small-molecule MDM2 inhibitor to reach the clinic. The 4-chlorophenyl ring was found to be occupied by the Trp23 and Leu26 pockets in the crystal structure, while the ethoxy group was clearly visible in the Phe19 pocket 341 The Kd for RG7112 was 2.9 nm. It efficiently inhibits the binding of p53 to MDM2, hence promoting apoptosis and cell cycle arrest in cancer. RG7112's capacity to cause cancer cells to undergo apoptosis, however, varies greatly. MDM2 gene-amplified osteosarcoma cell lines and xenografts had the best responses.

(vi) AM232

AMG32, a piperidone analogue with a Kd of 0.045 nms., potently inhibits the MDM2-p53 interaction. According to a structural study, the "cleft" in Gly58 might help the tiny molecule attach MDM2 even more. The isopropyl group, C6 aryl group, and C5 aryl group, respectively, occupy the three main binding pockets of p53, which are Trp23, Leu26, and Phe19, according to the crystal structure. Concurrently, the His96 imidazole group generated hydrogen bonds with carboxylate and engaged in π-π stacking interactions with the C5 aryl group. Furthermore, the isopropyl group penetrates into Gly58's "cleft," establishing CH2O-type interactions with Gly58 and strengthening bonds with surrounding hydrophobic residues. AMG232 increases p53 activity in tumor cells expressing high levels of MDM2, which in turn stops tumor growth.

(v) SAR405838

An optimized spiro-oxide molecule, SAR405838 (MI77301), with a Ki value of 0.88 nm., inhibits the MDM2-p53 interaction and stops p53 degradation. Like other MDM2 inhibitors, 354 SAR405838 mimics the three essential amino acid residues of p53 and makes hydrogen bonds with the His96 residue of MDM2 at various chemical groups to produce π-π stacking. The structural variations include the fact that SAR405838 interacts with Val14 and Thr16 extensively and facilitates the refolding of the MDM2 N-terminus. Additionally, a hydrogen bond is formed between the hydroxy-cyclohexyl group and Lys94354. SAR405838 is able to achieve tight binding and excellent specificity for MDM2 because of these structural characteristics. SAR405838 causes total tumor regression in SJSA-1 osteosarcoma xenograft mice by activating the p53 pathway, upregulating PUMA and P21 expression, and creating these effects.

(vi) NVP-CGM097

Via virtual screening and structural optimization, NVP-CGM097, a new dihydroisoquinoline-like MDM2 inhibitor, was discovered with a Ki of 1.3 nm. The isopropyl ether group creates water-mediated hydrogen bonds with Tyr100, Gln24, and Phe55 in addition to the three essential amino acids that replicate the contact between p53 and MDM2, as demonstrated by the cocrystal structure. Phe55 had a conformational shift brought on by NVP-CGM097, which allowed Phe55 to develop a π-π stacking contact with the dihydroisoquinolone core. When applied to colorectal cancer and osteosarcoma cells that express wild-type p53, NVP-CGM097 demonstrates strong antiproliferative activity and great selectivity for wild-type p53.In combination with MEK inhibitors, NVP-CGM097 enhances the MAPK signaling pathway, thereby reducing the cell burden of acute myelogenous leukemia (AML). In neuroblastoma or ER-positive breast cancer cells, NVP-CGM097 causes cell death when paired with BET or Cdk4/6 inhibitors.

