

MDM2 Inhibitors for Cancer Therapy: The Past, Present, and Future

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Non-Standard Abbreviations

3qw, Three times per week; ABCG2, ATP-binding cassette sub-family G member 2; AEs, Adverse events; ALL, Acute lymphoblastic leukemia; AML, Acute myeloid leukemia; AR, Androgen receptor; ATF3, activating transcription factor 3; ATF4, activating transcription factor 4; Bad, BCL2-associated agonist of cell death; Bax, BCL2-associated X; Bcl-2, B-cell lymphoma 2; Bcl-xL, B-cell lymphoma-extra large; BET, Bromodomain and extra-terminal motif; Bid, BH3 interacting domain death agonist; B-raf, V-raf murine sarcoma viral oncogene homolog B1; c-Cbl, Casitas B lymphoma; CCND1, Cyclin D1; CD133, prominin-1; CDK, Cyclin-dependent kinases; CLL, Chronic lymphocytic leukemia; Cmax, Maximum observed concentration; CML, Chronic myeloid leukemia; CR, Complete remission; CRc, Composite complete remission; CRC, Colorectal cancer; D1D8Q4W, Days 1 and 8 of a 28-day cycle; DCR, Disease control rate; DDLPS, Dedifferentiated liposarcoma; DHRS2, Dehydrogenase/reductase member 2; DLBCL, Diffuse large B cell lymphoma; DLTs, Dose limiting toxicities; DNMTs, DNA methyltransferases; E2F1, E2F transcription factor 1; EGFR, Epidermal growth factor receptor; Emax, Maximum effect; EMT, Epithelial-mesenchymal transition; EZH2, Enhancer of zeste homolog 2; ER, Estrogen receptor; FDA, Food and drug administration; FGFR, Fibroblast growth factor receptor; FLT3, Fms-like tyrosine kinase 3; FMRP, Fragile X mental retardation protein; FOXO4, Forkhead box O4; FXS, IGF-Fragile X syndrome; GBM, Glioblastoma; GPRC5A, G Protein-coupled receptor class C group 5 member A; GSPT1, G1 to S phase transition 1; HDAC3, Histone deacetylase 3; HLA, Human leukocyte antigen; HOX, Homeobox; HOXA13, Homeobox A13; HPD, Hyperprogressive disease; HSCs, Hematopoietic stem cells; HTS, High-throughput screening; ICIs, Immune checkpoint inhibitors; IDH, Isocitrate dehydrogenase; IFN- γ , interferon-gamma; IGF-1R, insulin-like growth factor 1 receptor; IL-15,

interleukin-15; ING3, Inhibitor of growth family member 3; INSM1, Insulinoma-associated 1; iPSCs, Induced pluripotent stem cells; JAKi, Janus kinase inhibitor; KRAS, Kirsten rat sarcoma virus; LPS, Liposarcoma; MAPK, Mitogen-activated protein kinase; MCC, Merkel cell carcinoma; MDM2, Mouse double minute 2; MDMX, Murine double minute X; MDS, Myelodysplastic syndrome; MEFs, murine embryonic fibroblasts; MEK, Mitogen-activated protein kinase kinase; MGMT, O-6-methylguanine-DNA methyltransferase; MHC-II, Major histocompatibility complex class II molecules; MIC-1, Macrophage inhibitory cytokine 1; MSCs, mesenchymal stem cells; MTF2, Metal response element binding transcription factor 2; MTD, Maximum tolerated dose; MT-ND6, NADH-dehydrogenase 6; mTOR, Mammalian target of rapamycin; NB, Neuroblastoma; Nbs1, Nijmegen breakage syndrome protein 1; NDUFS1, NADH:ubiquinone oxidoreductase 75 kDa Fe-S protein 1; NFATc2, Nuclear factor of activated T cells, cytoplasmic 2; NF- κ B, Nuclear factor kappa B; NMR, nuclear magnetic resonance; NSCLC, Non-small cell lung cancer; OS, Overall survival; p53WT, Wild-type p53; PARP, poly-ADP ribose polymerase; P-gp, P-glycoprotein; Ph⁺ CML, Philadelphia chromosome-positive chronic myeloid leukemia; PD, Pharmacodynamics; PD-1, Programmed cell death 1; PD-L1, Programmed death-ligand 1; PDX, Patient-derived xenograft; PFS, Progression-free survival; PK, Pharmacokinetics; PKM2, pyruvate kinase M2; PI3K, Phosphatidylinositol-3-kinase; PMF, Primary myelofibrosis; Post-ET-MF, Post-essential thrombocythemia myelofibrosis; Post-PV-MF, Post-polycythemia vera myelofibrosis; PPIX, Protoporphyrin IX; PR, Partial response; PRC2, Polycomb repressor complex 2; PROTAC, Proteolysis targeting chimera; PV, Polycythemia vera; Q3W, Once every 3 weeks; QD, Once daily; QOD, Every other day; R/R, Relapsed or refractory; RDE, Recommended dose for expansion; RNF2, RING finger protein 2; ROS, Reactive oxygen species; RP2D, Recommended phase 2 dose; SCF, Skp1-cullin-F box;

SD, Stable disease; STAT5, Signal transducer and activator of transcription 5; SVR, Spleen volume reduction; SUZ12, Suppressor of zeste 12 homolog; TAA, Tumor-associated antigen; TKIs, Tyrosine kinase inhibitors; TNF- α , Tumor necrosis factor alpha; TSS, Total symptom score; VEGF, Vascular endothelial growth factor; WD/DD, Well-differentiated/dedifferentiated; WDLPS, Well-differentiated liposarcoma; WT, Wild-type.

Abstract

Since its discovery over 35 years ago, MDM2 has emerged as an attractive target for the development of cancer therapy. MDM2's activities extend from carcinogenesis to immunity, to the response to various cancer therapies. Since the report of the first MDM2 inhibitor more than 30 years ago, various approaches to inhibit MDM2 have been attempted, with hundreds of small molecule inhibitors evaluated in preclinical studies and numerous molecules tested in clinical trials. Although many MDM2 inhibitors and degraders have been evaluated in clinical trials, there is currently no FDA-approved MDM2 inhibitor on the market. Nevertheless, there are several current clinical trials of promising agents that may overcome the past failures, including agents granted FDA orphan drug or fast-track status. We herein summarize the research efforts to discover and develop MDM2 inhibitors, focusing on those that induce MDM2 degradation and exert anticancer activity, regardless of the p53 status of the cancer. We also describe how preclinical and clinical investigations have moved towards combining MDM2 inhibitors with other agents, including immune checkpoint inhibitors. Finally, we discuss the current challenges and future directions to accelerate the clinical application of MDM2 inhibitors. In conclusion, targeting MDM2 remains a promising treatment approach, and targeting MDM2 for protein degradation represents a novel strategy to downregulate MDM2 without the side effects of the existing agents blocking p53-MDM2 binding. Additional preclinical and clinical investigations are needed to finally realize the full potential of MDM2 inhibition in treating cancer and other chronic diseases where MDM2 has been implicated.

Keywords: MDM2, p53, cancer therapy, drug resistance, drug discovery, pharmacokinetics, pharmacodynamics.

Significance Statement

Overexpression/amplification of the MDM2 oncogene has been detected in various human cancers and is associated with disease progression, treatment resistance, and poor patient outcomes. Herein, we review the previous, current and emerging MDM2-targeted therapies and summarize the preclinical and clinical studies combining MDM2 inhibitors with chemotherapy and immunotherapy regimens. The findings of these contemporary studies may lead to safer and more effective treatments for patients with cancers overexpressing MDM2.

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1. Introduction

The mouse double minute 2 (MDM2) oncogene was first identified by researchers investigating the DNA sequences that were associated with double minutes (Cahilly-Snyder et al., 1987) (**Figure 1**). It was quickly noted that MDM2 plays critical roles in carcinogenesis via its down-regulation of the p53 tumor suppressor via its E3 ligase activity (Fakharzadeh et al., 1991; Honda et al., 1997; Levine, 2020; Momand et al., 1992; Oliner et al., 1992). It was demonstrated that dysregulated MDM2 functions as an oncogenic protein that regulates proliferation and apoptosis by altering p53-mediated death and survival signaling (Freedman et al., 1999). Beyond these effects on proliferation and apoptosis, MDM2 functionally regulates metastasis and the epithelial-mesenchymal transition (EMT) (Tang et al., 2019; Tonsing-Carter et al., 2015), and is associated with genomic instability, a hallmark of carcinogenesis. MDM2 is now known to exert a wide variety of effects, many via p53-independent mechanisms (Li et al., 2020b). The relationships among cancer stem cells, p53, and MDM2 have been illustrated by numerous studies (Gadepalli et al., 2014; Vummidi Giridhar et al., 2019; Wienken et al., 2016). MDM2 is also a key contributor to the resistance of cancer cells to tyrosine kinase inhibitors (TKIs), conventional chemotherapy, and radiotherapy (Hou et al., 2019). Recently, MDM2 was reported to be associated with the development of hyperprogressive disease (HPD) after immunotherapy, as has been observed for immune checkpoint inhibitor (ICI)-based therapies (Fuentes-Antras et al., 2018). This has been further supported by the observation that pharmacological inhibition of MDM2 enhanced the response of cancer cells to ICIs (Fang et al., 2019). Studies have also suggested that MDM2 contributes to other human diseases, such as chronic inflammation, neurological conditions, and autoimmune disorders, via alterations in inflammation or cell signaling (Wang et al., 2020).

Clinical studies have also provided evidence that there is overexpression and amplification of MDM2 in different cancer types, and overexpression of MDM2 is associated with a poor prognosis for all of these cancers (Momand et al., 1998; Onel and Cordon-Cardo, 2004; Ware et al., 2014). Additionally, the expanding network of MDM2 pathways reveals that MDM2 has pivotal functions under both physiological and pathological conditions (Fahraeus and Olivares-Illana, 2014). Together, these observations suggest that targeting MDM2 represents a potentially effective approach for preventing or treating various pathological conditions, but with particular utility for cancer.

A few years after MDM2 was discovered, we and others proposed targeting MDM2 as a new approach to cancer therapy. We initially developed an antisense approach to targeting MDM2, one of the first attempts to explore the potential anti-tumor efficacy of MDM2 inhibitors (Wang et al., 1999). That work demonstrated that inhibiting MDM2 not only led to anticancer activity *in vitro* and *in vivo*, but also sensitized cancer cells to DNA damaging agents. During the next 20-plus years, various strategies were validated to target MDM2. Most of these were intended to block the interaction between MDM2 and p53 to reactivate p53 in tumors harboring wild-type p53 (p53WT) (Chen et al., 1993; Kussie et al., 1996; Rusiecki et al., 2019; Shi et al., 2021). However, following the discovery that MDM2 has p53-independent functions (Bohlman and Manfredi, 2014; Klein et al., 2021), small molecule inhibitors, protein destabilizers/degradation enhancers, and proteolysis targeting chimeras (PROTAC)s have been explored to directly target MDM2, with promising data obtained for several different molecules (Fang et al., 2020b; Zhu et al., 2022). Unfortunately, Phase I trials with most of the small molecule MDM2 inhibitors have demonstrated limited effectiveness and notable thrombocytopenia as a dose-limiting toxicity associated with persistent MDM2 inhibition (**Table**

1). Nevertheless, several small molecule MDM2 inhibitors are currently undergoing Phase II/III clinical trials for the treatment of p53WT tumors, which are outlined in **Table 2** (Konopleva et al., 2020; Shi et al., 2021).

Despite MDM2 being the subject of intensive study for several decades, and being considered a highly promising target, there are no Food and Drug Administration (FDA)-approved products that have reached the market. The primary obstacle lies in the MDM2-p53 interaction, which is essential for normal cell regulation (Momand et al., 1992). Crafting drugs that achieve the desired anti-cancer effects without causing other unwanted effects is a complex task. Additionally, safety and toxicity issues, particularly those associated with p53 activation, present significant difficulties in achieving a balance between the therapeutic benefits and potential side effects. The diverse nature of cancers further complicates this scenario.

The effectiveness of MDM2 inhibitors varies considerably depending on the specific type, stage, and genetic makeup of the cancer (Haronikova et al., 2021; Konopleva et al., 2020). This diversity demands a highly tailored approach during both drug development and clinical testing, significantly complicating the path to market approval. These multifaceted challenges underline the complexity involved in the development and approval of MDM2 inhibitors, highlighting the need for continued research and innovation in this promising field of cancer therapy. Notably, the FDA recently granted orphan drug designation to KT-253, a novel MDM2 degrader, for the treatment of acute myeloid leukemia (AML). If this agent is successful in its clinical trials, it will likely be fast-tracked for acceptance. Nevertheless, numerous MDM2 inhibitors have been investigated in clinical trials without success, suggesting that further refinement and evaluation are needed to optimize the translation of these agents to routine clinical application.

The investigation of MDM2 and its interactions with p53 and other critical partners represents one of the hottest topics in the cancer research community. Several critical and comprehensive reviews have been published recently, and interested readers are directed to those excellent publications (Beloglazkina et al., 2020; Dobbstein and Levine, 2020; Fang et al., 2020b; Klein et al., 2021; Konopleva et al., 2020; Levine, 2020; Liu et al., 2019; Wang et al., 2020). The present review will focus on recent advances in developing MDM2 inhibitors, including preclinical and clinical research on various inhibitory strategies. We will also discuss the challenges associated with targeting MDM2 and suggest future research directions and opportunities, including the design of molecules to inhibit specific MDM2 functions, using dual-target inhibitors, developing combination treatment strategies with other agents, and the identification of biomarkers that may be used to guide the application of MDM2 inhibitors. In addition, we will also discuss strategies that can improve the application of MDM2 inhibitors either as single agents or in combination with other targeted therapies.

2. The Rationale for Targeting MDM2 for Molecular Targeted Therapy

2.1. The Oncogenic Roles of MDM2

MDM2 was initially discovered as a negative regulator of p53 (Bieging et al., 2014). Both molecules are short-lived proteins, so the balance between MDM2 and p53 maintains the normal functions of cells under different conditions, allowing cells to rapidly respond to stresses and repair DNA damage to prevent genomic instability (Nag et al., 2013). Overactive MDM2 negatively regulates p53's stability and/or transcriptional activity (Haupt et al., 1997), contributing to genome instability and carcinogenesis. Amplification or overexpression of MDM2 and/or loss of p53 function has been detected in many cancer types, including lung,

breast, liver, esophagogastric, and colorectal cancer (CRC), as well as sarcomas, melanoma, leukemia, lymphoma, and glioblastoma (GBM) (Wade et al., 2013). In transgenic mouse models, upregulation of MDM2 is associated with spontaneous lung tumors in G protein-coupled receptor class c group 5 member A (GPRC5A) knockout mice, which suggests that MDM2 plays a role during tumor development (Song et al., 2019). Transgenic mice with overexpression of MDM2 are predisposed to spontaneous tumor development, which occurs in a p53-independent manner (Jones et al., 1998).

Transgenic mice with tissue-specific MDM2 overexpression also show polyploidy of mammary epithelial cells, indicating that MDM2 is involved in genomic instability (Lundgren et al., 1997). A similar phenomenon was observed in B cells (Wang et al., 2008). The correlation between MDM2 and genomic instability can be explained by another report, in which MDM2 was found to promote genomic instability by interacting with Nijmegen breakage syndrome protein 1 (Nbs1), a subunit of the MRN complex (Mre11-Rad50-Nbs1), to delay DNA repair (Alt et al., 2005). It should be noted that this occurs independently of MDM2's effects on p53.

Interestingly, MDM2 knockout in mice bearing $p53^{515C/515C}$, which prevents p53-mediated apoptosis but maintains its ability to arrest the cell cycle, led to dysfunctional hematopoietic stem cells (HSCs) and progenitor cells in postnatal bone marrow (Abbas et al., 2010), indicating that MDM2 affects the stemness properties of these cells. Furthermore, Wienken et al. compared $p53^{-/-}$ murine embryonic fibroblasts (MEFs) with $p53^{-/-}$ MDM2 $^{-/-}$ double knockout MEFs and demonstrated that the absence of MDM2 strongly reduced the efficiency of induced pluripotent stem cells (iPSCs) generation (Wienken et al., 2016). In the same report, Wienken et al. showed that MDM2 promoted stemness independent of p53, and the lack of MDM2 increased the expression of homeobox (HOX) genes, which govern cell type

differentiation and specification. They also demonstrated that MDM2 physically associates with Enhancer of zeste homolog 2 (EZH2) and Suppressor of zeste 12 homolog (SUZ12), the subunits of the chromatin-modifying factor, Polycomb repressor complex 2 (PRC2) (Wienken et al., 2016). Wen et al. reported that MDM2 and p53 form a ternary complex with RING finger protein 2 (RNF2), a member of the Polycomb repressor complex 1 (PRC1), which results in increased MDM2 stability and promotes p53 MDM2-mediated ubiquitination (Wen et al., 2014). This MDM2 binding to the PRCs mediates transcriptional repression by enhancing histone H2A ubiquitination at K119, as well as histone H3 trimethylation at K27 in stem cells and tumor cells (Minsky and Oren, 2004; Wienken et al., 2016; Wienken et al., 2017). Furthermore, Wienken et al. also reported that depletion of MDM2 increased the osteoblastic differentiation of human mesenchymal stem cells (MSCs) and diminished the clonogenic survival of HCT116 p53^{-/-} (colon carcinoma) cells, MCF7 (breast carcinoma) cells, p53-depleted SJSA (osteosarcoma) cells, and p53-mutant Panc-1 (pancreatic carcinoma) cells (Wienken et al., 2016). Taken together, these studies indicate that high expression of MDM2 in cancer cells not only antagonizes the inhibitory effects of p53 on cell growth, but also maintains a stem cell phenotype, independent of the effects of p53. This helps explain why cancer cells appear to require MDM2 even when p53 is absent or mutant. This is further supported by the identification of cancers that simultaneously show amplifications of the MDM2 gene and mutations of p53 (Jain and Barton, 2016).

MDM2 also plays a critical role in regulating the stability and ubiquitination of various proteins. Ubiquitination affects the stability and functions of proteins, influencing critical processes such as the growth, survival, and chemoresistance of cancer cells. MDM2 was first recognized for its roles in facilitating the ubiquitylation and subsequent proteasomal degradation of p53 (Haupt et al., 1997; Honda et al., 1997; Kubbutat et al., 1997), but it has since been

demonstrated that MDM2 interacts with and modifies a wide variety of other targets. A study by Choi et al. demonstrated that MDM2 directly interacts with histone deacetylase 3 (HDAC3), significantly enhancing its monoubiquitination and stability. This direct interaction is essential for cell migration (Choi et al., 2019). Another study identified MDM2 as a novel E3 ligase for forkhead box O4 (FOXO4), demonstrating that MDM2 directly catalyzes FOXO4's (multi)mono-ubiquitination in a manner similar to its regulation of p53. Furthermore, MDM2's ubiquitination of FOXO4 was shown to significantly influence FOXO4's transcriptional activity (Brenkman et al., 2008). MDM2 also acts as a ligase for insulin-like growth factor 1 receptor (IGF-1R) ubiquitination, leading to its subsequent degradation via the proteasome pathway (Girnit et al., 2003). In addition, MDM2 downregulates other E3 ligases, such as SCF^{Skp2} and casitas B-lineage lymphoma (c-Cbl) and stabilizes their downstream targets, E2F transcription factor 1 (E2F1) and signal transducer and activator of transcription 5 (STAT5), respectively (Zhang et al., 2005; Zhou et al., 2021a). A recent study indicated that MDM2 alters the transcription factor inhibitor of growth protein 3 (ING3) through the ubiquitination-proteasome degradation pathway, diminishing ING3 protein stability and consequently fostering CRC cell growth and chemoresistance (Zhang et al., 2023).

MDM2 is renowned for its pivotal role in regulating cellular growth, apoptosis, DNA repair, and metastasis in cancer cells (Oliner et al., 2016; Shaikh et al., 2016; Zafar et al., 2023). However, it has recently garnered attention for its involvement in cancer metabolism, further emphasizing the diversity of its biological roles in cancer. Research has shown that MDM2 targets chromatin to regulate amino acid metabolism and maintain the redox balance in cancer cells, independent of p53 (Riscal et al., 2016). This process, influenced by activating transcription factor 3/4 (ATF3/4) and modulated by pyruvate kinase M2 (PKM2) under

conditions like oxidative stress and serine/glycine scarcity, points to a nuanced regulatory mechanism (Riscal et al., 2016). Depleting MDM2 in p53-deficient cells also disrupts the balance of NAD⁺/NADH, and affects glutathione recycling, highlighting a novel function of chromatin-bound MDM2 in cancer cell metabolism (Riscal et al., 2016). Another study revealed that MDM2 regulates the metabolism of serine and glycine, and fosters the growth of liposarcomas (LPS) by enhancing new nucleotide synthesis (Cissé et al., 2020). Disrupting MDM2's role in the production of purines and pyrimidines diminished the proliferation and survival of LPS cells, ultimately affecting their ability to form tumors (Cissé et al., 2020). Under conditions of oxidative stress and hypoxia, there is increased import of MDM2 into the mitochondria, independent of p53 (Arena et al., 2018). This mitochondrial MDM2 downregulates NADH-dehydrogenase 6 (MT-ND6) transcription, impacting the activity of respiratory complex I and boosting the production of mitochondrial reactive oxygen species (ROS) (Arena et al., 2018). MDM2 interacts with NADH:ubiquinone oxidoreductase 75 kDa Fe-S protein 1 (NDUFS1), destabilizing the Complex I supercomplex, which in turn enhances ROS production (Elkholi et al., 2019). Additionally, MDM2's negative regulation of NDUFS1 leads to diminished mitochondrial respiration, increased oxidative stress, and triggers the mitochondrial apoptosis pathway, independent of p53 (Elkholi et al., 2019).

MDM2 also plays a notable role in modulating the immune response within the tumor microenvironment. Interestingly, research has identified MDM2 as a tumor-associated antigen (TAA) in chronic lymphocytic leukemia (CLL), which is recognized by CD8⁺ autologous T lymphocytes (Mayr et al., 2006). This discovery earmarks MDM2 as a potential target for immunotherapy, including clinical vaccination trials and adoptive T-cell transfer for CLL (Mayr et al., 2006). Moreover, in tumors harboring wild-type p53, the application of the MDM2

inhibitor HDM201 resulted in a significant increase in dendritic cells, an enhanced population of Tbet+Eomes+ CD8+ T cells, and an improved CD8+/Treg ratio (Wang et al., 2021b). Further emphasizing its role in immune modulation, a mouse tumor model with conditional MDM2 knockout in T cells demonstrated accelerated tumor progression, accompanied by a decrease in the survival and function of tumor-infiltrating CD8+ T cells (Zhou et al., 2021a). Additionally, MDM2 enhances STAT5 protein expression in T cells and regulates T cell function via c-Cbl. MDM2 inhibits c-Cbl's binding to STAT5, reducing STAT5 degradation and stabilizing STAT5 expression in tumor-infiltrating CD8+ T cells (Zhou et al., 2021a). Moreover, MDM2 inhibition has been observed to induce tumor necrosis factor alpha (TNF- α) and interferon-gamma (IFN- γ) production in T cells (Ho et al., 2022), whereas it leads to the induction of interleukin-15 (IL-15) and major histocompatibility complex class II molecules (MHC-II) in melanoma cells (Langenbach et al., 2023). This diversity in responses highlights the intricate and cell type-specific actions of MDM2 in the immune landscape of cancers, presenting a complex yet promising avenue for therapeutic intervention. Understanding and harnessing these varied responses of MDM2 could pave the way for more effective cancer treatments and immunotherapies for other diseases.

2.2. The Functions of MDM2 in the Resistance to Cancer Therapy

The above sections clearly demonstrate that MDM2 is involved in carcinogenesis via the aforementioned mechanisms. However, it also regulates the resistance to various types of anti-tumor treatments (**Figure 2**).

2.2.1. Chemo- and Radioresistance

MDM2 directly drives the malignant behavior of cancer cells and modulates both intrinsic and acquired drug resistance. As early as 1995, the MDM2 protein was confirmed to regulate cisplatin-induced apoptosis in brain tumor cells (Kondo et al., 1995). The MDM2/p53 interaction also regulates the expression of O-6-methylguanine-DNA methyltransferase (MGMT), which promotes the resistance of glioma cells to temozolomide by maintaining cancer stem cell populations (Sato et al., 2011). Gemcitabine and cisplatin treatment can increase MDM2 expression in pancreatic cancer cells via the upregulation of RNA binding protein Musashi-2 (Sheng et al., 2017), resulting in acquired resistance to these chemotherapeutic agents. The sensitivity of gastric cancer cells to fluorouracil treatment negatively correlates with the expression of homeobox A13 (HOXA13), which increases the expression of MDM2 by downregulating dehydrogenase/reductase member 2 (DHRS2), a negative regulator of MDM2 (Han et al., 2018). High expression of MDM2 has been detected in doxorubicin-resistant breast cancer cells (Suzuki et al., 1998). An analysis of clinical data has also shown an association of MDM2 overexpression with chemo- and radioresistance in esophageal squamous cell carcinoma (Okamoto et al., 2013). Although downregulation of p53 was initially thought to be the primary reason why MDM2 was involved in drug resistance, enhanced expression of p65 by MDM2 could directly increase nuclear factor kappa B (NF- κ B) signaling and induce doxorubicin resistance in a p53-independent manner in acute lymphoblastic leukemia (ALL) (Gu et al., 2002). Another study reported that the MDM2-p53 feedback loop upregulates p73 expression to induce cisplatin resistance in squamous cell carcinoma cells (Hayashi et al., 2006). However, it is important to note that multiple studies have found that p73 upregulation, particularly in the context of inactivated p53, can actually induce apoptosis in cancer cells following treatment with cisplatin or

other anticancer agents (Cai et al., 2022). Therefore, the role of p73 in the response to cancer therapy is complex and may vary depending on the specific cellular context.

MDM2 can also promote the EMT and upregulate cancer stem cell properties to increase the resistance to chemotherapy (Sun and Tang, 2016). A correlation between MDM2 and the EMT has been reported in many cancer types, including lung cancer (Tang et al., 2019), ovarian cancer (Chen et al., 2017b), and breast cancer (Hauck et al., 2017). Gemcitabine treatment can enhance the expression of MDM2 and increase mesenchymal properties in pancreatic and breast cancer (Ahmad et al., 2020). Recent studies have demonstrated that MDM2 promotes the EMT via MDM2/p53/14-3-3 signaling mediated by v-raf murine sarcoma viral oncogene homolog B1 (B-raf) activity (Ou et al., 2021). It may also be enhanced by MDM2/protein kinase B (Akt)/androgen receptor (AR) signaling, suggesting another pathway linking MDM2 and the EMT to drug resistance (Singh et al., 2013).

MDM2 amplification is also associated with stem cell marker prominin-1 (CD133) in melanoma cells (Gil-Benso et al., 2012) and CD34 in chronic myeloid leukemia (CML) (Carter et al., 2015), providing additional evidence that MDM2 is involved in maintaining the stemness properties of cancer cells. It has been demonstrated that MDM2 expression is elevated during the transition of bone marrow stromal cells to cancer stem cells (He et al., 2016). MDM2 is also involved in the generation of induced pluripotent stem cells (iPSCs) from murine embryonic fibroblasts (Wienken et al., 2016), wherein MDM2 interacts with Polycomb repressor complex 2 (PRC2) to repress lineage-specific genes to maintain the pluripotency of stem cells. These activities further support the roles of MDM2 in treatment resistance since the presence of cancer stem cells have been considered a major cause of treatment failure (Li et al., 2021).

The downregulation of p53 by MDM2 has long been considered a major signaling mechanism that reduces the anti-tumor efficacy of radiotherapy. This concept was supported by a study in a transgenic mouse model that showed sensitization of cancer cells to irradiation when MDM2 was inhibited (Ringshausen et al., 2006). This idea has been validated in various cancer types using several novel inhibitors targeting MDM2. For example, inhibition of MDM2 with AMG232 enhanced the sensitivity of multiple cancer cell lines to radiation (Werner et al., 2015). Another MDM2 inhibitor, APG-115, was shown to enhance the response of gastric adenocarcinoma cells to irradiation (Yi et al., 2018). The mechanism underlying this enhanced sensitivity to radiation is postulated to be mainly due to MDM2 inhibition leading to the reactivation of p53 and subsequent apoptosis.

2.2.2. Tyrosine Kinase Inhibitor (TKI) Resistance

MDM2 also mediates at least some of the resistance of cancer cells to TKIs. Amplification and high expression of MDM2 are associated with epidermal growth factor receptor (EGFR) TKI resistance in lung cancer (Dworakowska et al., 2004; Sun et al., 2020; Yamaura et al., 2020), and alteration of the MDM2/p53 axis is considered to be the major reason why inhibition of MDM2 can sensitize cancer cells to TKIs. This has also been documented in other cancer types, including lung and prostate cancer (Bianco et al., 2004) and neuroblastoma (NB) (Wang et al., 2017). One mechanism-focused study reported that the NF- κ B signaling pathway is involved in TKI resistance in non-small cell lung cancer (NSCLC) (Wu et al., 2011). Whether MDM2 drives this resistant phenotype by directly activating NF- κ B needs to be addressed in future studies. Another report indicated that the combination of MDM2 and BCR-ABL1 inhibitors reduced the leukemia burden and increased survival in a mouse model of CML with intrinsic resistance to BCR-ABL1

inhibition (Carter et al., 2020). The combination was thought to function by decreasing the CML stem cell frequency. Most studies have focused on combining MDM2 inhibition with EGFR TKIs in lung cancer. However, because TKIs are becoming widely used for targeted therapy, deeper and broader investigations are needed to clearly define the roles of MDM2 during TKI resistance and to determine the optimal application of MDM2 inhibitors for patients with these cancers.

2.2.3. Immune Checkpoint Inhibitor Resistance

Despite significant advancements in cancer immunotherapy during the past several years, immunotherapy continues to have limited efficacy for most patients. One reason is that hyperprogressive disease has been reported after an initial response to immunotherapy. Several studies have shown that MDM2 is associated with HPD and can serve as a marker to indicate the risk of HPD in cancer patients (Adashek et al., 2020). It has been demonstrated that MDM2 inhibition can significantly increase the response of cancer cells to ICIs treatment (Wang et al., 2021b; Zhou et al., 2021b). For example, treatment with the MDM2 inhibitor ALRN-6924 significantly promoted T cell infiltration and enhanced the anti-tumor efficacy of immune checkpoint blockade (Zhou et al., 2021b). In that study, an immune response similar to that initiated by a viral infection and an inflammatory pattern of gene expression were detected after MDM2 inhibition in melanoma patients, suggesting that MDM2 inhibitors can boost anti-tumor immunity. Another study demonstrated that MDM2 negatively regulates T cell activation through the degradation of the nuclear factor of activated T cells, cytoplasmic 2 (NFATc2, also known as NFAT1), a transcription factor involved in the activation of T cells (Zou et al., 2014). Therefore, the combination of MDM2 inhibition and ICIs may overcome the resistance or insensitivity of patients to antitumor immunotherapy by activating cytotoxic T cells and blocking the immune

checkpoint. We previously reported that NFAT1 regulates the expression of MDM2 in cancer cells (Zhang et al., 2012), and MDM2 and NFAT1 may form a similar feedback loop as MDM2/p53 to balance the functions of MDM2/NFAT1. Further investigations are needed to address how this feedback loop regulates the efficacy of ICIs.

3. Major Strategies for Targeting MDM2

Over the past several decades, many strategies have been developed to target MDM2, including the use of peptides, antisense oligonucleotides, and a number of small molecules with different core structures (reviewed in (Beloglazkina et al., 2020; Fang et al., 2020a; Liu et al., 2019)). The initial approach used to target MDM2 via small molecules was focused on blocking the interaction between MDM2 and p53 and preventing the MDM2-mediated degradation of p53. The crystal structure of the MDM2/p53 complex revealed that several amino acid residues localized on the N-terminal of p53 maintain an α -helix which interacts with the hydrophobic cleft of MDM2 (Kussie et al., 1996). These key amino acids have provided a structural foundation to develop compounds targeting MDM2, including those based on cis-imidazoline, spiro-oxindole, pyrrolidone, piperidinones, pyrrolidonoimidazole, β -carboline, dihydro-isoquinolinone, and benzodiazepinedione (Beloglazkina et al., 2020; Fang et al., 2020b; Liu et al., 2019). Representative MDM2 inhibitors of different types are shown in **Figure 3**.

3.1. Blocking the MDM2-p53 Interaction

Strategies intended to block the binding between p53 and MDM2 were the first attempts at MDM2 inhibition (**Figure 1**). These early inhibitors had limited efficacy, and also often had serious side effects in clinical trials (Abdul Razak et al., 2022; Andreeff et al., 2016; Daver et al.,

2023; de Weger et al., 2019b; Erba et al., 2019; Gounder et al., 2023; Konopleva et al., 2022; Mascarenhas et al., 2022; Moschos et al., 2022; Ray-Coquard et al., 2012; Sekiguchi et al., 2023). Nevertheless, they have provided some insight into different strategies that might be used and into the impact of different structures on MDM2 and its targets.

3.1.1. Peptide-Based MDM2 Inhibitors

The potential of targeting MDM2 for molecular therapy was first demonstrated by gene knockdown/knockout strategies, including antisense and RNAi. Subsequently, p53-derived peptides were used to block the interaction between MDM2 and p53 (Garcia-Echeverria et al., 2000). These peptides were modified to mimic the alpha-helix of p53, resulting in more potent peptide inhibitors, such as the retroinverso p53 peptide (Sakurai et al., 2004) and beta-hairpin peptide (Fasan et al., 2004). However, although peptide-based inhibitors were designed to mimic the interaction motif of p53 and bind to MDM2 to allow the (re)activation of p53, the binding of these peptides to MDM2 was low due to the conformational differences between the peptides and the whole protein (Garcia-Echeverria et al., 2000). Cyclic-helical peptides have emerged as a potential alternative to stabilize targets based on hydrocarbon interactions (Sawyer et al., 2018). For example, the α -helix cyclic peptide ATSP-7041 was developed as a selective dual inhibitor of MDM2 and MDMX (Murine double minute X, also named MDM4; another inhibitory protein that leads to the degradation of p53) that effectively activated the p53 pathway in tumors *in vitro* and *in vivo* (Chang et al., 2013b). The modified version of ATSP-7041, ALRN-6924, also blocked the binding of MDM2 and MDMX to p53, suggesting that it can serve as a dual inhibitor of MDM2 and MDMX (Carvajal et al., 2018).

3.1.2. Small Molecule Inhibitors Blocking the MDM2-p53 Interaction

Single Ring Core Derivatives

Non-peptide small molecule inhibitors mimicking the key residues of p53, such as Phe19, Trp23, and Leu26, have also been developed to target MDM2. The Nutlins (Nutlin-1, -2, and -3) were the first potent and selective non-peptidic small molecule MDM2 inhibitors. Studies with Nutlins were among the first to provide mechanistic proof-of-concept that targeting the p53–MDM2 interaction had therapeutic potential for cancer. Nutlins are cis-imidazoline analogs that were identified via high-throughput screening (HTS) by scientists at Hoffman-La Roche (Vassilev et al., 2004). Nutlins bind to the three key subpockets of the hydrophobic cleft at the N-terminus of MDM2 and effectively disrupt the p53–MDM2 interaction (Tovar et al., 2006). Nutlins stabilize p53 and activate the p53 pathway in human cancer cells with p53WT, but not in cells with mutant p53, activating p53 target genes, cell-cycle arrest, and apoptosis (Tovar et al., 2006; Vassilev et al., 2004). Nutlin-3a is a Nutlin-3 enantiomer ((-)-Nutlin-3) and is the most biologically active among the Nutlin analogs that have been reported to date. However, its pharmacologic properties were inadequate for clinical development. Guided by further structural biology insights, including X-ray and nuclear magnetic resonance (NMR) analyses, Nutlin-3a was optimized to yield the 2,4,5-triaryl imidazoline analog RG7112 (RO5045337, Roche). This derivative has seen extensive application in both pre-clinical and clinical studies (Vu et al., 2013).

A new generation of MDM2 inhibitors was developed based on the spiro-oxindole core structure (Ding et al., 2005). The MDM2 inhibitors with spiro-oxindole core structures were initially discovered by Wang and coworkers at the University of Michigan by applying a structure-based design and employing a 1,3-di-polar cycloaddition synthetic strategy to mimic the same triad in p53 (Wang et al., 2014a; Yu et al., 2009). MI-77301 (SAR405838) is Sanofi's

MDM2 inhibitor obtained via further optimization of MI-219, a first-generation spiro-oxindole MDM2 inhibitor. It showed efficacy following oral administration in mouse xenograft models of cancer (Wang et al., 2014a). The oxindole in MI-219 mimics Trp23 of p53 as well as the spiro-pyrrolidine core, where the 3-chlorophenyl and neopentyl groups mimic the Phe19 and Leu26 to fit the hydrophobic pocket of MDM2 (Ding et al., 2005). This pyrrolidine could be a useful scaffold core to develop another class of MDM2 inhibitors. MI-77301 showed more than 10-fold activity enhancement in binding to MDM2 ($K_i = 0.88$ nM vs 13.6 nM for MI-219) and in activation of p53 in tumor cells with wild-type p53 (p53WT) compared to MI-219 (Wang et al., 2014a).

The high-affinity binding of MI-77301 to MDM2 is attributed to its ability to capture all of the critical hydrogen bonding and hydrophobic contacts among the three p53 key binding residues (Leu26, Trp23, and Phe19) with MDM2 (Kussie et al., 1996). It also had additional interactions with MDM2 that were not observed in the p53:MDM2 (Kussie et al., 1996) or Nutlin:MDM2 (Vassilev, 2005) cocrystal structures. These interactions induce refolding of the unstructured extreme N-terminus of MDM2 (residues 10–25) which further enhances its binding affinity (Wang et al., 2014a). However, MI-77301 undergoes epimerization at C2 and C3 via a slowly reversible pyrrolidine ring-opening and retro-Mannich reaction, which caused its activity to be unstable (Zhao et al., 2013). Structural optimization of MI-77301 led to the discovery of APG-115 (AA-115, alrizomadlin), a potent ($K_i < 1$ nM), selective, and stable spiro-oxindole-based MDM2 inhibitor with optimal oral pharmacokinetics (PK) (Aguilar et al., 2017b). APG-115 has already been granted fast-track designation by the US FDA for the treatment of relapsed or refractory (R/R) unresectable or metastatic melanoma, and orphan drug designations for gastric cancer, acute myeloid leukemia, soft tissue sarcoma, and retinoblastoma, as well as stage IIB-IV

melanoma and neuroblastoma. APG-115 is currently being investigated alone or in combination in ongoing phase I and II studies (**Table 2**). Milademetan (DS-3032, DS-3032b, Rain-32) is another potent spiro-oxindole-based inhibitor of the MDM2-p53 interaction licensed by Rain Therapeutics from Daiichi Sankyo. It showed antitumor efficacy and has been tested in clinical trials (Arnhold et al., 2018).