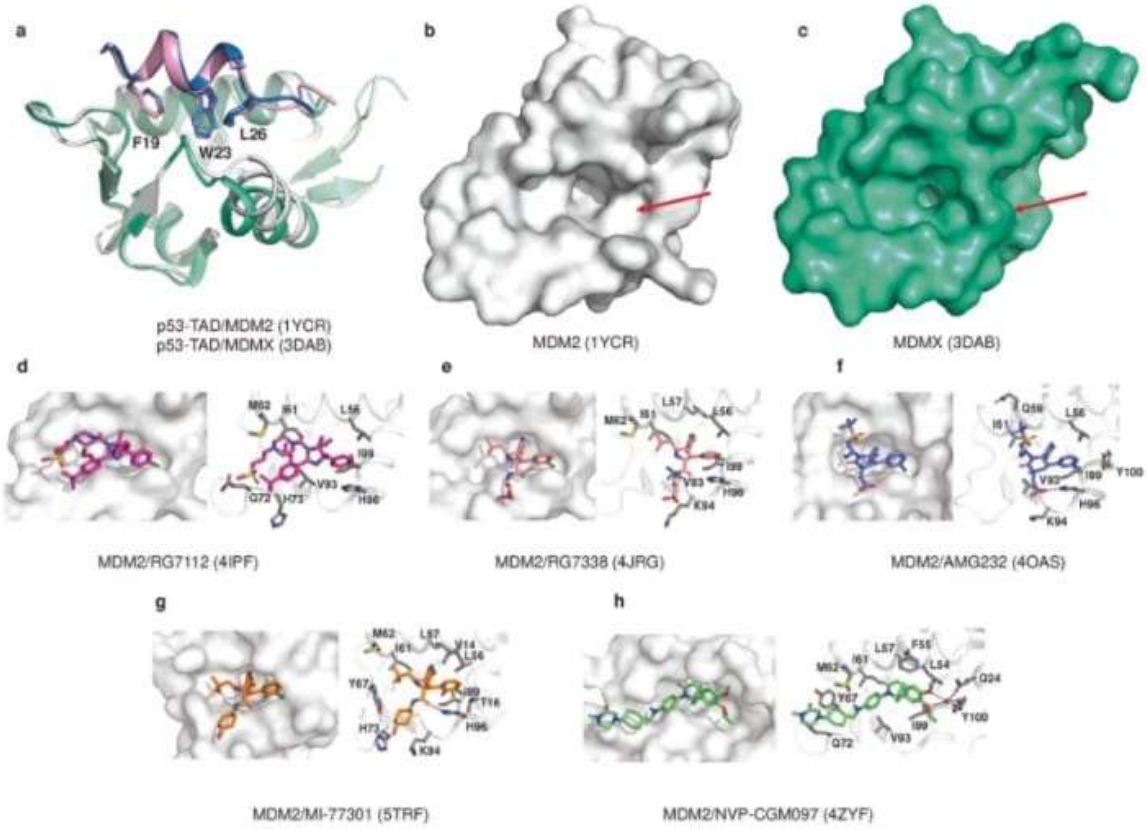

Fig.2. **Structure of MDM2/X with small molecules. an Overlay of the crystal structures of MDM2/p53-TAD (white, sky blue), MDMX/p53-TAD (green, cyan, pink) and the three residues of p53-TAD (F19, W23, L26) are shown as sticks. b MDM2 is shown as a surface. c MDMX is shown as surface. d MDM2/RG7112 (PDB: 4IPF). e MDM2/RG7388 (PDB: 4JRG). f MDM2/AMG 232 (PDB: 4OAS). g MDM2/MI-77301 (PDB: 5TRF). h MDM2/ NVP-CGM097 (PDB: 4ZYF). Water molecules are red spheres, and hydrogen bonds are black lines. The interacting amino acid residues are shown as sticks (colored gray).**

(vii) MDM2/MDMX-p53inhibitor

The MDMX-p53 structure retains the major characteristics of the MDM2-p53 interaction; however, the p53 peptide binding's core hydrophobic cleft in MDMX is smaller and has a different shape than in MDM2. As a result, many small-molecule medications that target MDM2 have poor MDMX binding.

(viii) ALRN-6924

As of right now, no inhibitors have been found that specifically target MDMX; however, ALRN-6924, a stapled peptide, can bind to MDM2 (Kd = 10.9 nm.) and MDMX (Kd = 57 nm.). By inducing cell cycle arrest and death in AML cells, ALRN-6924 considerably increases the survival time of AML model mice. In phase I clinical studies, ALRN-6924 is well tolerated by patients with solid tumors and lymphomas that have wild-type p53. However, it appears from molecular simulations that ATSP-7041, an analogue of ALRN-6924, may bind to and isolate free p300 from the p53 coactivator, hence decreasing p53's transcriptional activity. Additional research is necessary to fully understand this mechanism.