Roche developed the cyanopyrrolidine analog, RG7388 (Idasanutlin, RO5503781), a more potent and selective follow-up compound to the cis-imidazoline RG7112 and the spirooxindole MDM2 inhibitor, MI-219, with improved stereochemical and conformational properties (Ding et al., 2005; Ding et al., 2013; Yu et al., 2009), but it is not currently in clinical trials. The “trans” configuration of the aryl rings in the pyrrolidine core of this molecule is the major difference compared with cis-imidazoline (Nutlins) and spiro-oxindole (MI-219). In addition to the occupation of the Trp23, Leu26, and Phe19 pockets by the 4-chlorophenyl ring, 3-chlorophenyl, and neopentyl, the C α -carbonyl of pyrrolidine interacts with the NH of His96 via a hydrogen bond. RG7775 (RO6839921) is an inactive PEGylated intravenous prodrug of RG7388 that was designed to decrease the variability in exposure and dose-limiting gastrointestinal toxicity seen with oral RG7388 and to improve its PK properties (Abdul Razak et al., 2020; Uy et al., 2020).

Piperidinones, which have a 6-membered ring, have been investigated as another scaffold to develop another category of MDM2 inhibitors. AMG232 (Navtemadlin, KRT232) developed by Amgen and is now acquired by Kartos Therapeutics, is a representative of this group. Similar to the Nutlins, the chlorophenyl groups at C5 and C6 mimic Leu26 and Trp23 of p53 and occupy the binding pocket of MDM2 (Rew and Sun, 2014; Rew et al., 2012; Sun et al., 2014).

Bicyclic and Multicyclic Core Derivatives

Bicyclic core inhibitors were discovered during a screen of about 50,000 compounds for inhibitory activity (Gessier et al., 2015). One compound, CGM097 (NVP-CGM097), was designed and developed by Novartis after structural optimization of the dihydro-isoquinolinone virtual screening hit (Holzer et al., 2015). The central valine of MDM2 (V93) was shown to have a critical role in binding the inhibitor within van der Waals distance (Furet et al., 2016). Compared with 6-membered rings, a 5-membered lactam bicyclic scaffold generates a flat core and forces substituents into an obligatory pseudo-equatorial orientation. Novartis subsequently developed HDM201 (NVP-HDM201, Siremadlin) to inhibit the interaction between MDM2 and p53, representing a new class of pyrrolidonoimidazole-based MDM2 inhibitors (Jeay et al., 2018).

Boehringer Ingelheim shifted the nitrogen of the pyrrolidine ring one atom closer to the oxindole and incorporated a fused ring system to capture the known interactions with the MDM2 pocket, yielding BI 0252 and BI 907828 (Brigimadlin), a new class of spiro-oxindole MDM2 inhibitors that are not prone to epimerization (Gollner et al., 2016; Gollner et al., 2019). MK-8242 (SCH 900242) is a first-generation MDM2–p53 inhibitor that was developed by Merck based on a geminally-disubstituted piperidine hit that was identified via an in-house HTS (Bogen et al., 2016; Ma et al., 2014a; Ma et al., 2014b). MK-8242 development was not further pursued since it has a high molecular weight, high lipophilicity (CLog P = 5.2), and required a relatively high dose for efficacy; the recommended phase 2 dose (RP2D) was 400 mg twice a day in a phase I study in p53WT advanced solid tumors (NCT01463696) (Wagner et al., 2017). However, in subsequent HTS, Merck identified MK-4688, a more drug-like and low molecular weight novel purine carboxylic acid-derived MDM2 inhibitor with an estimated human dose requirement of 38 mg twice daily (Reutershan et al., 2021).

Imidazo-indoles are another class of potent inhibitors with a multicyclic core that blocks the interaction between MDM2 and p53 (Popowicz et al., 2010). Several compounds, including WK23 and WK298, were developed based on the optimization of these imidazo-indoles. The first isoindolinone-based inhibitor (NU8231) was developed using computational methods (Hardcastle et al., 2005). *In silico* screening and small library synthesis led to the development of isoindolinone scaffold inhibitors. The structure of the complex formed between MDM2 and an isoindolinone inhibitor provided another layer of evidence to support that isoindolinones can be potent inhibitors of MDM2 (Riedinger et al., 2011). Modifying isoindolinone with 2,3-substituted ester derivatives has been reported to provide additional binding sites for His96 of MDM2, in addition to the Phe19, Trp23, and Leu26 pockets (Grigoreva et al., 2017), resulting in stronger binding.

Using thermal shift screening with compound libraries, benzodiazepinediones such as compound (S,S)-15 (also known as TDP222669) have been identified by two independent groups as compounds able to bind MDM2 (Grasberger et al., 2005; Koblish et al., 2006; Raboisson et al., 2005). Thio-benzodiazepine and other derivatives, such as sulfamidebenzodiazepine and triazolebenzodiazepine, have subsequently been reported to potently bind MDM2 and more strongly inhibit its biological functions than Nutlin-3 (Guo et al., 2012; Yu et al., 2014).

Other Core Structures

A National Cancer Institute (NCI) anticancer drug screen identified the small molecule, 2,5-bis(5-hydroxymethyl-2-thienyl) furan, to be the most potent thiophene derivative (Rivera et al., 1999). This small molecule, later named RITA, was originally reported to block the interaction between p53 and MDM2 (Issaeva et al., 2004). However, research has shown that

RITA's effects are not limited to p53-dependent mechanisms, because the compound also exhibits biological activity in the absence of p53 (Weilbacher et al., 2014; Zhao et al., 2010). This suggests that RITA has a broader mechanism of action than initially thought. Further research is essential to understand RITA's full range of interactions and to determine its optimal use in treating cancers, especially those with altered or absent p53.

Derivatives of chalcone were initially designed to inhibit tumor growth. Their potential to re-activate p53 has been evaluated based on their putative function as a small molecule targeting MDM2 (Stoll et al., 2001). Compared with other selective inhibitors that directly bind to MDM2, there is no structural evidence that the chalcone derivatives are trapped in the binding pocket of MDM2. Studies are ongoing to evaluate whether the anti-tumor activity of the chalcone derivatives depends on their blocking the interaction between MDM2 and p53 and reactivating p53 (Alaaeldin et al., 2021; Moreira et al., 2021).

3.2. Small Molecules Directly Targeting MDM2

As noted above, mimicking p53 to block the interaction between p53 and MDM2 is the main strategy that has been used to target MDM2. Although small molecule MDM2-p53 interaction inhibitors work efficiently in reactivating/stabilizing p53, their effectiveness is limited and typically restricted to tumors harboring wild-type p53. Some MDM2 inhibitors that activated p53 had elevated levels of MDM2 protein, raising concerns about whether other functions of MDM2 might be induced. Furthermore, because MDM2 has a variety of functions and interactions with other molecules, blocking the binding between MDM2 and p53 may only affect some of MDM2's functions. Thus, direct negative regulation of MDM2 could be an alternative way to not only activate p53, but also to inhibit other functions of MDM2 by decreasing its

expression, inhibiting its enzymatic activity, and/or inducing the degradation of the MDM2 protein. This might be achieved by the direct binding of small molecules or by using a PROTAC to introduce an E3 ligase to digest MDM2.

Although antisense phosphorothioate oligodeoxynucleotides were shown to inhibit MDM2 expression effectively (Wang et al., 1999), their clinical application has yet to be explored. Targeting the RING domain of MDM2 may directly inactivate its ligase activity. A structure-activity relationship analysis showed that a 5-deazaflavin derivative could bind to the RING domain of MDM2 (Dickens et al., 2013). There are other compounds that have also been reported to potentially decrease the ubiquitination of MDM2. However, it is unclear whether the ubiquitination level of MDM2 correlates with its E3 ligase activity or its degradation (Klein et al., 2021), and these inhibitors binding the RING domain have not yet been explored in clinical trials.

Makaluvamine analogs were initially designed to inhibit topoisomerase II (Barrows et al., 1993). Our lab found that a synthetic makaluvamine analog has cytotoxic activity in prostate cancer cells, which is at least partly due to its inducing the degradation of MDM2 (Wang et al., 2009). MA242, a more recently developed makaluvamine analog, has shown highly selective and potent inhibition of MDM2 by inducing its auto-degradation (Wang et al., 2018). Our group has also studied SP141, which was developed based on the crystal structure of the human p53-MDM2 complex and computational modeling, as well as a screen for changes in p21 expression. SP141 is a pyrido[3,4-b] indole class (β -carboline) inhibitor, which not only blocks the interaction between MDM2 and p53 but also directly induces the degradation of MDM2 (Patil et al., 2017; Wang et al., 2014b).

There have been a few reports showing a correlation between inhibitor treatment and the downregulation of MDM2, but more evidence is needed to determine whether the inhibitors are

directly or indirectly affecting MDM2. For example, Adriamycin (doxorubicin) treatment downregulates MDM2 at the protein level and induces DNA damage, but does not directly inhibit the transcription of MDM2 (Ma et al., 2000). It has been shown that orphan receptor TR3 inhibits MDM2 expression, but there is not yet any pharmacological inhibitor available to target TR3. A β -carboline-based chalcone, CPI-7c, has been demonstrated to induce the degradation of MDM2 (Singh et al., 2016). Although MDM2 is not the only target of CPI-7c, this observation supports the possibility that β -carboline-based chalcones can be used as degradation-inducers for MDM2, similar to SP141. In line with the findings for other potent MDM2 degraders, the anti-cancer drug SQ0814061 has been shown to downregulate MDM2, but whether the inhibitor directly causes this downregulation or whether the downregulation is just correlated with other anti-cancer effects is currently unclear (Xu et al., 2016). Together, these studies suggest that inducing the degradation of MDM2 may represent an effective strategy and may be more beneficial than just blocking the interaction between p53 and MDM2.

3.3. Proteolysis-Targeting Chimeras (PROTACs)

PROTACs were developed using chimeric small molecules that guide proteins to the Skp1-cullin-F box (SCF) complex for ubiquitination-mediated degradation (Sakamoto et al., 2001). More than 20 years after their initial development, PROTACs have been widely applied preclinically to downregulate different targets for cancer treatment (Bekes et al., 2022; Sun et al., 2019). Some PROTACs are currently being evaluated in clinical trials (Mullard, 2021). Inhibitors of MDM2 have been used along with other ligands to recruit MDM2 to induce its degradation by PROTACs (Bricelj et al., 2021). Representative PROTAC-based MDM2 inhibitors are shown in **Figure 4**.

The Wang lab at the University of Michigan designed and developed the first PROTAC MDM2 degraders, MD-222 and MD-224, by using MDM2 inhibitor MI-1061 and a cereblon ligand, lenalidomide, which recruits the cereblon E3 ubiquitin ligase to MDM2 and induces its degradation (Li et al., 2019). This innovation led to a 100-fold increase in cell potency compared to MI-1061 alone. At concentrations as low as 1 nM, MD-222 and MD-224 induced complete degradation of the MDM2 protein, accumulation of p53 protein, and induced apoptosis in p53WT human leukemia cells. Building on this success, the Wang research group further optimized MD-224, resulting in the development of AA-265. AA-265 is more potent than its precursor, with an IC_{50} of 0.72 nM compared to 1.5 nM for MD-224 in RS4;11 ALL cells. Currently, it is undergoing advanced preclinical evaluation in preparation for progression to clinical trials.

The same research team further modified MD-222 by removing the benzamide substituent from its MDM2 inhibitor moiety (MI-1061), which resulted in the identification of MG-277 (Yang et al., 2019). Unlike MD-222, MG-277 only moderately degrades MDM2 and does not activate p53 in cancer cells. However, it effectively inhibits the growth of cancer cells regardless of their p53 status (Yang et al., 2019). Interestingly, MG-277's inhibitory effects on cell growth rely on its binding to cereblon rather than MDM2 or p53. Mechanistically, MG-277 acts as a molecular glue instead of functioning like a standard PROTAC MDM2 degrader. It plays a crucial role in bringing the GSPT1 (G1 to S phase transition 1) protein, which is a key factor in translation termination, into proximity with cereblon and Cullin 4A. This interaction facilitates the ubiquitination of GSPT1, leading to its subsequent degradation (Yang et al., 2019).

Applying a similar strategy, the Tang group at the University of Wisconsin-Madison made significant advancements in the field of PROTAC MDM2 degraders with the discovery of

WB156 and WB214. WB156 was derived from RG7112 (originally derived from a Nutlin), and the cereblon ligand, lenalidomide. WB156 has remarkable potency, being nearly 1,000 times more effective than RG7112 in inhibiting cell growth. It degrades MDM2, activating wild-type p53, and inducing apoptosis in RS4;11 leukemia cells (Wang et al., 2019a). The other candidate, WB214, optimized from WB156, degrades both MDM2 and p53 in RS4;11 cells. Interestingly, it also acts as a molecular glue. Notably, Wang et al. revealed that co-treating RS4;11 cells with WB214 and MDM2-p53 interaction inhibitors led to the rescue of p53 from degradation, but not MDM2. This indicates that the p53 degradation induced by WB214 occurs due to its direct association with MDM2. These observations suggest that WB214 does not bind to the p53 binding pocket on MDM2 (Wang et al., 2021a). Moreover, WB214 was also found to degrade GSPT1 independently of both MDM2 and p53 degradation (Wang et al., 2021a).

KT-253 is also a heterobifunctional MDM2 degrader (structure undisclosed) developed by Kymera Therapeutics that has shown remarkable efficacy, with greater than 200-fold improvements in *in vitro* cell growth inhibition compared to small molecule MDM2 inhibitors (Chutake et al., 2022). Recent studies by Kymera indicate that just a single dose of KT-253 led to rapid apoptosis and sustained tumor regression in a MV4;11 mouse xenograft model of AML and in mice bearing RS4;11 xenograft tumors (Chutake et al., 2022). Administering 1 mg/kg of KT-253 once every three weeks resulted in tumor regression in three patient-derived xenograft (PDX) models of AML (CTG-2227, CTG-2240, and CTG-2700). Similarly, an intermittent dosing schedule of KT-253 in combination with a B-cell lymphoma 2 (BCL-2) inhibitor, venetoclax, achieved a durable tumor regression in a venetoclax-resistant xenograft model of AML (MOLM13) (Mayo et al., 2022). KT-253 has received the FDA orphan drug designation for the treatment of AML and is currently being investigated in patients with R/R high-grade

myeloid malignancies, ALL, R/R lymphoma, and R/R solid tumors in a Phase I trial (NCT05775406).

Marcellino et al. reported the development of a PROTAC von Hippel–Lindau (VHL)-recruiting MDM2 degrader, MS3227, for p53WT leukemia. This compound is based on an AMG-232 analog with a piperazine sulfonyl group and includes a ligand for the VHL E3 ubiquitin ligase. VHL was chosen as the E3 ligase due to its higher expression in AML cells compared to other cancer types and normal tissues, providing greater specificity toward MDM2 inhibition in leukemic cells over other cell types (Marcellino et al., 2023). Another potent MDM2-targeted PROTAC, YX-02-030, which induces MDM2 degradation by recruiting VHL E3 ligase. This results in apoptosis in p53 mutant or deleted triple negative breast cancer cells across diverse models, including 2D and 3D cultures, patient-derived explants, and tumor xenografts, through the activation of TAp73 (Adams et al., 2023).

Recently, the “suicide” cleavage of MDM2 was proposed as a new concept that utilizes a homo-PROTAC strategy (He et al., 2021). This involves linking two Nutlin-3 molecules to degrade MDM2 by harnessing its own E3 ligase activity. Currently, most MDM2-targeting PROTACs, with KT-253 as an exception, are in preclinical testing. It's anticipated that more PROTACs targeting MDM2 will advance to clinical trials, employing either traditional ligases like cereblon and VHL, or innovative strategies like the 'suicide' cleavage approach.

3.4. Dual Inhibitors

While MDMX does not have E3 ligase activity on its own, it binds to the N-terminus of p53 and neutralizes its transactivational activity (Shvarts et al., 1996). Additionally, MDMX forms a heterodimer with MDM2, enhancing its stability and amplifying its ability to

ubiquitinate p53 (Leslie et al., 2015; Marine et al., 2006). Thus, targeting both MDM2 and MDMX will more effectively activate p53. In addition to developing potent and selective inhibitors of MDM2, developing dual functional inhibitors to target both MDM2 and MDMX has been explored. For instance, Stockwell's group identified MEL23 and MEL24 as small-molecule MDM2-MDMX E3 ligase activity inhibitors using a high-throughput cell-based MDM2 ubiquitination screening assay (Herman et al., 2011). MEL23 and MEL24 inhibit the E3 ligase activity of the MDM2-MDMX complex and prevent the degradation of MDM2, MDMX, and p53, thus increasing the stability of both MDM2 and p53 in cells. This results in an increase in the transcription of p53 target genes p21, Bcl2-associated X (Bax), and Puma. MEL23 showed synergy with the DNA-damaging agent, camptothecin, and Nutlin-3 in reducing the viability of both p53WT and p53-null cells *in vitro* (Herman et al., 2011). DIMP53-1, a small molecule inhibitor, was identified by a yeast-based screening assay to bind p53 and block both MDM2- and MDMX-mediated degradation (Soares et al., 2017). Hoffmann-La Roche screened another library of small molecules and reported that indolyl hydantoins show potential functions as MDM2/MDMX antagonists. Indolyl hydantoin RO-2443 inhibits the binding of both MDM2 ($IC_{50}=33$ nM) and MDMX ($IC_{50}=41$ nM) to p53. Further structural optimization (substitution of a diol-containing carboxamide at the methyl position of the benzyl group of RO-2443) led to the discovery of RO5963, which exhibited increased potency and improved solubility. RO-5963 showed a similar p53-MDM2 inhibitory activity as Nutlin-3a but ~400-fold better MDMX inhibition than Nutlin-3a (Graves et al., 2012). ATSP-7041 and ALRN-6924 are two stapled α -helical peptides with enhanced cell permeability that bind to both MDMX and MDM2 and block their interaction with p53 (Carvajal et al., 2018; Chang et al., 2013a). Another chemical, protoporphyrin IX (PPIX), the precursor of heme, inhibits both the p53/MDM2 and p53/MDMX

interaction (Jiang et al., 2019). Even though PPIX is an endogenous metabolite, as a photosensitizer, PPIX accumulation is associated with severe pain symptoms upon sun exposure, potentially limiting its clinical applications.

Besides the dual inhibition of MDM2 and MDMX, other proteins can be simultaneously targeted using small molecule inhibitors. A few years ago, our group developed MA242, an MDM2 inhibitor that also targets NFAT1, a key transcription factor that regulates cytokine expression. Preclinical studies suggested that MA242 exerts anti-tumor effects by targeting both MDM2 and NFAT1 in liver and pancreatic cancer (Wang et al., 2019b; Wang et al., 2018). Bcl-2 is a pro-survival protein that inhibits pro-apoptotic molecules such as Bax, Bcl2-associated agonist of cell death (Bad), and BH3 interacting domain death agonist (Bid) to promote cellular survival (Ashkenazi et al., 2017). The combined treatment of AML with Bcl-2 inhibition and an MDM2 inhibitor leads to synergistic effects, suggesting that dual Bcl-2/MDM2 inhibitors might represent a potential new treatment strategy (Pan et al., 2017). One such α -helix-mimicking dual inhibitor was designed, developed, and confirmed to show potent antitumor activity (Wang et al., 2016). All of these studies indicate a new path for the future development of MDM2 inhibitors. Although the selectivity of small molecule inhibitors is a major concern that will need to be addressed to rule out off-target issues, the use of dual or poly-molecule targeting agents may represent both a more potent strategy and a way to overcome drug resistance mediated via a single pathway or molecule (Ramsay et al., 2018).

4. Preclinical Studies of MDM2 Inhibitors

During the past twenty years, MDM2 inhibitors have been developed and tested in preclinical models of many diseases. In the sections below, we will discuss the preclinical investigations performed to assess the potential use of MDM2 inhibitors for cancer treatment.

However, it is worth mentioning that while most of the work has focused on cancer, there have also been investigations on non-cancer diseases. For example, Nutlin-3 showed therapeutic efficacy for Fragile X Syndrome (FXS). Patients with FXS have inherited loss of function of the fragile X mental retardation protein (FMRP), which negatively regulates Mdm2 mRNA stability. Treatment with an MDM2 inhibitor can reduce MDM2 expression and induce the differentiation of neural stem cells to functional neurons (Li et al., 2016). RG-7112 selectively kills senescent intervertebral disc cells through apoptosis and has been used as a senotherapeutic drug for patients with intervertebral disc degeneration and low back pain (Cherif et al., 2020). These have been reviewed elsewhere (Beloglazkina et al., 2020; Liu et al., 2019; Munisamy et al., 2021; Rusiecki et al., 2019; Zafar et al., 2021).

4.1. Efficacy

The first studies targeting MDM2 by an antisense approach showed that this inhibition could lead to anti-cancer effects (Wang et al., 2001; Wang et al., 1999). Various preclinical studies have since demonstrated that MDM2 inhibition leads to significant anti-tumor effects both *in vitro* and *in vivo*.

Peptides were designed to mimic the binding motif of p53 to disrupt the interaction between MDM2 and p53, but their low binding affinity precluded the development of this category of MDM2 inhibitors. Recently, a stapled α -helical peptide was synthesized that can form a stable structure with a cyclic ring that strongly binds to both MDM2 and MDMX. Treatment with ALRN-6924, the latest version of this class of cyclic peptides, has shown anti-tumor activity in models of several cancer types, including leukemia (Carvajal et al., 2018), lymphoma (Ng et al., 2018), and ER-positive breast cancer (Pairawan et al., 2021).

As the first generation of small molecule inhibitors, Nutlins have been widely used in preclinical studies to block the interaction between MDM2 and p53 (Vassilev et al., 2004). The latest Nutlin derivative, RG7112, has been validated in preclinical studies and showed efficacy against many cancer types, including NB (Al-Ghabkari and Narendran, 2019), leukemia (Richmond et al., 2015), ovarian carcinoma (Makii et al., 2016), and GBM (Verreault et al., 2016). In sphere cultures of GBM, MDM2 inhibition with RG7112 induced significant cell death, especially in the p53WT cells (Her et al., 2018).

MI-77301 is another inhibitor of MDM2/p53, this one based on a spirooxindole core. MI-77301 treatment resulted in the activation of p53 signaling and robust tumor regression in preclinical models of advanced adenoid cystic carcinoma (Warner et al., 2016). Notably, although the distribution of MI-77301 in the brain is low, a mechanistic study has identified that p-glycoprotein (P-gp), a transporter protein, limits MI-77301 efflux to prevent brain distribution (Kim et al., 2019). If P-gp can be pharmacologically inhibited, MI-77301 may be useful for treating brain cancer. In addition, two independent groups have reported that MI-77301 has anti-tumor effects against NB (Chen et al., 2021; Lu et al., 2016a), in which p53-mediated apoptosis is observed. Although insulinoma-associated 1 (INSM1), a molecule downstream of p53, was identified as being upregulated, there was only a correlation between INSM1 and tumor inhibition, with no direct role demonstrated. MI-77301 treatment also enhanced the protein level of p53 and decreased the expression of the Polycomb ring finger proto-oncogene BMI1, a marker of cancer stem cells, and decreased the population of ALDH⁺CD44⁺ cancer stem cells in mice harboring mucoepidermoid carcinoma xenografts, suggesting a potential mechanism wherein MDM2 inhibition reduces tumor growth and drug resistance via reducing the number of cancer stem cells (Andrews et al., 2019). A similar mechanism has been observed in models of adenoid cystic

carcinomas where treatment with MI-77301 to eliminate tumor recurrence apparently worked by reducing the population of cancer stem cells and sensitizing the cells to cisplatin (Nor et al., 2017).

A further optimized spiro-oxindole-based inhibitor, APG-115, has shown activity against AML (Fang et al., 2021) and dedifferentiated papillary thyroid cancer cells (Chen et al., 2017a). APG-115 treatment not only releases active p53 in cancer cells by blocking the binding of MDM2 to p53, but also augments MDM2 expression in tumor-infiltrating CD8⁺T cells. This leads to competition with c-Cbl, a negative regulator of STAT5, resulting in stabilization of STAT5 and boosted anti-tumor immunity (Zhou et al., 2021a). That study demonstrated that an MDM2 inhibitor could rescue anti-tumor immunity and indicated that combining MDM2 inhibition and immune checkpoint blockade may be a promising therapeutic strategy for cancer. Milademetan, a compound similar to APG-115, has been shown to reduce the growth of clear cell ovarian carcinoma with p53WT (Kawata et al., 2020) and to reactivate p53 signaling in NB cells, thus representing a therapeutic approach for high-risk NB (Arnhold et al., 2018).

The lead MDM2 inhibitor under development by Boehringer Ingelheim, BI0252, has an MDM2 binding affinity IC₅₀ of 4 nM and achieved *in vivo* tumor regression in an SJSA-1 osteosarcoma xenograft model even when given as a single dose (Gollner et al., 2016). BI 907828 is an optimized analog of BI-0252 that has high permeability, good bioavailability across species, low systemic clearance, and a low predicted human efficacious dose that enables intermittent oral dose schedules. *In vitro*, BI 907828 reduced the viability of p53WT GBM patient-derived brain tumor stem cell lines with picomolar IC₅₀ concentrations. *In vivo*, BI 907828 monotherapy showed significant anti-tumor activity and improved survival in orthotopic brain tumor stem cell xenograft models of GBM, and this was improved by use in combination with temozolomide, a DNA alkylating agent (Hao et al., 2023). BI 907828 also demonstrated preclinical efficacy in two

PDX mouse models of dedifferentiated liposarcoma (DDLPS) with MDM2 amplification. In fact, in one of the models, a 2.5 mg/kg or 10 mg/kg dose of BI 907828 induced a complete tumor regression, and no tumor regrowth was observed 37 days post-treatment (Cornillie et al., 2020).

Daily doses of 50 and 100 mg/kg of Merck's MDM2 inhibitor, MK-4688, for seven consecutive days, followed by a 2-week drug holiday, induced 11% and 82% tumor regression by day 14 in a MDM2-amplified SJSA-1 xenograft model. MK-4688 is still in the preclinical stage of development (Reutershan et al., 2021), but further demonstrated the potential efficacy of limited or short-term treatment with an MDM2 inhibitor.

There have been many other preclinical studies of different categories of MDM2 inhibitors in multiple cancer types. For example, RG7388 treatment activated p53 and inhibited cell proliferation in nasopharyngeal carcinoma (Fan et al., 2019). It also induced an apoptotic gene signature in chronic lymphocytic leukemia cells (Ciardullo et al., 2019). MI-219 was demonstrated to regulate p53 by enhancing MDM2 autoubiquitination and degradation in human malignant B-cell lymphomas (Sosin et al., 2012), pancreatic cancer (Azmi et al., 2010), and CML (Peterson et al., 2017; Peterson et al., 2011). AMG232 has similarly shown anti-tumor effects in multiple cell lines of different cancer types both *in vitro* and *in vivo* (Canon et al., 2015). AMG232 also has potent effects against GBM cells (Her et al., 2018).

Our group has developed two structurally diverse selective inhibitors that lead to the degradation of MDM2. These molecules, MA242 and SP141, show potent antitumor efficacy in different cancer types, including breast cancer, NB, hepatocellular carcinoma, and pancreatic cancer (Wang et al., 2019b; Wang et al., 2019c; Wang et al., 2018; Wang et al., 2014b; Wang et al., 2014c).

4.2. Pharmacology and Toxicology of Agents Targeting MDM2

In addition to studies of the anti-tumor efficacy of the various MDM2 inhibitors, the toxicity, PK, and pharmacodynamics (PD) have also been assessed in preclinical studies.

ATSP-7041, a compound related to the newer ALRN-6942, has been administered intravenously to mice, rats, and monkeys to evaluate the PK/PD profiles, and the results showed favorable metabolism and PK/PD for the compound (Chang et al., 2013b).

The first pharmacokinetic study of Nutlin-3a was performed in mice to provide information to guide the dose and schedule for further preclinical investigations (Zhang et al., 2011a). PK profiling of RG7112, another Nutlin, in an intracranial PDX model of GBM demonstrated that the compound significantly crosses both the blood-brain and blood-tumor barriers (Verreault et al., 2016).

A PK/PD study of RG7388 was performed in a SJSA1 osteosarcoma xenograft model. The PD was assessed after both a single dose and after five days of dosing. The highest feasible single dose was determined to be 200 mg/kg, with an effective concentration lasting up to 48 hours. The dose for the 5-day schedule was determined to be 80 mg/kg, with a maximal effect on day 3 after the last administration of the drug (Higgins et al., 2014). In addition, a PK analysis was performed for the prodrug of RG7338, RG7775, in an orthotopic mouse model of SHSY5Y-Luc neuroblastoma (p53WT cells). These mice were treated intravenously with a single dose of RG7775 or RG7775 combined with temozolomide (Chen et al., 2019b). RG7775 showed a favorable pharmacokinetic profile with a half-life of 3.2 ± 0.5 h in plasma and 6 h in tumor based on the detection of free RG7338. The levels of MDM2, p53, p21, and MIC-1 (a potential PD biomarker of MDM2 inhibitor activity in clinical trials) were sustained until 6 h post-treatment (Rew et al., 2012). The PK and metabolism of AMG 232, another single ring inhibitor, have

been assessed in several mammals, including rats, dogs, and monkeys. AMG 232 has shown a low turnover rate and moderate to high oral bioavailability in mice, rats, and monkeys. However, there was high clearance and low oral exposure in dogs. AMG 232 is extensively metabolized by biotransformation in the liver of rats. A small amount of ^{14}C -labeled AMG 232 could be recovered in bile (Ye et al., 2015). There were no significant effects on cardiovascular variables observed in rats (Rew and Sun, 2014). An evaluation of the PD profile of APG-115 was performed in an SJSA-1 xenograft mouse model. It has been observed that p53 target genes show upregulation upon APG-115 treatment, indicating that there is activation of p53 (Aguilar et al., 2017a). A preclinical *in vivo* PK study of MI-219, conducted in CD-1 mice, rats, dogs, and monkeys, used multi-exponential allometric scaling (ME), in vitro-in vivo extrapolation (IVIVE), and Oie-Tozer methods to predict human pharmacokinetics. These accurate predictions support the potential of MI-219 for first-in-human studies. (Zou et al., 2012).

Bicyclic and multicyclic core derivatives are other categories of MDM2 inhibitors. A preclinical *in vivo* PK/PD study of HDM201 was performed to determine the optimal dose and schedule in tumor-bearing rats. Both intermittent high-dose and continuous low-dose administration of HDM201 achieved complete and sustained tumor regressions in the SJSA-1 xenograft model and HSAX2655 liposarcoma (LPS) PDX models in rats (Jeay et al., 2018). This is in accordance with the observation that fractionated low-dose of HDM201 induced p21 expression and delayed the accumulation of apoptotic cells, while high-dose pulses of HDM201 were associated with a rapid and dramatic induction of the mRNA and/or protein levels of p53-dependent PUMA *in vitro* and *in vivo*, as well as rapid onset of apoptosis and downregulation of B-cell lymphoma-extra large (Bcl-xL) (Jeay et al., 2018). A similar compound, CGM097, was evaluated in mice bearing SJSA-1 tumors to determine the maximum effect (Emax) and plasma

maximum observed concentration (C_{max}) to profile the preclinical PK/PD (Bauer et al., 2021). SP141, which was developed by our group, has been examined for toxicity in a xenograft mouse model bearing human breast cancer cells. No significant overt toxicity was observed, and no apparent organ-specific effects were detected in the treatment groups (Wang et al., 2014b).

4.3. MDM2 Inhibition-Based Combination Therapies to Overcome Drug Resistance

MDM2 inhibitors have been combined with various targeted therapies, chemotherapies, and immunotherapies in many cancer types. This section will summarize these combination strategies.

4.3.1. Combined Use with Chemotherapy and Radiotherapy

The principal goal of both chemotherapy and radiotherapy is to induce cell death. MDM2 is a key molecule involved in cell death by providing a pro-survival signal that counteracts the pro-apoptotic role of p53. Logically, inhibition of MDM2 will enhance the death signals in cancer cells induced by both chemo- and radiotherapy. Indeed, Nutlin-3 has been found to sensitize NB to chemotherapy (Barbieri et al., 2006). Combining RG7388 with multiple chemotherapeutic agents also showed synergistic anti-tumor effects in NB cells (Chen et al., 2015). A combination of RG7775, the prodrug of RG7338, and temozolomide showed better survival and greater anti-tumor efficacy than either agent alone in a xenograft model of NB (Chen et al., 2019b). MI-77301 decreased the viability of NB cells, induced apoptosis, and augmented the anti-tumor effects of doxorubicin (Lu et al., 2016a). Targeting MDM2 with Nutlin-3a enhanced the sensitivity of AML cells to chemotherapy (Maganti et al., 2018). In that study, refractory AML with a deficiency of metal response element binding transcription factor 2

(MTF2), a cofactor of PRC2, had upregulated MDM2 due to a loss of the normal suppressive function of PRC2. MDM2 inhibitors sensitized these MTF2-deficient AML cells to standard chemotherapy. Nutlin-3 also increased the vulnerability of sarcoma cells to radiation therapy via induction of senescence in polyploid cells (Das, 2020). In another report, Nutlin derivative RG7112 significantly synergized with trabectedin in MDM2-amplified LPS cells, representing a promising therapeutic strategy for sarcomas with MDM2 amplification (Obrador-Hevia et al., 2015). The acquired resistance of liver cancer HepG2 cells to doxorubicin could also be reversed by MI-77301 treatment (Guo et al., 2020). Another of the MI series compounds, MI-219, sensitized prostate cancer cells to radiation therapy and improved the outcomes of mice bearing high-risk prostate cancer (Feng et al., 2016). Other MI series compounds, such as MI-43, were found to block the interaction of MDM2 and p53, increasing the sensitivity of lung cancer cells to etoposide (Sun et al., 2008). The combination of Nutlin-3a and mitoxantrone (a chemotherapeutic agent) showed synergistic effects in breast cancer cells with high ATP-binding cassette sub-family G member 2 (ABCG2) expression (Zhang et al., 2011b). Nutlin-3a enhances mitoxantrone's efficacy by inhibiting ABCG2's transport function, suggesting that this combination may be promising for treating cancers with stem cell-like traits and high ABCG2 levels (Zhang et al., 2011b).

More than 90% of ovarian cancer cells exhibit p53 mutations or inactivation. The combination of a MDM2 inhibitor, RG-7388, and nuclear export inhibitor, Selinexor, reduced cell viability and induced apoptosis, outperforming the individual therapies and upregulating cancer suppressor proteins like p53 and p21 (Alzahrani et al., 2022). Nutlin-3 synergized with cisplatin to enhance the cytotoxicity in both cisplatin-sensitive ovarian cancer A2780 cells and cisplatin-resistant 2780CP/CI-16 and A2780/CI-24 tumor cells (Xie et al., 2020). This provides

further evidence that combination therapies may be useful for reversing the resistance of malignant cells to various chemotherapeutic agents.

The exploration of MDM2's role in radiation resistance forms a significant part of contemporary cancer research. The upregulation of MDM2 contributes to the development of resistance against radiation therapy both by inhibiting p53 and via its interactions with various other molecules (Hou et al., 2019; Perry, 2004). During the early development of MDM2 inhibitors, our group found that antisense oligonucleotides targeting MDM2 could serve as radiosensitizers to improve the effects of radiation therapy during cancer treatment (Zhang et al., 2004). It was later demonstrated that AMG232 enhanced the radiation response in a variety of human tumor cell lines and xenograft mouse models harboring functional p53, including breast, colorectal, melanoma, lung, and sarcoma (Prabakaran et al., 2017; Werner et al., 2015). In preclinical models of glioblastoma multiforme (GBM), MDM2 inhibitors like Nutlin-3 (Luo et al., 2013) and RG7388 (Berberich et al., 2019) reduced tumor growth and increased radiation sensitivity when used with radiation therapy, especially in tumors resistant to standard chemotherapy. The efficacy of KRT-232 also increased when it was combined with radiation therapy in patient-derived GBM models, suggesting a broader applicability of this therapeutic approach (Mladek AC, 2019).

Another MDM2 inhibitor, Navtemadlin, effectively halted the growth of B16-F10 melanoma cells *in vitro* with minimal apoptosis, but exhibited increased apoptosis when combined with radiotherapy. The combination of Navtemadlin with radiation significantly reduced B16-F10 melanoma growth in mice, demonstrating the model's value in testing p53-MDM2 inhibitors and identifying effective combination therapies (Ingelshed et al., 2022). A recent study revealed that Nutlin-3 upregulated p53 and RB, while reducing DNA

methyltransferases (DNMTs) in chemoradiation-resistant p53WT ESCC cells (Chang et al., 2023). While the upregulation of MDM2 is a recognized mechanism contributing to the radiation resistance, the role of MDM2 inhibitors in countering this resistance is still being investigated, and using MDM2 inhibition to improve the response to radiation is a promising and active area of research.

4.3.2. The Use of an MDM2 Inhibitor in Combination with Targeted Therapy

MDM2 inhibition not only sensitizes cancer cells to chemo- and radiotherapy, but can also enhance the anti-tumor efficacy of targeted therapies. For example, using an agent targeting the PI3K pathway in combination with RG7112 to target MDM2 might represent a promising strategy for treating clear cell ovarian carcinoma (Makii et al., 2019). It has been demonstrated that Kirsten rat sarcoma virus (KRAS) mutant NSCLCs and CRCs are unresponsive to mitogen-activated protein kinase kinase (MEK) inhibitors (Niemantsverdriet et al., 2018). However, combined treatment with a MEK inhibitor (pimasertib) and the MDM2 inhibitor MI-77301 had synergistic anti-tumor effects and induced the expression of apoptotic proteins such as PUMA and BIM, resulting in apoptosis and cell growth arrest. The findings for this combination provide useful evidence to support the introduction of a MDM2-targeting therapeutic approach for cancer patients whose tumors are insensitive to MEK inhibitors (Hata et al., 2017). MI-77301 treatment activated p53WT and induced cell cycle arrest in PDX models (Lu et al., 2016b). Endocrine-resistant breast cancer is a subgroup of estrogen receptor (ER)-positive breast cancer that is insensitive to endocrine treatments, such as tamoxifen.