VIII. TARGETING P53 FOR CANCER TREATMENT

There are now several methods for treating cancer that target p53 in both its wild-type and mutant variants. One of the most promising treatments for tumors containing mutant TP53 is to encourage the reactivation of the mutant protein to a version that resembles the natural type. However, the strategy that is now receiving the greatest research for tumors that still have the wild type gene entails stopping the wild-type protein's breakdown in order to extend its cellular half-life. The next section discusses the most extensively studied molecules from each of these approaches that have advanced to clinical trials.

(i) Reactivation mutant p53

There are various reasons why targeting mutant p53 for cancer treatment is appealing. First off, wild type p53 is often found in low concentrations in unstressed normal cells, but mutant p53 builds up in cancer cells. There should be an increase in selectivity as a result of the distinct expression of malignant and normal cells. The fact that mutations in mutant p53 tend to arise early in carcinogenesis and are clonal in a variety of tumor types makes it an appealing target for anticancer therapeutics. Clonal mutations typically manifest early in the carcinogenesis process and are found in all or the majority of the malignant cells within a tumor. Even though mutant p53 is concentrated and clonal (at least in some tumor types), it has proven to be challenging to target. In fact, like RAS and MYC, p53 has historically been thought of as "undruggable" or challenging to manipulate pharmacologically. While EGFR, HER2, and BRAF are examples of cancer-driver oncoproteins that have been successfully targeted for treatment, p53 does not have enzymatic activity and hence cannot be blocked by low-molecular-weight catalytic inhibitors. Moreover, the majority of

p53's subcellular location is nuclear, making it inaccessible to large molecular weight medications like monoclonal antibodies. That's right—aside from ligand-activated transcription factors like the androgen and estrogen receptors, targeting nuclear proteins for cancer treatment has proven challenging. Many missense mutations affecting mutant p53 have long been recognized to change the protein's structure and cause it to unfold. It is possible to anticipate that reversing this mutant conformation will restore the wild-type form and its wild-type activity. Thousands of chemicals have been examined for this capacity using either cell-based screening or in silico techniques, but only a few have been found to appear to be able to reverse the mutant conformation. The restoration of particular wild-type functions to mutant p53, such as the induction of apoptosis, the stimulation of cell cycle arrest, and the prevention of cancer cell proliferation, served as confirmation of this "mutant reactivation." **(**Table.1.)

Method/Approach	Example of drug
Prevention of WT p53 degradation by blocking interaction with MDM2/ MDM4	Nutlin-3a, **RG7112, RG7388, RG 7775, MI-77301, AMG232, MK-8242, CGM097, DS-3032b, ALRN-6924**
Targeted degradation of MDM2 using PROTAC	IMiD-based MDM2 PROTAC 8, A1874*
Reactivation of mutant p53 to a form with WT properties	MIRA1, STIMA1, PRIMA-1, **APR-246,** PK110007, PK7088, **COTI2,** HO-3867
Depletion of mutant p53 using HSP inhibitors or statins	**Ganetespib, onalespib, luminespib (HSPs)** **atorvastatin, lovastatin (statins)**
Gene replacement (gene therapy)	Gendicine (Ad-53)**
Restoration of zinc to zinc-deficient p53 mutants	ZMC1, ZMC2, ZMC3, ZN-1
Promotion of readthrough of premature termination	Geneticin, gentamicin, G418
Use of oncolytic viruses that selectively replicates in some mutant p53 cells	ONYX-015
Inhibition of mevalonate pathway	Statin drugs (atorvastatin)
Blocking mutant p53 aggregation	PRIMA-1

Table.1. Strategies used for targeting p53. Drugs shown in bold have undergone or are currently undergoing clinical trials. WT, wild-type; PROTAC, proteolysis-targeting chimera; HSP, heat shock protein. *An MDM-recruiting BRDR-degrading PROTAC; **Gendi Cine is commercially available (Shenzhen SiBiono GeneTech Co); Data reviewed from refs.