Other MDM2 inhibitors, RG7388 and AMG232, have been shown similar synergistic anti-tumor effects as MI-77301 when combined with a MEK inhibitor (trametinib) in NB cells

(Berberich et al., 2019) and in a PDX model of NSCLC carrying KRAS mutations (Zhang et al., 2020). In metastatic melanoma, AMG232 treatment enhanced the anti-tumor response to MEK/BRAF inhibitors (navitoclax and dabrafenib) in PDX models of melanoma with a BRAF^{V600E} mutation (Shattuck-Brandt et al., 2020). When RG7388 was combined with a fibroblast growth factor receptor (FGFR) inhibitor, erdafitinib, it led to synergistic anti-tumor effects in DDLPS (Dadone-Montaudie et al., 2020). Notably, RG7388 treatment upregulates the activity of the extracellular signal-regulated kinase in DDLPS cells, implying that combining RG7388 with inhibitors targeting ERK may be a useful approach for DDLPS (Roy et al., 2020). RG7388 also showed synergistic tumor reduction in ovarian cancer models when combined with the poly-ADP ribose polymerase (PARP) inhibitor rucaparib (Zanjirband et al., 2017). Although the combination of RG7388 and metformin could inhibit growth and induce apoptosis in ovarian cancer cells via the phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway, there was an increase in reactive oxygen species (ROS) by metformin observed in that study, which may detract from the clinical development of this combination (Cui et al., 2020).

MDM2 inhibitors have also been used in combination with various other targeted therapies, and these combinations showed significant anti-tumor effects in different cancer types. A screening study has identified a synergy between RG7388 and the Bcl-2 inhibitor venetoclax in NB (Van Goethem et al., 2017) and AML (Lehmann et al., 2016). Another MDM2 inhibitor, HDM201 (Novartis), has been shown to have a similar anti-tumor synergy when combined with Bcl-2 inhibitor ABT263 in uveal melanoma cells (Decaudin et al., 2020). It also effectively inhibited the growth of fms-like tyrosine kinase 3 (FLT3)-ITD-positive and p53WT AML when it was used in combination with a kinase inhibitor targeting FLT3, midostaurin (Seipel et al., 2018).

CGM097 could sensitize endocrine-resistant ER-positive breast cancer cells to endocrine therapy. It also showed synergistic inhibition of tumor growth when combined with a cyclin-dependent kinase (CDK)4/6 inhibitor (Portman et al., 2020). The use of CGM097 in combination with the bromodomain and extra-terminal motif (BET) inhibitor, OTX015, led to reduced tumor growth and increased cell death in NB (Maser et al., 2020), suggesting that MDM2 has crosstalk with various proteins, including acetylated proteins such as histones or transcription factors that play roles through protein-protein interactions via bromodomains. Another screening study using a panel of uveal melanoma cell lines identified that the combination of a protein kinase C inhibitor, AEB071, with CGM097 showed promising inhibition of cancer cell growth (Carita et al., 2016).

4.3.3. The Combination of MDM2 Inhibition with Immunotherapy

Immunotherapy has recently started to be applied for cancer treatment. After MDM2 was confirmed to be associated with HPD and resistance to ICIs (Fang et al., 2019; Fuentes-Antras et al., 2018), subsequent investigations demonstrated that inhibition of MDM2 could sensitize cancer cells to immunotherapy. For example, inhibition of MDM2 by ALRN-6924 improved the anti-tumor efficacy of immunotherapy, apparently via re-activation of p53 (Zhou et al., 2021b). Interestingly, APG-115 has been found to sensitize cancer cells to programmed cell death 1 (PD-1) blockade (Fang et al., 2019), providing further evidence to support the role of MDM2 in enhancing anti-tumor immunity. Similar phenomena have been reported for HDM201, wherein MDM2 inhibition correlated with the response to adaptive immunity, and the response was increased by disruption of the PD-1/programmed death-ligand 1 (PD-L1) interaction,

demonstrating that combining MDM2 inhibitors and ICIs represents an effective new approach for cancer therapy (Wang et al., 2021b).

The combination of Nutlin-3 and a therapeutic vaccine containing an MDM2-derived peptide enhanced the antitumor T cell responses by increasing human leukocyte antigen (HLA) expression (Kono et al., 2021). A recent study demonstrated that inhibition of MDM2 by Nutlin-3a improved the activity of natural killer cells in NB (Veneziani et al., 2021). Nutlin-3 treatment also decreased the expression of PD-L1, further suggesting that MDM2 inhibition can boost anti-tumor immunity (Li et al., 2020a)

In addition to being directly involved in regulating the immune checkpoint blockades, RG7388 treatment has shown increased efficacy against B-cell lymphoma when combined with an anti-CD20 antibody (Herting et al., 2016). AMG232 treatment also reduces the expression of interleukin-6 and enhances the T-cell-mediated killing of cancer cells (Sahin et al., 2020). One study demonstrated that using the combination of an anti-CD20 antibody, Obinutuzumab, along with a Bcl-2 inhibitor and RG-7388 in mouse models of non-Hodgkin's lymphoma had potent anti-tumor effects (Herting et al., 2018).

To date, all evidence supports a correlation of the MDM2 expression with the immune response. Both pre-clinical and clinical studies suggest that inhibiting MDM2 will potentially overcome the resistance to immune checkpoint inhibitors and/or reduce the development of hyper-progressive disease during or following immunotherapy. However, further investigation is needed to better understand how MDM2 regulates the immune response with regard to anti-tumor immunotherapy.

5. Clinical Trials of MDM2 Inhibitors

Many MDM2 inhibitors from different pharmaceutical companies have been tested in clinical trials to evaluate their safety, PK, PD, and efficacy. These trials are summarized in Table 1.

5.1. Pharmacology and Safety Evaluations

RG7112 (Roche), a derivative of Nutlin-3a, was the first small molecule MDM2 inhibitor to be introduced into clinical trials. RG7112 has a higher potency, a stronger binding ability to MDM2, and better PK parameters than Nutlin-3a (Tovar et al., 2013; Vu et al., 2013). Several completed Phase I clinical trials of RG7112 have been performed to evaluate the safety, maximum tolerated dose (MTD), and PK in patients with hematological neoplasms, advanced solid tumors (NCT00559533), and in LPS patients who were eligible for debulking surgery (NCT01143740). A study of the combination of RG7112 and doxorubicin has been conducted in patients with soft tissue sarcoma to evaluate the safety, PK, and efficacy of the treatment (NCT01605526). The combination of RG7112 with cytarabine was examined in another trial in leukemia patients (NCT01635296). Although RG7112 improved the expression level of p53 and its downstream target, p21, in phase I clinical trials, RG7112 displayed variable exposures at the MTD, poor tolerability, and relatively severe hematological and gastrointestinal toxicities at the higher doses. While RG7112 activated p53 in AML patients, with a complete response seen in some R/R patients and durable remission achieved in patients with acute leukemia and chronic lymphocytic leukemia/small cell lymphocytic lymphoma, it showed obvious gastrointestinal toxicity (NCT00623870) (Andreeff et al., 2016). Another Phase I trial was performed to characterize the pharmacology of RG7112 with high-fat and low-fat meals and new formulations (crystalline and amorphous) in patients with advanced solid tumors (NCT01164033). The results

showed that high-dose daily treatment for 3 to 5 days was better than weekly and low-dose longer daily regimens. The most commonly observed adverse events (AEs) were grades 1 and 2 drug-related gastrointestinal distress, indicating that RG7112 is well tolerated overall but is associated with gastrointestinal toxicities (Patnaik et al., 2015). To further evaluate the safety of RG7112, patients participating in previous studies were examined to determine the percentage of participants with any AEs and serious AEs during 24 months of treatment (NCT01677780). However, the outcomes of this evaluation have not been publicly disclosed.

RG7388, a more potent and selective follow-up compound to RG7112, was developed by Roche to improve upon the stereochemical and conformational properties of RG7112 and the spirooxindole MDM2 inhibitor MI-219. Phase I trials were performed to evaluate the bioequivalence or bioavailability following oral administration in participants with solid tumors (NCT03362723) and patients with polycythemia vera (PV) (NCT02407080). RG7388 was also assessed in combination with the Bcl-2 inhibitor venetoclax in difficult-to-treat patients with R/R AML in a Phase Ib trial (NCT02670044). The clinical activity observed with the RG7388-venetoclax combination in the dose escalation phase was moderate, with the combined rates for the antileukemic response of 40% and composite complete remission (CRc) of 26%. In line with prior experience with RG7388, the common AEs included diarrhea (87.3% of patients), nausea (74.5%), vomiting (52.7%), hypokalemia (50.9%), and febrile neutropenia (45.5%). The MTD was 200 mg of RG7388 + 600 mg of venetoclax. However, the dosing schedule optimization phase was not completed because of study termination after the MIRROS trial failed to meet its primary survival endpoint, and the RP2D was not determined (Daver et al., 2023). Another Phase I trial evaluated the safety, PK, PD, and efficacy of dose-escalating for this agent (NCT01462175) and demonstrated that the MTD was 3200 mg when it was given every week for 3 weeks, 1000

mg daily for 3 days in the 28-day cycle (QD3) or 500 mg daily for 5 days in a 28-day cycle (QD5). The QD5 schedule was used for further trials. Exposure-dependent hematological toxicity was noted in a PK/PD analysis. There was no apparent effect of food on the activity of RG7388 (Italiano et al., 2021). In another Phase I trial, a single arm evaluated the excretion, metabolism, and oral bioavailability of a single dose of ¹⁴C-labeled Idasanutlin and a single intravenous dose of ¹³C-labeled RG7388 in patients with solid tumors (NCT02828930). The results revealed a moderate (40.1%) absolute bioavailability of RG7388. RG7388 and its major inactive metabolite were the main compounds found in plasma. The excretion of RG7388 was primarily via the fecal route, with a small amount of RG7388 also detected in urine (Papai et al., 2019). RG7775, a pegylated product of RG7388, was tested in a Phase I study to investigate its safety and PK/PD in patients with solid tumors or AML (NCT02098967). The MTD was 110 mg for the patients with solid tumors, with 8% of patients experiencing dose limiting toxicities (DLTs), and was found to be 200 mg for AML patients (0 DLTs in 7 patients) (Abdul Razak et al., 2020; Uy et al., 2020).

Early Phase I trials of the MDM2 inhibitor milademetan (Rain Therapeutics) were conducted in healthy participants to evaluate the effects of food on the single-dose PK (NCT03647202) or to evaluate the single-dose PK when the agent was combined with itraconazole or posaconazole (NCT03614455). Two phase I trials of milademetan were completed in patients with R/R AML (NCT03671564, JapicCTI-184054), where it was given at a single dose as a single agent to evaluate its safety, tolerability, and PK. Dose escalation and dose expansion studies were included in a subsequent trial (NCT01877382). That trial assessed the maximum plasma concentration, area under the curve, time to reach C_{max}, apparent clearance, and PD, as assessed by measuring the serum macrophage inhibitory cytokine 1 (MIC-1) levels of extended/continuous or intermittent dosing schedules of milademetan in patients with advanced

solid tumors and lymphomas. Mild-to-moderate nonhematological AEs were observed regardless of the dosing schedule, whereas the severity of hematological abnormalities, particularly thrombocytopenia, was dependent on the dose density. Thrombocytopenia, nausea, fatigue, and anemia were the most common drug-related all-grade AEs. Notably, the occurrence and severity of thrombocytopenia and other hematological events were markedly reduced with intermittent dosing compared to extended or continuous schedules (Gounder et al., 2023). The recommended intermittent dose of milademetan was 260 mg on days 1-3 and 15-17 every 28 days. This schedule significantly reduced thrombocytopenia and on-target toxicities associated with MDM2 inhibitors compared with more continuous dosing regimens. This dosing schedule allowed time for bone marrow recovery while maintaining efficacy, as evidenced by the elevated serum growth differentiation factor-15 (GDF15) level, which is a biomarker of p53 reactivation, together with increased tumor expression of p53 and downstream gene products (p21 and MDM2) (Gounder et al., 2023). The combination of milademetan and low-dose cytarabine, with or without venetoclax, was associated with noticeable gastrointestinal toxicity (50% patients \geq grade 3) in a phase I clinical trial (NCT03634228) in patients with R/R or newly diagnosed AML (Senapati et al., 2023). However, a Phase I clinical trial of milademetan registered in Japan showed that it was well-tolerated and had potential antitumor activity in patients with solid tumors (JapicCTI-142693) when it was given at 90 mg daily for 21 days in a 28-day cycle (Takahashi et al., 2021). Thus, the dosing regimen appears to be a major factor influencing both the efficacy and tolerability of MDM2 inhibitors, particularly when given as part of combination treatments.

ALRN-6924 (Aileron), the only peptide inhibitor tested in clinical trials, was evaluated in dose escalation and dose expansion studies, and was well tolerated. The recommended dose for subsequent Phase I/IIa studies was 3.1 mg/kg on days 1, 8, and 15 in a 28-day cycle for p53WT

solid tumors and lymphomas (NCT02264613) (Saleh et al., 2021). Another completed Phase I trial evaluated the safety, tolerability, PK, and PD of ALRN-6924 alone or in combination with cytarabine, an antimetabolic agent, in patients with AML and myelodysplastic syndrome (MDS) (NCT02909972). A middle-term report demonstrated that the combination was generally well tolerated with transient, self-resolving Grade 3 and 4 neutropenia, pulmonary embolism, thrombocytopenia, leukopenia, and increased ALT in the 12 enrolled patients (F. Meric-Bernstam¹, 2019), but the final results are not yet available. Aileron also announced interim data from its Phase Ib trial on preventing chemotherapy-induced side effects (NCT04022876) in patients with advanced p53-mutated NSCLC undergoing treatment with first-line carboplatin plus pemetrexed. Patients treated with ALRN-6924 stayed on chemotherapy longer, successfully completing 93% of the initial 4 cycles of carboplatin/pemetrexed, in contrast to the 78% completion rate observed in the placebo plus carboplatin/pemetrexed group. The proportion of patients who completed 6 cycles of treatment was also higher in those treated with ALRN-6924 (79%) compared to those on placebo (57%). However, ALRN-6924-treated patients demonstrated only 56% of cycles free from Grade ≥ 3 hematologic toxicities (neutropenia, thrombocytopenia, and anemia) compared to 50% on placebo. Thus, Aileron has terminated the NSCLC trial.

Only one phase I trial of single-dose treatment has been completed for an oral MDM2 inhibitor APG-115 (Ascentage Pharma) in patients with advanced solid tumors or lymphoma (NCT02935907) to determine the MTD, DLTs, and recommended dose for a future phase II trial. APG-115 was well tolerated with manageable adverse drug events. The MTD/RP2D of APG-115 (every other day (QOD) for 21 days of a 28-day cycle) has been determined to be 100 mg (Drew W. Rasco, 2019).

A phase I study of MI-77301 (Sanofi-Aventis) combined with the MEK inhibitor, pimasertib, was conducted in patients with locally advanced or metastatic solid tumors (NCT01985191). The MTD was determined to be 200 mg of MI-77301 daily plus 45 mg of pimasertib two times a day (de Weger et al., 2019a). Another Phase I trial investigated the MTD, safety, and PK/PD of MI-77301 in patients with advanced solid tumors (NCT01636479). That trial determined that the MTD was 300 mg MI-77301 once daily (QD) because two patients treated with 400 mg MI-77301 QD developed thrombocytopenia. One patient had nausea with an 1800 mg twice-weekly dose. Treatment with MI-77301 was associated with increased plasma MIC-1, a marker for activation of p53 (de Jonge et al., 2017).

AMG232 (Kartos) is a leading inhibitor being tested in many clinical trials as either a single drug or in combinations with other agents in patients with solid tumors, hematological malignancies, Merkel cell carcinoma (MCC), small cell lung cancer, and myelofibrosis (Table 1). Open-label Phase I studies evaluated the safety, PK, and MTD of AMG232 in patients with R/R AML (NCT02016729). The MTDs were 360 mg for single agent treatment or 60 mg when the agent was combined with trametinib (Erba et al., 2019). Another Phase I dose-expansion trial of AMG232 used alone in advanced p53WT solid tumors or multiple myeloma (NCT01723020) indicated that the MTD was 240 mg when the drug was given every 3 weeks (Gluck et al., 2020). The intermittent dosing of patients with AMG232 (240 mg, Day 1-7 of a 28-day cycle) demonstrated a tolerable safety profile when it included the use of prophylaxis for nausea and vomiting in a phase II study (NCT03662126) in patients with R/R myelofibrosis (MF). The most frequently reported AEs were gastrointestinal (e.g., diarrhea, nausea, vomiting) and hematological (e.g., thrombocytopenia, anemia, neutropenia) (Verstovsek et al., 2022). Recently, a Phase Ib

clinical study was initiated to focus on the side effects of combining AMG 232 and radiation therapy for the treatment of soft tissue sarcoma (NCT03217266).

A phase I study of CGM097 (Novartis) evaluated different dosing regimens and assessed the safety of the compound in patients with advanced solid tumors (NCT01760525). Patients with p53WT advanced solid tumors received CGM097 via two different dosing regimens: a continuous three times a week (3qw) and an alternative 3qw, 2 weeks on and 1 week off regimen, to allow bone marrow recovery. The continuous 3qw dosing of the agent at 300 mg showed a disease control rate (DCR) of 39%, including one patient with malignant melanoma who achieved a partial response (PR) and 19 patients with stable disease (SD). However, the continuous 3qw dosing was not well tolerated with delayed-onset thrombocytopenia, lymphopenia, and neutropenia as the most common treatment-related grade 3 or 4 AEs (Bauer et al., 2021). Novartis strategically decided to stop developing CGM097 and prioritize the clinical development of HDM201, another MDM2 inhibitor.

HDM201 is an imidazolopyrrolidinone analog that demonstrated improved potency, physicochemical properties, and a more favorable PK profile compared to CGM097 (Holzer, 2017). Pulsed high-dose and fractionated low-dose regimens of HDM201 were compared in a phase I clinical study (NCT02143635) in patients with p53WT advanced solid tumors or R/R AML or ALL (Stein et al., 2022). The recommended dose for expansion (RDE) was determined by a dose-escalation study that indicated the RDEs to be 250 mg on day 1 with a 21-day cycle (1A regiment); 120 mg on days 1 and 8 with a 28-day cycle (1B regiment); and 45 mg on days 1 to day 7 with a 28-day cycle (2C regiment) (Stein et al., 2021; 2022). The safety profile for HDM201 was manageable and consistent with the other MDM2 inhibitors. Delayed-onset thrombocytopenia, tumor lysis syndrome (in patients with hematological malignancies, but not in

those with solid tumors), neutropenia, anemia, and gastrointestinal disorders were the most common grade 3/4 AEs suspected to be related to treatment. However, short-term high-dose HDM201 treatment intervals could be beneficial to mitigate the occurrence of severe myelosuppression that would otherwise be associated with prolonged continuous administration of HDM201, potentially widening the therapeutic window for MDM2 inhibition (Stein et al., 2022).

Intermittent administration of BI 907828 (on day 1 of 21-day cycles (once every 3 weeks, or Q3W) or days 1 and 8 of 28-day cycles (D1D8Q4W)) showed a manageable safety profile in patients with advanced solid tumors (NCT03449381). The MTDs were 60 mg and 45 mg in the D1Q3W arm and the D1D8Q4W arm, respectively, and the RDE for the phase Ib dose-expansion study was chosen to be 45 mg Q3W. The exceptionally long half-life (30–60 hours) of BI 907828 allowed for the intermittent administration schedule (every 21 days), which increased the patient convenience and treatment adherence and contributed to the manageable thrombocytopenia and safety profile of BI 907828 (LoRusso et al., 2023).