(ii) COTI-2

COTI-2{E)-N'-(6,7-dihydroquinolin-8(5H)-lysidine)-4-(pyridin-2-yl) piperazine-1-carbothiohydrazide} is a thiosemicarbazone compound that was found by using an in-silico machine learning system that is said to be able to predict target biological activities based on molecular structures. Similar to APR-246, COTI-2 caused the mutant p53 conformation to revert to the wild-type form when p53 was stained with antibodies specific to p53 conformation. Moreover, it has been demonstrated that COTI-2 binds to mutant p53, normalizes the production of the wild-type p53 target gene, and restores the wild-type DNA binding characteristics. Like APR-246, COTI-2 also seems to have the ability to function independently of p53. It was discovered that the substance causes replication stress, induces DNA damage, inhibits the mTOR pathways, and activates AMPK. These p53-independent properties of COTI-2 are probably going to add to its anticancer effects because it has been shown that inducing DNA damage and replication can cause cell death, and blocking mTOR signaling is a tried-and-true method of treating cancer. In line with the aforementioned actions, COTI-2 slowed the development of several cancerous cell lines and tumor models in animals. It was discovered that breast cancer cell lines harboring mutant p53 were more sensitive to COTI-2 than those with wild-type p53. The administration of COTI-2 was found to be well tolerated in the examined animal models, with no signs of morbidity or weight loss [81, 85]. In a phase I clinical trial, COTI-2 is now being evaluated for the treatment of recurring gynecological malignancies (NCT02433626). A preliminary assessment of the compound's pharmacokinetics revealed that oral treatment resulted in a T-max after 15 to 90 minutes and a half-life of 8 to 10 hours.

(iii) Potential predictive biomarkers for mutant p53-reactivating compounds

Predictive biomarkers are being tested more often before anticancer medicines are administered in order to improve efficacy and reduce toxicity. Given that numerous compounds that reactivate mutant p53 do so, at least in part, by binding to and activating mutant p53, it stands to reason that malignant cells that express the mutant protein would be more susceptible to these compounds than cells that express wild type p53**.** According to Synnott et al., three distinct p53-reactivating compounds—APR-246, PK11007, and COTI-2—had a greater effect on mutant p53 breast cancer cell lines than on p53-wild-type cells. Additionally, there was a substantial correlation found between the reaction to these drugs and the levels of endogenous p53 protein, which serves as a surrogate marker for the presence of mutant p53. This means that when baseline p53 protein levels grew, so did the IC50 values for these compounds. If these compounds are eventually used in clinical settings, our findings imply that the existence of mutations or elevated amounts of p53 protein may serve as predictive biomarkers for certain mutant p53-reactivating drugs. While tumor cells containing mutant p53 might respond more strongly to medications like APR-246, response is not always prevented in the absence of such mutations. In fact, as was already mentioned, a number of investigations have demonstrated that the medication can also affect tumor cells with wild-type or null p53.In esophageal as well as in a mixed collection of cancer cell lines, SLC7A11 expression (a component of the cystine/glutamate antiporter, system XC−) was also found to correlate with responsiveness to APR-246, i.e., the lower the SLC7A11 expression, the more sensitive cells were to APR-246 . Findings showing SLC7A11 overexpression in

mutant p53 cells resulted in resistance, whereas SLC7A11 knockdown in p53-null cells sensitized cells to APR-246 supported a causal connection between SLC7A11 and responsiveness to APR-246. These findings imply that SLC7A11 may potentially function as a predictive biomarker for APR-246 sensitivity. Lastly, it was discovered that increased susceptibility to APR-246 was linked to a deficit of the SWI/SNF family member Arid1A, which regulates SLC7A11 expression.SLC7A11 expression, a part of the cystine/glutamate antiporter system XC−, was also observed to correlate with responsiveness to APR-246 in esophageal as well as in a mixed collection of cancer cell lines . That is, the lower the SLC7A11 expression, the more susceptible the cells were to APR-246. Findings showing SLC7A11 overexpression in mutant p53 cells resulted in resistance, whereas SLC7A11 knockdown in p53-null cells sensitized cells to APR-246 supported a causal connection between SLC7A11 and responsiveness to APR-246. These findings imply that SLC7A11 may potentially function as a predictive biomarker for APR-246 sensitivity. Lastly, it was discovered that increased susceptibility to APR-246 was linked to a deficit of the SWI/SNF family member Arid1A, which regulates SLC7A11 expression.