Most clinical trials of MDM2 inhibitors have focused on cancer patients. However, the safety and tolerability of UBX0101, a p53/MDM2 interaction inhibitor developed by Unity Biotechnology, were evaluated in osteoarthritis patients (NCT04229225 and NCT03513016). Results from NCT04229225 have not been published. In contrast, NCT03513016 demonstrated that the intra-articular administration of UBX0101 had a significant, dosage-dependent effect on pain and function in knee osteoarthritis patients (Lane et al., APRIL 2021).

5.2. Clinical Efficacy

Although all agents tested clinically are initially evaluated for their safety profile, there are several MDM2 inhibitors that have also been evaluated for efficacy as cancer therapeutics. For example, ALRN-6924 showed good anti-tumor efficacy in Phase I/II trials, with 41 evaluable patients with p53WT having a DCR of 59% (NCT02264613) (Saleh et al., 2021).

In a Phase I/Ib trial (NCT01773408), RG7388 was evaluated alone and in combination with cytarabine in patients with AML and demonstrated tolerable safety and encouraging clinical activity (CRc rates were 18.9% with RG7388 alone and 35.6% with combination therapy). The most common AEs were diarrhea, febrile neutropenia, and nausea (Yee et al., 2021). However, in a phase III study (the MIRROS trial; NCT02545283), the addition of RG7388 to cytarabine failed to improve the overall survival (OS) rate (median, 8.3 vs. 9.1 months with RG7388-cytarabine vs placebo-cytarabine) or the complete remission (CR) rate (20.3% vs 17.1%) in patients with p53WT R/R AML.

The prodrug of RG7388, RG7775, was administered intravenously and compared with oral RG7388 in Phase I studies in patients with advanced solid tumors and AML (NCT02098967) (Abdul Razak et al., 2020; Uy et al., 2020). SD was observed in 14 patients (34%) with solid tumors, and the DCR was 42% in patients with AML. However, although RG7775 also showed improved interpatient variability compared with RG7388, its adverse event profile was similar to RG7388, with neutropenia, thrombocytopenia, and stridor as dose-limiting toxicities in patients with advanced solid tumors; and QT interval prolongation, colitis, stomatitis, and diarrhea being dose-limiting toxicities in patients with AML. Thus, there was insufficient evidence of improved efficacy or safety to support the continued development of the prodrug given its toxicity.

Although Phase I trials typically focus on the safety and PK/PD of the drug, one study of MI-77301 showed preliminary anti-tumor efficacy in patients with locally advanced or metastatic

solid tumors (NCT01985191), with one patient (4%) with an endometrial tumor having a PR (one out of 24 efficacy-evaluable patients) and 15 (63%) patients having SD when MI-77301 was used in combination with pimasertib, a MEK inhibitor (de Weger et al., 2019a). Notably, the preliminary antitumor efficacy of this combination in the first-in-human study is consistent with the preclinically suggested benefit of inhibiting the Mitogen-activated protein kinase (MAPK) pathway while restoring p53 activity for cancers that harbor p53WT and MAPK mutations (Hata et al., 2017). Single agent treatment with MI-77301 in another Phase I trial (NCT01636479) also showed promising anti-tumor efficacy, with a response rate of 58%, with these patients all showing SD, and 32% of the patients remained progression-free at 3 months (de Jonge et al., 2017).

A Phase I study (NCT02016729) not only evaluated the safety, PK, and MTD of AMG232 in R/R AML patients, but also showed that 1 of the 30 patients evaluable for a response who received AMG232 combined with the MEK inhibitor trametinib achieved CR, 4 patients achieved a morphologic leukemia-free state, and 1 patient achieved a PR (Erba et al., 2019). Gastrointestinal AEs were the most common treatment-related toxicities in both the melanoma and leukemia studies; however, more serious and frequent thrombocytopenia, and leukopenia occurred in the leukemia study (Moschos et al., 2022).

Another completed phase I/II clinical trial investigated AMG232 in combination with a BRAF inhibitor (dabrafenib) and a MEK inhibitor (trametinib), or trametinib alone (NCT02110355) in patients with p53WT metastatic cutaneous melanoma with or without BRAFV600 mutations, and without prior treatment with BRAF or MEK inhibitors (Moschos et al., 2022). The overall objective response rate was 80% (2 CR and 6 PR) in the 10 patients who received the combination of AMG232, dabrafenib, and trametinib. On the other hand, the overall

objective response rate was only 15% (3 PR) in patients who received the AMG232 and trametinib combination arm (20 patients) (Moschos et al., 2022). Importantly, in a phase II study (NCT03662126) of AMG232 in patients with primary myelofibrosis (PMF), post-polycythemia vera myelofibrosis (Post-PV-MF), or post-essential thrombocythemia myelofibrosis (Post-ET-MF) who were R/R to Janus kinase inhibitor (JAKi) treatment, intermittent QD dosing with 240 mg of AMG232 (Days 1-7 of a 28-day cycle) led to the best spleen volume reduction ((SVR) $\geq 35\%$ in 16% of patients), as well as the best total symptom score (TSS) response ($> 50\%$ in 30% of patients), and an 87% reduction in the number of CD34+ cells in the peripheral blood at week 24. Therefore, AMG232 received a fast track designation for the treatment of JAKi R/R MF, and it is currently being compared with best available therapy for patients with PMF, Post-PV-MF, or Post-ET-MF who are R/R to JAKi treatment in a global phase III clinical trial (NCT03662126; the BOREAS trial) (Verstovsek et al., 2022). There are also several phase Ib or Ib/II studies of AMG232 as a single agent or combined with chemotherapy or radiation that are active and/or recruiting participants (Table 2).

A Phase I study also showed that CGM097 was effective in patients with advanced solid tumors (NCT01760525). The trial's response rate was 39%, including one PR and 19 patients with SD (Bauer et al., 2021). HDM201 was also evaluated in a Phase I trial and showed a 10.3% response rate in patients with solid tumors, 4 PRs and 38 SD, and response rates ranging from 4.2%-22.2% based on the regimen. The 2C regimen (days 1-7 on a 28-day cycle) gave the best results; 5 patients with AML across all dosing cohorts achieved CRs (NCT02143635) (Stein et al., 2021).

In another phase Ib/II (NCT02343172) clinical trial in patients with locally advanced/metastatic well-differentiated/dedifferentiated (WD/DD) LPS, high-dose, pulsed

regimens of HDM201 in combination with ribociclib, an inhibitor of cyclin D1(CCND1)/cyclin-CDK4/6 was more efficacious than the low-dose daily regimen. Although no CR was achieved, 3 PRs and 27 cases of SD were reported in high-dose, pulsed regimens versus 11 patients with SD in the group with the low-dose daily regimen (Abdul Razak et al., 2022). Inspired by these results, HDM201 is currently being investigated/planned for investigation in early-phase clinical trials (phase I and I/II) in combination with other agents to potentially broaden its efficacy (Table 2).

Combining BCL2 inhibition (venetoclax) with MDM2 inhibition (milademetan) resulted in only minimal clinical responses in a Phase I trial in patients with R/R AML (NCT03634228) (Senapati et al., 2023). Similarly, milademetan monotherapy did not translate into meaningful clinical responses in another study in Japanese patients with R/R AML (NCT03671564) (Sekiguchi et al., 2023), despite an earlier study in Japan showing potential benefits (JapicCTI-142693; (Takahashi et al., 2021). In patients with R/R AML or high-risk MDS (NCT02319369), milademetan monotherapy resulted in a reduction in bone marrow blasts in 15 of 38 patients and 3 CR, 2 in patients with AML and 1 in a patient with MDS (DiNardo et al., 2016). In another phase I study in patients with advanced solid tumors or lymphomas (NCT01877382), milademetan given once daily as part of extended/continuous or intermittent schedules had single-agent efficacy across all cohorts (N =107 patients); the ORR was 4.7%, DCR was 45.8%, and median progression-free survival (PFS) was 4.0 months. Interestingly, in the overall DDLPS cohort (N = 53 patients); the ORR, DCR, and median PFS were 3.8%, 58.5% and 7.2 months, respectively (Gounder et al., 2023). Based on these studies, Milademetan has been given orphan drug status by the U.S. FDA for patients with LPS and is currently being evaluated in an ongoing Phase III clinical trial in patients with WD/DD LPS that have progressed on at least one prior systemic therapy, including an anthracycline (MANTRA; NCT04979442). For that study, 175

patients will be randomly assigned in a 1:1 ratio to receive milademetan or trabectedin, an alkylating agent and standard of care for WD/DD LPS. Additionally, a Phase 2 tumor-agnostic basket study (MANTRA-2; NCT05012397) is enrolling participants with advanced or metastatic solid tumors refractory or intolerant to the standard of care therapy that exhibit p53WT and a MDM2 copy number ≥ 8 using prespecified biomarker criteria to evaluate the safety and efficacy of milademetan.

Treatment with BI 907828 (Boehringer Ingelheim) also showed encouraging preliminary efficacy in a Phase I trial (NCT03449381) in patients with advanced/metastatic solid tumors; 6 of 54 patients achieved a PR (overall response rate of 11.1%) and 34 patients achieved SD as the best response, giving a DCR of 74.1%. Interestingly, 4 of 7 patients with well-differentiated liposarcoma (WDLPS) achieved a durable PR (responses lasting ≥ 12 months to up to 2 years) and 3 patients achieved SD, giving a 100% DCR. Similarly, 9 of the 12 patients with DDLPS achieved SD (75.0% DCR). Two more PRs were seen, one in a patient with intrahepatic cholangiocarcinoma and another in a patient with pancreatic cancer (LoRusso et al., 2023).

The phase Ib dose expansion part of this study is ongoing with two cohorts: one for patients with p53WT, MDM2-amplified sarcoma and one for patients with p53WT, MDM2-amplified NSCLC, urothelial carcinoma, gastric carcinoma, biliary tract carcinoma, or pancreatic ductal adenocarcinoma. BI 907828 is also now being investigated further in a phase II/III study (NCT05218499, Brightline-1) in patients with advanced/metastatic DDLPS to evaluate whether it is superior to doxorubicin as first-line treatment (Schoffski et al., 2023). Moreover, a phase IIa/IIb clinical trial (NCT05512377, Brightline-2) is recruiting participants to investigate the efficacy of BI 907828 as monotherapy for locally advanced or metastatic, MDM2 amplified, p53WT biliary tract adenocarcinoma, pancreatic ductal adenocarcinoma, urothelial bladder cancer, and lung

adenocarcinoma. The agent is also being investigated in several ongoing phase I studies in patients with advanced or metastatic solid tumors (NCT05613036 and NCT05372367) and in patients with newly-diagnosed GBM (NCT05376800).

So far, most of the efficacy studies of MDM2 inhibitors have been part of Phase I trials. Of note, several clinical phase II-III trials of first-generation small molecule MDM2 inhibitors blocking p53-MDM2 binding have shown disappointing efficacy and extensive adverse effects. Two Phase III studies were terminated by the sponsors. A phase II trial to evaluate the efficacy in patients with non-cancer diseases assessed the efficacy of a single dose of UBX0101 in patients with knee osteoarthritis (NCT04129944), but did not show any beneficial effects. Despite these disappointing results, there are still numerous clinical trials that are ongoing and actively recruiting patients with multiple cancer types for clinical evaluation, including several Phase III trials summarized in Table 2.

6. Challenges and Future Directions

Targeting MDM2 has been attempted using different strategies for more than 20 years. Despite the significant progress that has been made in this field, there are still many unmet challenges, such as the selectivity of inhibitors, efficacy against cancer or other diseases, and the identification of biomarkers for preclinical studies and clinical trials. However, there are excellent reviews pointing out some potential issues to guide future research (Dobbelstein and Levine, 2020; Klein et al., 2021). We will provide another perspective to discuss what needs to be addressed during the next steps of development, as well as potential new opportunities for targeting MDM2.

6.1. Blocking the MDM2/p53 Protein-Protein Interaction versus Directly Targeting MDM2

The original rationale for targeting MDM2 was to release p53 from the MDM2/p53 complex and reactivate p53 to induce cell death. Most preclinical and clinical studies have emphasized the role of MDM2 inhibitors in a subpopulation of patients carrying tumors with p53WT. However, extensive evidence indicates that the functions of MDM2 are more complicated than just regulating p53 (Klein et al., 2021). In addition, more than half of human cancers have p53 mutations or loss of p53 function (Nishikawa and Iwakuma, 2023; Xu et al., 2021), and not all patients with p53WT respond to MDM2 inhibitor treatment (Ishizawa et al., 2018). Perhaps more importantly, patients with p53 mutations still often respond to MDM2 inhibitors (Andreoff et al., 2016). This suggests that MDM2 inhibition suppresses tumor growth not only due to MDM2-mediated p53 activation but also through other MDM2-mediated signals. Elucidating the full spectrum of these other MDM2-mediated effects is critical to guide preclinical studies and stratify patients for clinical evaluations. Moreover, it may be more effective to target MDM2 using MDM2 degradation inducers or MDM2 PROTACs, or direct MDM2 inhibitors that do not require p53 for their mechanism of action. In addition, it is possible that the outcomes of clinical trials might differ if different patient populations were recruited (i.e., if p53WT is not a requirement for eligibility in the trial).

6.2. Dual Inhibitors of MDM2 and MDMX

MDMX functions as a partner of MDM2 to regulate p53 (Manfredi, 2021; Wade et al., 2013; Zhang et al., 2014). MDMX can also downregulate p53 in the absence of MDM2. This implies that targeting both MDM2 and MDMX will be more effective than targeting MDM2

alone if p53 restoration is the target of treatment. Based on this concept, dual inhibitors for MDM2/MDMX have been considered as new agents to re-activate p53. The stapled peptide inhibitor ALRN-6924, which targets both MDM2 and MDMX, showed promising preclinical and clinical results (Pairawan et al., 2021; Saleh et al., 2021). Although the body of evidence supports the roles of MDM2 and MDMX in various disease conditions, questions still need to be answered to completely understand how MDM2 and MDMX regulate each other and their targets, including p53, p63, and p73. Of particular importance are the functional differences between MDM2 and MDMX and the precise conditions leading to the selective activation of one protein over the other, as well as whether non-p53 functions can explain the observed effects of dual inhibition. It is also currently unclear how the fact that MDMX is missing the nuclear location signal present in MDM2 affects its functions and stability. A comprehensive investigation of the molecular mechanisms involving MDM2 and MDMX will help guide the development of inhibitors targeting MDM2 and/or MDMX and identify their optimal clinical applications.

6.3. Screening to Identify More Potent and Selective Compounds

So far, the screening for compounds targeting MDM2 has generally been based on the regions involved in the binding between MDM2 and p53, which were initially published about 25 years ago. Numerous peptide-based inhibitors and small molecule inhibitors with different core structures were designed to mimic the critical residues of the p53 binding motif, including Phe19, Trp23, and Leu26 (Shangary and Wang, 2009). Several serine residues of MDM2 are phosphorylated in response to cell growth signals (Meek and Knippschild, 2003). For example, IGF and ATM stimulate the phosphorylation of serine 166 or serine 394, respectively (Feng et al., 2004; Gannon et al., 2012). Whether these modifications affect the binding of MDM2 to p53 at

the cellular level needs to be evaluated. Several residues at the C-terminus of MDM2 can be modified under different conditions (Okoro et al., 2012), and these modifications can potentially affect the binding of compounds targeting MDM2. Therefore, cell-based screening that more accurately mimics the physiological conditions may provide more value than *in vitro* binding assays and structural modeling. Luciferase and fluorescence two-hybrid assays can detect protein interactions (Li et al., 2011; Yurlova et al., 2014), which could be used to screen for potent inhibitors of specific MDM2 interactions at the cellular level. In addition, studies focusing on the non-p53 target of MDM2 are needed to elucidate which one(s) is responsible for the optimal effects on cancer cells, ideally with minimal effects on normal cells.

6.4. Biomarkers of MDM2 Activity

As noted above, p53WT and cell death signaling have been considered the main biomarkers for the response to MDM2 inhibitors. Identifying the biomarkers correlated with the other functions of MDM2 will be critical for the clinical application of MDM2 inhibitors in the future. Gene expression profiling of patient samples can also help identify biomarkers to stratify patients based on their predicted response to MDM2 inhibitor treatment, and this can be used to guide clinical trials. For example, miR-10a has been identified as a potential biomarker of the response to combined treatment with Nutlin-3a and cytarabine in patients with AML (Bryant et al., 2012; Vu et al., 2021). One study tried to identify the gene signatures that occurred in response to MDM2 inhibitors (Jeay et al., 2015). However, conflicting results (Sonkin, 2015) raised questions about these gene signatures. Another concern is that the gene signatures identified for the response to a single inhibitor cannot provide information about the general response to other inhibitors due to differences in the structures of the inhibitors and the likelihood of off-target

effects. A comprehensive non-biased analysis using a variety of different inhibitors and genetic disruption, followed by systematic validation using cell lines and human clinical samples, will be needed to provide a complete understanding of the general response to MDM2 inhibition.

6.5. Combination Therapy Using MDM2 Inhibitors with Other Treatment Regimens

In simple terms, carcinogenesis is the result of cancer cells escaping death signals and immune surveillance. Cancer cells thus often have intrinsic resistance to treatments that induce cell death, and tumors may develop mechanisms to escape treatments due to their high proliferation and mutation rates. Because MDM2 is considered an oncogenic protein that plays a variety of functions during carcinogenesis and the response to treatment, it has long been considered a potential target for therapy. Gene signatures that predict the sensitivity to MDM2 inhibitors have been identified in AML and PDX models (Ishizawa et al., 2018). Many signaling pathways have cross-talk with MDM2 and may affect the efficacy of MDM2 inhibitors (Haronikova et al., 2021). Combination treatments may overcome both intrinsic and acquired drug resistance. For example, the p-gp transporter negatively regulated the distribution of an MDM2 inhibitor in the brain, resulting in a low response to the MDM2 inhibitor in patients with brain tumors (Kim et al., 2019). Simultaneously targeting both p-gp and MDM2 may reduce resistance to the MDM2 inhibitor. Although preclinical studies have established many effective MDM2 inhibitor-based combination strategies, it is critical to elucidate the underlying mechanisms and off-target effects to predict which combinations will be most effective, and to optimize the treatment regimens.

6.6. Nano-formulation of MDM2 inhibitors

With the rapid advancements that have been made in nanotechnology and nanomedicine, incorporating MDM2 inhibitors into nano-formulations represents a promising research area. These formulations are engineered for targeted delivery, concentrating the therapeutic actions of the inhibitors on malignant cells while mitigating systemic side effects. The possibility of enhancing the solubility and stability of MDM2 inhibitors in nanoparticulate forms may improve their bioavailability, a critical factor in clinical efficacy. A key advantage of these nano-formulations is their capability for controlled drug release, ensuring a sustained therapeutic level and potentially allowing for a reduced dosing frequency (Gautam et al., 2023). Moreover, the potential of nano-formulations to co-deliver MDM2 inhibitors alongside other therapeutic compounds or genetic materials represents a strategic approach to counteract drug resistance in cancer cells. Additionally, the ability to leverage the enhanced permeability and retention of compounds by nanoparticles can facilitate drug penetration and retention within tumor tissues (Gautam et al., 2023). This innovation in drug delivery aligns with the principles of personalized medicine, offering the prospect of tailoring cancer treatments to the unique molecular profiles of individual patients, thereby optimizing therapeutic outcomes.

Our team has developed a novel nano-oral delivery system for SP141, a potent MDM2 oncogene inhibitor. The drug's oral bioavailability and tumor targeting were enhanced when it was loaded in nanoparticles (called SP141FcNP). These SP141 nanoparticles had improved transepithelial transport and intestinal absorption compared to the unencapsulated SP141, leading to increased anti-tumor effects both *in vitro* and *in vivo*, without significant host toxicity in models of breast cancer (Qin et al., 2016). Another study employed a PAMAM-OH derivative (PAMSPF) to co-deliver a p53 plasmid and the MDM2 inhibitor, RG7388. The resulting nanoparticles (PAMSPF/p53/RG) had high drug loading and stability, and significantly increased

the p53 expression in breast cancer cell lines. Treatment with PAMSPF/p53/RG led to reduced cell proliferation and increased apoptosis, effectively inhibiting tumor growth in MDA-MB-435 and MCF-7/WT breast cancer xenograft models, and demonstrating synergistic anti-tumor activity (Chen et al., 2019a).

To address the suppression of ARF, an inhibitor of MDM2, in p53WT tumors, ARF-mimetic MDM2-targeting reassembly peptide nanoparticles (MtrapNPs) were developed. These nanoparticles form a nanofiber structure with MDM2, stabilizing and activating p53. Additionally, MtrapNPs have been used to deliver arsenic trioxide to treat p53-mutated tumors, and these showed significant therapeutic effects in both orthotopic and metastatic models, highlighting the potential of the MDM2-trap strategy to treat both p53WT and mutated tumors (Li et al., 2023). In addition, one study introduced ^{PMI}Bcr/Abl-R6, a novel protein-based peptide drug carrier derived from the Bcr/Abl oncogenic protein. This carrier, enhanced with a dodecameric peptide inhibitor targeting the p53-MDM2/MDMX interaction and a C-terminal Arg-repeating hexapeptide, effectively induced apoptosis in p53-positive cells and inhibited tumor growth in a HCT116 *p53*^{+/+} mouse model, showcasing its potential as a viable approach for cancer therapy (Ma et al., 2019).

6.7. Nucleic acid therapeutics targeting MDM2

The advent of nucleic acid therapeutics marked a revolutionary shift in the pharmaceutical industry, signaling a new epoch of personalized medicine and targeted therapy. These therapies, encompassing RNA-based drugs like siRNA and miRNA, as well as DNA-based agents, such as antisense oligonucleotides, have now extended to include advanced modalities like CRISPR gene editing and aptamers (Hu et al., 2020; Kong et al., 2023; Shigdar et al., 2021; Shojaei Baghini et al., 2022). This broadened scope has brought unparalleled

specificity to disease treatment by directly targeting disease-linked genes or gene products. CRISPR offers a precise method to edit or regulate specific genes (Shojaei Baghini et al., 2022), while aptamers, comprising short DNA or RNA sequences (Shigdar et al., 2021), can selectively bind to and inhibit target proteins or genetic sequences, enhancing the precision and effectiveness of molecular therapeutics. The ability to target what were once considered 'undruggable' entities marks a pivotal advancement. The progress in genome editing and RNA interference technologies has been instrumental in driving this field forward, opening up novel treatment possibilities for genetic disorders, various forms of cancer, and viral infections.

Focusing on cancer treatment, the use of nucleic acid therapeutics to target MDM2 has emerged as a promising strategy. As mentioned in the previous sections, our group's pioneering work in validating the anti-cancer effects of targeting MDM2 through an antisense approach represents a significant milestone in cancer research (Wang et al., 2001; Wang et al., 1999; Zhang et al., 2004). This approach stands out for its ability to combat drug resistance, a formidable challenge in cancer therapy. By inhibiting MDM2 protein synthesis, these therapeutics can provide high specificity and reduced off-target effects. The growing presence of these therapies in clinical trials and recent FDA approvals further attest to their potential.

Looking ahead, the scope of nucleic acid therapies is expected to broaden, extending from treatment to prevention in high-risk populations and even regenerative medicine. Despite their promise, challenges such as ensuring stability, effective delivery, and minimizing immune responses remain. These are being addressed through innovative strategies like lipid nanoparticles and targeted delivery systems (Gautam et al., 2023). In contrast, small molecule MDM2 inhibitors offer a direct and rapid means to disrupt the MDM2-p53 interaction, with advantages like oral bioavailability and stability, backed by a solid history of clinical use.

However, their potential for off-target effects and the complexity of developing specific, efficacious inhibitors for a range of cancer types remain significant hurdles. Both approaches, nucleic acid therapeutics and small molecule inhibitors, represent significant strides in cancer treatment, each with unique strengths and challenges, contributing to the ever-evolving landscape of oncology therapeutics.

7. Conclusion and Perspectives

In summary, targeting MDM2 represents a promising therapeutic strategy for cancer patients. Although the previous and current inhibitors targeting MDM2 have shown promising results in preclinical and some clinical trials, there are currently no approved MDM2 inhibitors marketed for any indication. It should be noted that most of the MDM2 inhibitors investigated in clinical trials were designed to block the interaction between MDM2 and p53, which may actually increase the level of MDM2 and even increase its oncogenic activity. This may at least partially explain the failure of such MDM2 inhibitors in clinical trials. In contrast, directly inhibiting MDM2 using agents such as MDM2 degradation inducers or PROTACs would lead to more effective treatment with a better safety profile, particularly if specifically-targeted delivery and timed inhibition can be employed. Dual inhibition of specific interactions or molecules and combination treatments would also help to overcome intrinsic and acquired drug resistance. More detailed analyses of patient gene and protein expression profiles are needed to individualize treatments allowing the patients to achieve a more robust and durable response. Biomarkers will be helpful in guiding such studies and can be used to establish more effective and successful MDM2-targeted therapies.

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Data Availability Statement

N/A, there are no datasets in the paper.

Conflicts of Interest

Drs. Xian and McKeon have financial interests in Tract Pharmaceuticals, Inc.

Author Contributions

Wrote or contributed to the writing of the manuscript: WW, YD, NA, JF, HS, WX, FM, WL, JZ, and RZ

References

- Abbas HA, Maccio DR, Coskun S, Jackson JG, Hazen AL, Sills TM, You MJ, Hirschi KK and Lozano G (2010) Mdm2 is required for survival of hematopoietic stem cells/progenitors via dampening of ROS-induced p53 activity. *Cell Stem Cell* **7**:606-617.
- Abdul Razak AR, Bauer S, Suarez C, Lin CC, Quek R, Hütter-Krönke ML, Cubedo R, Ferretti S, Guerreiro N, Jullion A, Orlando EJ, Clementi G, Sand Dejmek J, Halilovic E, Fabre C, Blay JY and Italiano A (2022) Co-Targeting of MDM2 and CDK4/6 with Siremadlin and Ribociclib for the Treatment of Patients with Well-Differentiated or Dedifferentiated Liposarcoma: Results from a Proof-of-Concept, Phase Ib Study. *Clin Cancer Res* **28**:1087-1097.
- Abdul Razak AR, Miller WH, Jr., Uy GL, Blotner S, Young AM, Higgins B, Chen LC and Gore L (2020) A phase 1 study of the MDM2 antagonist RO6839921, a pegylated prodrug of idasanutlin, in patients with advanced solid tumors. *Invest New Drugs* **38**:1156-1165.
- Adams CM, Mitra R, Xiao Y, Michener P, Palazzo J, Chao A, Gour J, Cassel J, Salvino JM and Eischen CM (2023) Targeted MDM2 Degradation Reveals a New Vulnerability for p53-Inactivated Triple-Negative Breast Cancer. *Cancer Discov* **13**:1210-1229.
- Adashek JJ, Subbiah IM, Matos I, Garralda E, Menta AK, Ganeshan DM and Subbiah V (2020) Hyperprogression and Immunotherapy: Fact, Fiction, or Alternative Fact? *Trends Cancer* **6**:181-191.
- Aguilar A, Lu J, Liu L, Du D, Bernard D, McEachern D, Przybranowski S, Li X, Luo R, Wen B, Sun D, Wang H, Wen J, Wang G, Zhai Y, Guo M, Yang D and Wang S (2017a) Discovery of 4-((3'R,4'S,5'R)-6"-Chloro-4'-(3-chloro-2-fluorophenyl)-1'-ethyl-2"-oxodispiro[cyclohexane-1,2'-pyrrolidine-3',3"-indoline]-5'-carboxamido)bicyclo[2.2.2]octane-1-carboxylic Acid (AA-115/APG-115): A Potent and Orally Active Murine Double Minute 2 (MDM2) Inhibitor in Clinical Development. *J Med Chem* **60**:2819-2839.
- Aguilar A, Lu J, Liu L, Du D, Bernard D, McEachern D, Przybranowski S, Li X, Luo R, Wen B, Sun D, Wang H, Wen J, Wang G, Zhai Y, Guo M, Yang D and Wang S (2017b) Discovery of 4-((3'R,4'S,5'R)-6"-Chloro-4'-(3-chloro-2-fluorophenyl)-1'-ethyl-2"-oxodispiro[cyclohexane-1,2'-pyrrolidine-3',3"-indoline]-5'-carboxamido)bicyclo[2.2.2]octane-1-carboxylic Acid (AA-115/APG-115): A Potent and Orally Active Murine Double Minute 2 (MDM2) Inhibitor in Clinical Development. *J Med Chem* **60**:2819-2839.
- Ahmad SM, Nayak D, Mir KB, Faheem MM, Nawaz S, Yadav G and Goswami A (2020) Par-4 activation restrains EMT-induced chemoresistance in PDAC by attenuating MDM-2. *Pancreatolgy* **20**:1698-1710.
- Al-Ghabkari A and Narendran A (2019) In Vitro Characterization of a Potent p53-MDM2 Inhibitor, RG7112 in Neuroblastoma Cancer Cell Lines. *Cancer Biother Radiopharm* **34**:252-257.
- Alaaeldin R, Abuo-Rahma GEA, Zhao QL and Fathy M (2021) Modulation of Apoptosis and Epithelial-Mesenchymal Transition E-cadherin/TGF-beta/Snail/TWIST Pathways by a New Ciprofloxacin Chalcone in Breast Cancer Cells. *Anticancer Res* **41**:2383-2395.
- Alt JR, Bouska A, Fernandez MR, Cerny RL, Xiao H and Eischen CM (2005) Mdm2 binds to Nbs1 at sites of DNA damage and regulates double strand break repair. *J Biol Chem* **280**:18771-18781.

- Alzahrani A, Natarajan U and Rathinavelu A (2022) Enhancement of MDM2 inhibitory effects through blocking nuclear export mechanisms in ovarian cancer cells. *Cancer Genet* **266-267**:57-68.
- Andreeff M, Kelly KR, Yee K, Assouline S, Strair R, Popplewell L, Bowen D, Martinelli G, Drummond MW, Vyas P, Kirschbaum M, Iyer SP, Ruvolo V, Gonzalez GM, Huang X, Chen G, Graves B, Blotner S, Bridge P, Jukofsky L, Middleton S, Reckner M, Rueger R, Zhi J, Nichols G and Kojima K (2016) Results of the Phase I Trial of RG7112, a Small-Molecule MDM2 Antagonist in Leukemia. *Clin Cancer Res* **22**:868-876.
- Andrews A, Warner K, Rodriguez-Ramirez C, Pearson AT, Nor F, Zhang Z, Kerk S, Kulkarni A, Helman JI, Brenner JC, Wicha MS, Wang S and Nor JE (2019) Ablation of Cancer Stem Cells by Therapeutic Inhibition of the MDM2-p53 Interaction in Mucoepidermoid Carcinoma. *Clin Cancer Res* **25**:1588-1600.
- Arena G, Cissé MY, Pyrdziak S, Chatre L, Riscal R, Fuentes M, Arnold JJ, Kastner M, Gayte L, Bertrand-Gaday C, Nay K, Angebault-Prouteau C, Murray K, Chabi B, Koechlin-Ramonatxo C, Orsetti B, Vincent C, Casas F, Marine JC, Etienne-Manneville S, Bernex F, Lombès A, Cameron CE, Dubouchaud H, Ricchetti M, Linares LK and Le Cam L (2018) Mitochondrial MDM2 Regulates Respiratory Complex I Activity Independently of p53. *Mol Cell* **69**:594-609.e598.
- Arnhold V, Schmelz K, Proba J, Winkler A, Wunschel J, Toedling J, Deubzer HE, Kunkle A, Eggert A, Schulte JH and Hundsdoerfer P (2018) Reactivating TP53 signaling by the novel MDM2 inhibitor DS-3032b as a therapeutic option for high-risk neuroblastoma. *Oncotarget* **9**:2304-2319.
- Ashkenazi A, Fairbrother WJ, Levenson JD and Souers AJ (2017) From basic apoptosis discoveries to advanced selective BCL-2 family inhibitors. *Nat Rev Drug Discov* **16**:273-284.
- Azmi AS, Philip PA, Aboukameel A, Wang Z, Banerjee S, Zafar SF, Goustin AS, Almhanna K, Yang D, Sarkar FH and Mohammad RM (2010) Reactivation of p53 by novel MDM2 inhibitors: implications for pancreatic cancer therapy. *Curr Cancer Drug Targets* **10**:319-331.
- Barbieri E, Mehta P, Chen Z, Zhang L, Slack A, Berg S and Shohet JM (2006) MDM2 inhibition sensitizes neuroblastoma to chemotherapy-induced apoptotic cell death. *Mol Cancer Ther* **5**:2358-2365.
- Barrows LR, Radisky DC, Copp BR, Swaffar DS, Kramer RA, Warters RL and Ireland CM (1993) Makaluvamines, marine natural products, are active anti-cancer agents and DNA topo II inhibitors. *Anticancer Drug Des* **8**:333-347.
- Bauer S, Demetri GD, Halilovic E, Dummer R, Meille C, Tan DSW, Guerreiro N, Jullion A, Ferretti S, Jeay S, Van Bree L, Hourcade-Potelleret F, Wuerthner JU, Fabre C and Cassier PA (2021) Pharmacokinetic-pharmacodynamic guided optimisation of dose and schedule of CGM097, an HDM2 inhibitor, in preclinical and clinical studies. *Br J Cancer* **125**:687-698.
- Bekes M, Langley DR and Crews CM (2022) PROTAC targeted protein degraders: the past is prologue. *Nat Rev Drug Discov*.
- Beloglazkina A, Zyk N, Majouga A and Beloglazkina E (2020) Recent Small-Molecule Inhibitors of the p53-MDM2 Protein-Protein Interaction. *Molecules* **25**.
- Berberich A, Kessler T, Thome CM, Pusch S, Hielscher T, Sahm F, Oezen I, Schmitt LM, Ciprut S, Hücke N, Ruebmann P, Fischer M, Lemke D, Breckwoldt MO, von Deimling A,

- Bendszus M, Platten M and Wick W (2019) Targeting Resistance against the MDM2 Inhibitor RG7388 in Glioblastoma Cells by the MEK Inhibitor Trametinib. *Clin Cancer Res* **25**:253-265.
- Bianco R, Caputo R, Caputo R, Damiano V, De Placido S, Ficorella C, Agrawal S, Bianco AR, Ciardiello F and Tortora G (2004) Combined targeting of epidermal growth factor receptor and MDM2 by gefitinib and antisense MDM2 cooperatively inhibit hormone-independent prostate cancer. *Clin Cancer Res* **10**:4858-4864.
- Bieging KT, Mello SS and Attardi LD (2014) Unravelling mechanisms of p53-mediated tumour suppression. *Nat Rev Cancer* **14**:359-370.
- Bogen SL, Pan W, Gibeau CR, Lahue BR, Ma Y, Nair LG, Seigel E, Shipps GW, Jr., Tian Y, Wang Y, Lin Y, Liu M, Liu S, Mirza A, Wang X, Lipari P, Seidel-Dugan C, Hicklin DJ, Bishop WR, Rindgen D, Nomeir A, Prosise W, Reichert P, Scapin G, Strickland C and Doll RJ (2016) Discovery of Novel 3,3-Disubstituted Piperidines as Orally Bioavailable, Potent, and Efficacious HDM2-p53 Inhibitors. *ACS Med Chem Lett* **7**:324-329.
- Bohlman S and Manfredi JJ (2014) p53-independent effects of Mdm2. *Subcell Biochem* **85**:235-246.
- Brenkman AB, de Keizer PL, van den Broek NJ, Jochemsen AG and Burgering BM (2008) Mdm2 induces mono-ubiquitination of FOXO4. *PLoS One* **3**:e2819.
- Bricelj A, Steinebach C, Kuchta R, Gutschow M and Sosic I (2021) E3 Ligase Ligands in Successful PROTACs: An Overview of Syntheses and Linker Attachment Points. *Front Chem* **9**:707317.
- Bryant A, Palma CA, Jayaswal V, Yang YW, Lutherborrow M and Ma DD (2012) miR-10a is aberrantly overexpressed in Nucleophosmin1 mutated acute myeloid leukaemia and its suppression induces cell death. *Mol Cancer* **11**:8.
- Cahilly-Snyder L, Yang-Feng T, Francke U and George DL (1987) Molecular analysis and chromosomal mapping of amplified genes isolated from a transformed mouse 3T3 cell line. *Somat Cell Mol Genet* **13**:235-244.
- Cai BH, Hsu YC, Yeh FY, Lin YR, Lu RY, Yu SJ, Shaw JF, Wu MH, Tsai YZ, Lin YC, Bai ZY, Shih YC, Hsu YC, Liao RY, Kuo WH, Hsu CT, Lien CF and Chen CC (2022) P63 and P73 Activation in Cancers with p53 Mutation. *Biomedicines* **10**.
- Canon J, Osgood T, Olson SH, Saiki AY, Robertson R, Yu D, Eksterowicz J, Ye Q, Jin L, Chen A, Zhou J, Cordover D, Kaufman S, Kendall R, Oliner JD, Coxon A and Radinsky R (2015) The MDM2 Inhibitor AMG 232 Demonstrates Robust Antitumor Efficacy and Potentiates the Activity of p53-Inducing Cytotoxic Agents. *Mol Cancer Ther* **14**:649-658.
- Carita G, Frisch-Dit-Leitz E, Dahmani A, Raymondie C, Cassoux N, Piperno-Neumann S, Nemati F, Laurent C, De Koning L, Halilovic E, Jeay S, Wylie A, Emery C, Roman-Roman S, Schoumacher M and Decaudin D (2016) Dual inhibition of protein kinase C and p53-MDM2 or PKC and mTORC1 are novel efficient therapeutic approaches for uveal melanoma. *Oncotarget* **7**:33542-33556.
- Carter BZ, Mak PY, Mak DH, Ruvolo VR, Schober W, McQueen T, Cortes J, Kantarjian HM, Champlin RE, Konopleva M and Andreeff M (2015) Synergistic effects of p53 activation via MDM2 inhibition in combination with inhibition of Bcl-2 or Bcr-Abl in CD34+ proliferating and quiescent chronic myeloid leukemia blast crisis cells. *Oncotarget* **6**:30487-30499.
- Carter BZ, Mak PY, Mu H, Wang X, Tao W, Mak DH, Dettman EJ, Cardone M, Zernovak O, Seki T and Andreeff M (2020) Combined inhibition of MDM2 and BCR-ABL1 tyrosine