(iv) Uncertainties and challenges in the use of mutant p53 reactivation compounds

Preclinical research indicates that a number of mutant p53-reactivating drugs have anticancer activity, but there are still a number of unknowns regarding the precise mechanisms behind these compounds' actions. apoptosis, or some other mechanism? In fact, it's unclear if various cell types have distinct -

- First off, it's not clear how much reactivation of the mutant p53 protein is necessary for a long-lasting anticancer response. For instance, would part of the mutant protein, as previously described, continue to drive the progression of cancer in a dominant-negative fashion, or would the implications depend on the relative amount of wild-type vs. mutant p53 protein?

- The efficiency of substances like APR-246 or COTI-2 against the various p53 mutation types, which are likely to have distinct functionalities, is another issue that has to be addressed. For example, while p53 contact mutations are thought to have little impact on overall protein structure, it is unknown how substances like APR-246 and COTI-2 function in cells with these mutations.

- The precise method by which drugs containing mutant p53 exhibit their anticancer effects after p53 reactivation is another important open subject. Is it through inducing cell cycle arrest, processes. Further research is needed to determine how much ROS production vs. mutant p53 reactivation contributes to APR-246's anti-cancer activity. Likewise, it is yet unknown how much each of COTI-2's several actions contributes to the protein's ability to prevent the growth of tumor cells.

- Lastly, it is unknown how p53-reactivating substances affect the various p53 isoforms or the paralogs p63 and p73 in terms of causing tumor regression.

(v) Blocking interaction between wild type p53 and MDM2/MDM4

As previously noted, interaction with negative regulators, particularly MDM2 and MDM4, which are overexpressed in particular tumor types, keeps levels of the corresponding protein low in many malignancies that preserve wild-type TP53. The ability of the wild-type protein to inhibit carcinogenesis is restricted at these low concentrations. It would be anticipated that inhibiting the interaction between p53 and MDM2, MDM4, or ideally both proteins would stop the degradation of wild type p53 and preserve its capacity to suppress tumor formation. However, small-molecule inhibitors can be challenging to block protein-protein interaction because their binding surfaces are typically too wide or flat to achieve high-affinity binding. Early research on the interaction between p53 and MDM2 revealed that three hydrophobic amino acids of p53, namely Phe19, Trp23, and Leu26, might bind into a deep pocket found in the N-terminus of MDM2. Based on this understanding, a number of substances that mimicked this interaction—both peptides and tiny molecular-weight molecules—were created and demonstrated to prevent MDM2 from attaching to p53. Two additional sites of interaction between p53 and MDM2 have been discovered recently. These are the carboxy-terminal region of p53 (amino acid residues 367–393) binding to the N-terminal region of MDM2, and the DNA-binding domain (amino acid residues 234–286) attaching to the central acidic domain in MDM2.The cis-imidazoline group of molecules known as nutlings, particularly nutlin-3a, was one of the first series of low-molecular-weight compounds demonstrated to disrupt the interaction between p53 and MDM2 . Nutlin-3a was not pursued for possible clinical use due to poor bioavailability, toxicity, and limited efficacy, even though preclinical studies demonstrated that it bound to the p53 N-terminal interaction site on MDM2, blocked p53 degradation, increased p53 levels, enhanced apoptosis, decreased immune suppression, and decreased tumorigenicity in p53 wild-type cancer cells. Instead, stronger p53-MDM2 inhibitors were discovered, some of which are already being tested in clinical trials. These inhibitors have better pharmacokinetic characteristics and increase anticancer activity. The nutlin-3a analogue RG7112 was among the first MDM2 inhibitors to enter clinical trials. Twenty liposarcoma patients received RG7112 from Ray-Counard et al. in an exploratory proof-of-mechanism trial. Of the 17 individuals that could be evaluated, 14 had tumors with the amplified MDM2 gene. After eight days of treatment, levels of p53 and p21 proteins rose by about five and fourfold, respectively, indicating that MDM2-mediated degradation of the tumor suppressor protein was inhibited. One patient had a documented partial response, and fourteen had stable disease out of the patients who were eligible for evaluation. Treatment with RG7112 produced a complete or partial response in 5/30 evaluable patients in a second phase I trial in patients with different forms of leukemia, while 9 suffered stable disease. The specific p53 target genes, BAX, PUMA, and CDKN1A (p21), were all upregulated, which provided evidence for RG7112-mediated p53 stabilization. Significant toxicity prevented RG7112 from being further studied for clinical use in spite of these findings. Then, disannulling (RG7388, RO5503781), a more powerful and selective nutling analogue, was synthesized.

While disannulling and RG7112 share a similar mechanism of action, disannulling is a more powerful and selective MDM2 inhibitor. Numerous clinical trials are presently studying RG7388, including a phase III experiment that pairs it with cytarabine for individuals with relapsed or refractory acute myeloid leukemia (NCT02545283). According to preliminary research, leukemic blasts' pre-treatment MDM2 protein levels and responsiveness are related. The following list includes other MDM2 inhibitors that are being evaluated in clinical studies (mainly phase I). The limited affinity of the low-molecular-weight MDM2 inhibitors now on the market for MDM4 is one of its issues. As a result, they probably won't work well on tumors that overexpress MDM4. Moreover, it is possible that MDM4 overexpression will give MDM2 inhibitors acquired resistance. As a result, efforts have been made to create dual MDM2-MDM4 antagonists or selective MDM4 antagonists. As a matter of fact, MDM4 targeting might be less problematic than MDM2 antagonist use. A few pure MDM4 inhibitors have been demonstrated to inhibit anticancer activity, including XI-001, which acts by blocking MDM4 expression, and SAH-p53-8, a cell-penetrating, stabilized α-helical peptide.

Inhibitor	Chemical class	Company	Phase	Tumor	Identifier
RG7112	Cis-imidazoline	Roche	I	Liposarcoma	NCT01143740
			I	Solid tumors	NCT01164033
			I	Leukemia	NCT00623870
				Leukemia	NCT01677780
RG7388	Cis-imidazoline	Roche	I	Solid tumors	NCT02828930
			I	Solid tumors	NCT0146175
			III	AML*	NCT02545283
AMG232	Piperidinone	Amgen	IB	AML**	NCT02016729
			IB/IIA	Melanoma	NCT02110355
MI-77301	Spirooxindole	Sanofi	I	Advanced cancers	NCT01636479
SAR405838***	Piperidinone	Sanofi	I	Advanced cancers	NCT01985191
DS3032b	Unknown	Daiichi Sankyo	I	Advanced tumors	NCT01877382
HDM201	Imidazopyrrolidinone	Novartis	I	Multiple	NCT02143635
CGN097	Dihydroisoquinolinone	Novartis	I	Multiple	NCT01760525
ALRN-6924	Stapled peptide	Aileron Therapeutics	I/IB	AML/MDS	NCT02909972
ALRN	Stapled peptide	Aileron Therapeutics	I/IIA	Solid tumors, lymphomas	NCT02264613

Table .2. MDM2 and MDM2/4 inhibitors that have either completed, are undergoing, or are recruiting for clinical trials: * in conjunction with pimasertib; ** in conjunction with trametinib; * in conjunction with cytarabine. MDS stands for myelodysplastic syndrome, and AML stands for acute myeloid leukemia.**

In a case report, administration of the ALRN-6924 to a patient with AML and myelodysplastic syndrome (MDS) resulted in an increase of p53 levels in CD34+ leukemic blast cells but not in healthy lymphocytes. Importantly, ALRN-6924 decreased the number of circulating CD34+ leukemic cells from 5 to 7% prior to administration to < 1% after 2/3 weeks of treatment. ALRN-6924 is currently undergoing further clinical evaluation in patients with AML, MDS, and lymphomas, as well as in patients with diverse solid cancers (NCT02909972, NCT02264613). Preliminary results suggest that ALRN-6924 is well tolerated, with the most common non-hematologic adverse effects being gastrointestinal symptoms Toms, fatigue, and headaches.

(vi) potential predictive and resistance bio marks for MDM2/4 inhibitor

Early research using a wide range of cancer cell lines revealed that MDM2 overexpression made cancer cells more susceptible to nutlin-3a therapy. Preliminary data from early-phase clinical studies indicated that leukemic cells' levels of MDM2 expression were connected with their responsiveness to RG7112 or idasanutlin, which was in line with these preclinical findings. Two multigene signatures have been shown to predict responses, at least in vitro, for the MDM2 inhibitor DS3032b. In a mixed panel of cancer cell lines, one of these signatures, comprising 175 genes, was found to correlate with response to DS303-2b, while a second signature, comprising 1532 genes, predicted response in AML cells. While preclinical and early clinical research indicate that the existence of p53 mutations predicts resistance to these compounds, high levels of MDM2 are linked to a likely response to MDM2 antagonists. Therefore, the most significant ($p < 1 \times 10{-}36$) drug-gene association among the 130 drugs tested in 639 cancer cell lines was found in the correlation between the presence of mutant p53 and response to nutlin-3a, meaning that p53 mutant cell lines were significantly more resistant to nutlin-3a than p53 wild-type cells. In a heterogeneous collection of cell lines, it was also discovered that the existence of mutant p53 predicted resistance to another MDM2 inhibitor, such as AMGMDS3.As the administration of MDM2 inhibitors has been demonstrated to cause the emergence of TP53 mutations in tumor cells with the TP53 wild-type phenotype, the capacity of mutant p53 to confer resistance to various MDM2 inhibitors may have significant therapeutic implications for the usage of these inhibitors. The development of acquired resistance to the current anti-MDM2 medication is one of the likely outcomes of these acquired mutations, but another is the potential for the acquired mutant p53 protein to accelerate the advancement of any existing tumors. Should the results above hold true, treatment with MDM2 antagonists may require a combination with medications that reactivate the mutant p53 gene.

(vii) uncertainties and challenges in the use of MDM2/MDM4 inhibitors

Treatment with MDM2/MDM4 inhibitors may pose a risk since, in addition to stabilizing p53 in tumor cells, these drugs are also anticipated to stabilize the protein in normal cells. The accumulation of wild type p53 in normal cells could lead to inappropriate cell death if this stabilization takes place. A plausible reason for the adverse effects of MDM2 inhibitors documented in the aforementioned early-phase clinical trials could be this increased cell death. The injection of MDM2/MDM4 inhibitors may cause the overexpression of non-MDM2/MDM4 E3 ubiquitin ligases, which is another possible issue. These non-MDM2/MDM4 ligases have the ability to degrade p53, which may lead to acquired resistance, as was previously described. However, little information is known about how these non-MDM2/MDM4 proteins are expressed in tumors or whether therapy with MDM2/MDM4 inhibitors causes a compensatory mechanism that raises their levels. A possible resistance mechanism to MDM2 inhibitors could involve the overexpression or amplification of MDM4. Analogously, MDM2 overexpression or amplification may lead to MDM4 inhibitor resistance. Lastly, as was already indicated, MDM2 has p53-independent activities, such as the capacity to control gene expression, take part in DNA repair, and alter chromatin structure. It's unknown how the available MDM2 inhibitors affect these processes, if at all.

IX. CONCLUSION

The most commonly mutated gene in humans is TP53. In the forty years since p53 was discovered, new understandings of its tumor-suppressive pathways and gene regulatory mechanisms have emerged. Although we now know of a sophisticated and intricate network of tumor suppression, we do not yet have a clear understanding of it. Numerous anticancer medications that target p53 have been created as a result of the connection found between the structure and function of p53 and the development of cancer. There aren't any licensed medications yet, though. Even though p53 malfunction in cancer was first identified in the late 1970s, clinical trials on the subject have not started for more than thirty years. Only three compounds—the MDM2 antagonist idasanutlin and the mutant p53-reactivating medications APR-246 and COTI-2—have advanced to phase III clinical trials, with the majority of active trials still in the preliminary stage. Therefore, it is too soon to say whether or not targeting p53 will be effective in treating cancer. Nonetheless, given the high frequency of p53 malfunction in human malignancies, a new era in cancer treatment is anticipated if any of the substances now undergoing clinical trial evaluations prove to have strong anticancer potential. To present, the majority of research utilizing p53 targeting for cancer treatment has entailed the use of medications that either stop wild type p53 degradation or revive mutant p53. Future research should concentrate on other tactics, including the promotion of read-through of premature termination, statin-mediated suppression of the mevalonate pathway (which results in mutant p53 degradation), and the use of PROTAC drugs to degrade mutant p53 or MDM2. Ultimately, predictive indicators for these anti-p53 medicines will be critical for therapy personalization and optimization, provided they find a clinical home.

X. References

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