- kinase targets chronic myeloid leukemia stem/progenitor cells in a murine model. *Haematologica* **105**:1274-1284.
- Carvajal LA, Neriah DB, Senecal A, Benard L, Thiruthuvanathan V, Yatsenko T, Narayanagari SR, Wheat JC, Todorova TI, Mitchell K, Kenworthy C, Guerlavais V, Annis DA, Bartholdy B, Will B, Anampa JD, Mantzaris I, Aivado M, Singer RH, Coleman RA, Verma A and Steidl U (2018) Dual inhibition of MDMX and MDM2 as a therapeutic strategy in leukemia. *Sci Transl Med* **10**.
- Chang WL, Hsieh CH, Kuo IY, Lin CH, Huang YL and Wang YC (2023) Nutlin-3 acts as a DNA methyltransferase inhibitor to sensitize esophageal cancer to chemoradiation. *Mol Carcinog* **62**:277-287.
- Chang YS, Graves B, Guerlavais V, Tovar C, Packman K, To K-H, Olson KA, Kesavan K, Gangurde P, Mukherjee A, Baker T, Darlak K, Elkin C, Filipovic Z, Qureshi FZ, Cai H, Berry P, Feyfant E, Shi XE, Horstick J, Annis DA, Manning AM, Fotouhi N, Nash H, Vassilev LT and Sawyer TK (2013a) Stapled α -helical peptide drug development: A potent dual inhibitor of MDM2 and MDMX for p53-dependent cancer therapy. *Proceedings of the National Academy of Sciences* **110**:E3445-E3454.
- Chang YS, Graves B, Guerlavais V, Tovar C, Packman K, To KH, Olson KA, Kesavan K, Gangurde P, Mukherjee A, Baker T, Darlak K, Elkin C, Filipovic Z, Qureshi FZ, Cai H, Berry P, Feyfant E, Shi XE, Horstick J, Annis DA, Manning AM, Fotouhi N, Nash H, Vassilev LT and Sawyer TK (2013b) Stapled alpha-helical peptide drug development: a potent dual inhibitor of MDM2 and MDMX for p53-dependent cancer therapy. *Proc Natl Acad Sci U S A* **110**:E3445-3454.
- Chen H, Luo D, Zhang L, Lin X, Luo Q, Yi H, Wang J, Yan X, Li B, Chen Y, Liu X, Zhang H, Liu S, Qiu M, Yang D and Jiang N (2017a) Restoration of p53 using the novel MDM2-p53 antagonist APG115 suppresses dedifferentiated papillary thyroid cancer cells. *Oncotarget* **8**:43008-43022.
- Chen J, Marechal V and Levine AJ (1993) Mapping of the p53 and mdm-2 interaction domains. *Mol Cell Biol* **13**:4107-4114.
- Chen K, Xin X, Qiu L, Li W, Guan G, Li G, Qiao M, Zhao X, Hu H and Chen D (2019a) Co-delivery of p53 and MDM2 inhibitor RG7388 using a hydroxyl terminal PAMAM dendrimer derivative for synergistic cancer therapy. *Acta Biomater* **100**:118-131.
- Chen L, Pastorino F, Berry P, Bonner J, Kirk C, Wood KM, Thomas HD, Zhao Y, Daga A, Veal GJ, Lunec J, Newell DR, Ponzoni M and Tweddle DA (2019b) Preclinical evaluation of the first intravenous small molecule MDM2 antagonist alone and in combination with temozolomide in neuroblastoma. *Int J Cancer* **144**:3146-3159.
- Chen L, Rousseau RF, Middleton SA, Nichols GL, Newell DR, Lunec J and Tweddle DA (2015) Pre-clinical evaluation of the MDM2-p53 antagonist RG7388 alone and in combination with chemotherapy in neuroblastoma. *Oncotarget* **6**:10207-10221.
- Chen Y, Wang DD, Wu YP, Su D, Zhou TY, Gai RH, Fu YY, Zheng L, He QJ, Zhu H and Yang B (2017b) MDM2 promotes epithelial-mesenchymal transition and metastasis of ovarian cancer SKOV3 cells. *Br J Cancer* **117**:1192-1201.
- Chen YL, Zhang ZM, Li XL, Tao YF, Wu SY, Fang F, Xie Y, Liao XM, Li G, Wu D, Wang HR, Zuo R, Cao HB, Pan JJ, Yu JJ, Zhang Z, Chu XR, Zhang YP, Feng CX, Wang JW, Lu J, Hu SY, Li ZH and Pan J (2021) MI-773, a breaker of the MDM2/p53 axis, exhibits anticancer effects in neuroblastoma via downregulation of INSM1. *Oncol Lett* **22**:838.

- Cherif H, Bisson DG, Mannarino M, Rabau O, Ouellet JA and Haglund L (2020) Senotherapeutic drugs for human intervertebral disc degeneration and low back pain. *Elife* **9**.
- Choi YM, An S, Bae S and Jung JH (2019) Mdm2 is required for HDAC3 monoubiquitination and stability. *Biochem Biophys Res Commun* **517**:353-358.
- Chutake Y, Mayo M, Chen D, Enerson B, Cho P, Filiatrault J, Brown C, Placke M, Adams M, Karnik R, Shaw J, Shi Y, Walther D, McDonald A, Qi F, Liu P, Growney JD, Sharma K, Walker D, Schalm S, Williams J and Weiss M (2022) Abstract 3934: KT-253, a highly potent and selective heterobifunctional MDM2 degrader for the treatment of wildtype p53 tumors with superior potency and differentiated biological activity compared to small molecule inhibitors (SMI). *Cancer Research* **82**:3934-3934.
- Ciardullo C, Aptullahoglu E, Woodhouse L, Lin WY, Wallis JP, Marr H, Marshall S, Bown N, Willmore E and Lunec J (2019) Non-genotoxic MDM2 inhibition selectively induces a pro-apoptotic p53 gene signature in chronic lymphocytic leukemia cells. *Haematologica* **104**:2429-2442.
- Cissé MY, Pyrdziak S, Firmin N, Gayte L, Heuillet M, Bellvert F, Fuentes M, Delpech H, Riscal R, Arena G, Chibon F, Le Gellec S, Maran-Gonzalez A, Chateau MC, Theillet C, Carrere S, Portais JC, Le Cam L and Linares LK (2020) Targeting MDM2-dependent serine metabolism as a therapeutic strategy for liposarcoma. *Sci Transl Med* **12**.
- Cornillie J, Wozniak A, Li H, Gebreyohannes YK, Wellens J, Hompes D, Debiec-Rychter M, Sciort R and Schoffski P (2020) Anti-tumor activity of the MDM2-TP53 inhibitor BI-907828 in dedifferentiated liposarcoma patient-derived xenograft models harboring MDM2 amplification. *Clin Transl Oncol* **22**:546-554.
- Cui Y, Zhou J and Rong F (2020) Combination of metformin and RG7388 enhances inhibition of growth and induction of apoptosis of ovarian cancer cells through the PI3K/AKT/mTOR pathway. *Biochem Biophys Res Commun* **533**:665-671.
- Dadone-Montaudie B, Laroche-Clary A, Mongis A, Chamorey E, Mauro ID, Chaire V, Finetti P, Schiappa R, Le Loarer F, Birtwisle-Peyrottes I, Michiels JF, Bertucci F, Pedetour F, Italiano A and Bianchini L (2020) Novel Therapeutic Insights in Dedifferentiated Liposarcoma: A Role for FGFR and MDM2 Dual Targeting. *Cancers (Basel)* **12**.
- Das S (2020) MDM2 Inhibition in a Subset of Sarcoma Cell Lines Increases Susceptibility to Radiation Therapy by Inducing Senescence in the Polyploid Cells. *Adv Radiat Oncol* **5**:250-259.
- Daver NG, Dail M, Garcia JS, Jonas BA, Yee KWL, Kelly KR, Vey N, Assouline S, Roboz GJ, Paolini S, Pollyea DA, Tafuri A, Brandwein JM, Pigneux A, Powell BL, Fenaux P, Olin RL, Visani G, Martinelli G, Onishi M, Wang J, Huang W, Green C, Ott MG, Hong WJ, Konopleva MY and Andreeff M (2023) Venetoclax and idasanutlin in relapsed/refractory AML: a nonrandomized, open-label phase 1b trial. *Blood* **141**:1265-1276.
- de Jonge M, de Weger VA, Dickson MA, Langenberg M, Le Cesne A, Wagner AJ, Hsu K, Zheng W, Mace S, Tuffal G, Thomas K and Schellens JH (2017) A phase I study of SAR405838, a novel human double minute 2 (HDM2) antagonist, in patients with solid tumours. *Eur J Cancer* **76**:144-151.
- de Weger VA, de Jonge M, Langenberg MHG, Schellens JHM, Lolkema M, Varga A, Demers B, Thomas K, Hsu K, Tuffal G, Goodstal S, Mace S and Deutsch E (2019a) A phase I study of the HDM2 antagonist SAR405838 combined with the MEK inhibitor pimasertib in patients with advanced solid tumours. *Br J Cancer* **120**:286-293.

- de Weger VA, de Jonge M, Langenberg MHG, Schellens JHM, Lolkema M, Varga A, Demers B, Thomas K, Hsu K, Tuffal G, Goodstal S, Macé S and Deutsch E (2019b) A phase I study of the HDM2 antagonist SAR405838 combined with the MEK inhibitor pimasertib in patients with advanced solid tumours. *British Journal of Cancer* **120**:286-293.
- Decaudin D, Frisch D, Leitz E, Nemati F, Tarin M, Naguez A, Zerara M, Marande B, Vivet-Noguer R, Halilovic E, Fabre C, Jochemsen A, Roman-Roman S and Alsafadi S (2020) Preclinical evaluation of drug combinations identifies co-inhibition of Bcl-2/XL/W and MDM2 as a potential therapy in uveal melanoma. *Eur J Cancer* **126**:93-103.
- Dickens MP, Roxburgh P, Hock A, Mezna M, Kellam B, Vousden KH and Fischer PM (2013) 5-Deazaflavin derivatives as inhibitors of p53 ubiquitination by HDM2. *Bioorg Med Chem* **21**:6868-6877.
- DiNardo CD, Rosenthal J, Andreeff M, Zernovak O, Kumar P, Gajee R, Chen S, Rosen M, Song S, Kochan J, Limsakun T and Olin R (2016) Phase 1 Dose Escalation Study of MDM2 Inhibitor DS-3032b in Patients with Hematological Malignancies - Preliminary Results. *Blood* **128**:593-593.
- Ding K, Lu Y, Nikolovska-Coleska Z, Qiu S, Ding Y, Gao W, Stuckey J, Krajewski K, Roller PP, Tomita Y, Parrish DA, Deschamps JR and Wang S (2005) Structure-based design of potent non-peptide MDM2 inhibitors. *J Am Chem Soc* **127**:10130-10131.
- Ding Q, Zhang Z, Liu JJ, Jiang N, Zhang J, Ross TM, Chu XJ, Bartkovitz D, Podlaski F, Janson C, Tovar C, Filipovic ZM, Higgins B, Glenn K, Packman K, Vassilev LT and Graves B (2013) Discovery of RG7388, a potent and selective p53-MDM2 inhibitor in clinical development. *J Med Chem* **56**:5979-5983.
- Dobbelstein M and Levine AJ (2020) Mdm2: Open questions. *Cancer Sci* **111**:2203-2211.
- Drew W, Rasco NJL, Yufeng Li, Lichuang Men, Hengbang Wang, Jiao Ji, Yuefen Tang, Zhiyan Liang, Alex Amaya, Kathy Estkowski, Joan Sun, Yingjie Huang, Dajun Yang, Yifan Zhai (2019) A phase I study of a novel MDM2 antagonist APG-115 in patients with advanced solid tumors. *Journal of Clinical Oncology* **37**: 3126-3126.
- Dworakowska D, Jassem E, Jassem J, Peters B, Dziadziuszko R, Zylicz M, Jakobkiewicz-Banecka J, Kobierska-Gulida G, Szymanowska A, Skokowski J, Roessner A and Schneider-Stock R (2004) MDM2 gene amplification: a new independent factor of adverse prognosis in non-small cell lung cancer (NSCLC). *Lung Cancer* **43**:285-295.
- Elkholi R, Abraham-Enachescu I, Trotta AP, Rubio-Patiño C, Mohammed JN, Luna-Vargas MPA, Gelles JD, Kaminetsky JR, Serasinghe MN, Zou C, Ali S, McStay GP, Pfleger CM and Chipuk JE (2019) MDM2 Integrates Cellular Respiration and Apoptotic Signaling through NDUFS1 and the Mitochondrial Network. *Mol Cell* **74**:452-465.e457.
- Erba HP, Becker PS, Shami PJ, Grunwald MR, Flesher DL, Zhu M, Rasmussen E, Henary HA, Anderson AA and Wang ES (2019) Phase 1b study of the MDM2 inhibitor AMG 232 with or without trametinib in relapsed/refractory acute myeloid leukemia. *Blood Adv* **3**:1939-1949.
- F. Meric-Bernstam¹ NS, S.G. DuBois³, E.E. Ileana Dumbrava¹, G.I. Shapiro⁴, M.R. Patel⁵, S. Goel⁶, T. Bauer⁷, D. Pinchasik⁸, A. Annis⁹, M. Aivado¹⁰, V. Vukovic¹¹, M. Saleh¹² (2019) A Phase 2a clinical trial combining ALRN-6924 and palbociclib for the treatment of patients with tumors harboring wild-type p53 and MDM2 amplification or MDM2/CDK4 co-amplification. *Annals of Oncology* **30** (suppl_5):v159-v193.
- Fahraeus R and Olivares-Illana V (2014) MDM2's social network. *Oncogene* **33**:4365-4376.

- Fakharzadeh SS, Trusko SP and George DL (1991) Tumorigenic potential associated with enhanced expression of a gene that is amplified in a mouse tumor cell line. *EMBO J* **10**:1565-1569.
- Fan X, Wang Y, Song J, Wu H, Yang M, Lu L, Weng X, Liu L and Nie G (2019) MDM2 inhibitor RG7388 potently inhibits tumors by activating p53 pathway in nasopharyngeal carcinoma. *Cancer Biol Ther* **20**:1328-1336.
- Fang DD, Tang Q, Kong Y, Rong T, Wang Q, Li N, Fang X, Gu J, Xiong D, Yin Y, Deng J, Yang D and Zhai Y (2021) MDM2 inhibitor APG-115 exerts potent antitumor activity and synergizes with standard-of-care agents in preclinical acute myeloid leukemia models. *Cell Death Discov* **7**:90.
- Fang DD, Tang Q, Kong Y, Wang Q, Gu J, Fang X, Zou P, Rong T, Wang J, Yang D and Zhai Y (2019) MDM2 inhibitor APG-115 synergizes with PD-1 blockade through enhancing antitumor immunity in the tumor microenvironment. *J Immunother Cancer* **7**:327.
- Fang Y, Liao G and Yu B (2020a) Small-molecule MDM2/X inhibitors and PROTAC degraders for cancer therapy: advances and perspectives. *Acta Pharmaceutica Sinica B* **10**:1253-1278.
- Fang Y, Liao G and Yu B (2020b) Small-molecule MDM2/X inhibitors and PROTAC degraders for cancer therapy: advances and perspectives. *Acta Pharm Sin B* **10**:1253-1278.
- Fasan R, Dias RL, Moehle K, Zerbe O, Vrijbloed JW, Obrecht D and Robinson JA (2004) Using a beta-hairpin to mimic an alpha-helix: cyclic peptidomimetic inhibitors of the p53-HDM2 protein-protein interaction. *Angew Chem Int Ed Engl* **43**:2109-2112.
- Feng FY, Zhang Y, Kothari V, Evans JR, Jackson WC, Chen W, Johnson SB, Luczak C, Wang S and Hamstra DA (2016) MDM2 Inhibition Sensitizes Prostate Cancer Cells to Androgen Ablation and Radiotherapy in a p53-Dependent Manner. *Neoplasia* **18**:213-222.
- Feng J, Tamaskovic R, Yang Z, Brazil DP, Merlo A, Hess D and Hemmings BA (2004) Stabilization of Mdm2 via decreased ubiquitination is mediated by protein kinase B/Akt-dependent phosphorylation. *J Biol Chem* **279**:35510-35517.
- Freedman DA, Wu L and Levine AJ (1999) Functions of the MDM2 oncoprotein. *Cell Mol Life Sci* **55**:96-107.
- Fuentes-Antras J, Provencio M and Diaz-Rubio E (2018) Hyperprogression as a distinct outcome after immunotherapy. *Cancer Treat Rev* **70**:16-21.
- Furet P, Masuya K, Kallen J, Stachyra-Valat T, Ruetz S, Guagnano V, Holzer P, Mah R, Stutz S, Vaupel A, Chene P, Jeay S and Schlapbach A (2016) Discovery of a novel class of highly potent inhibitors of the p53-MDM2 interaction by structure-based design starting from a conformational argument. *Bioorg Med Chem Lett* **26**:4837-4841.
- Gadepalli VS, Deb SP, Deb S and Rao RR (2014) Lung cancer stem cells, p53 mutations and MDM2. *Subcell Biochem* **85**:359-370.
- Gannon HS, Woda BA and Jones SN (2012) ATM phosphorylation of Mdm2 Ser394 regulates the amplitude and duration of the DNA damage response in mice. *Cancer Cell* **21**:668-679.
- Garcia-Echeverria C, Chene P, Blommers MJ and Furet P (2000) Discovery of potent antagonists of the interaction between human double minute 2 and tumor suppressor p53. *J Med Chem* **43**:3205-3208.
- Gautam RK, Mittal P, Goyal R, Dua K, Mishra DK, Sharma S and Singla RK (2023) Nanomedicine: Innovative Strategies and Recent Advances in Targeted Cancer Therapy. *Curr Med Chem*.

- Gessier F, Kallen J, Jacoby E, Chene P, Stachyra-Valat T, Ruetz S, Jeay S, Holzer P, Masuya K and Furet P (2015) Discovery of dihydroisoquinolinone derivatives as novel inhibitors of the p53-MDM2 interaction with a distinct binding mode. *Bioorg Med Chem Lett* **25**:3621-3625.
- Gil-Benso R, Monteagudo C, Cerda-Nicolas M, Callaghan RC, Pinto S, Martinez-Romero A, Pellin-Carcelen A, San-Miguel T, Cigudosa JC and Lopez-Gines C (2012) Characterization of a new human melanoma cell line with CD133 expression. *Hum Cell* **25**:61-67.
- Girnita L, Girnita A and Larsson O (2003) Mdm2-dependent ubiquitination and degradation of the insulin-like growth factor 1 receptor. *Proc Natl Acad Sci U S A* **100**:8247-8252.
- Gluck WL, Gounder MM, Frank R, Eskens F, Blay JY, Cassier PA, Soria JC, Chawla S, de Weger V, Wagner AJ, Siegel D, De Vos F, Rasmussen E and Henary HA (2020) Phase 1 study of the MDM2 inhibitor AMG 232 in patients with advanced P53 wild-type solid tumors or multiple myeloma. *Invest New Drugs* **38**:831-843.
- Gollner A, Rudolph D, Arnhof H, Bauer M, Blake SM, Boehmelt G, Cockcroft XL, Dahmann G, Ettmayer P, Gerstberger T, Karolyi-Oezguer J, Kessler D, Kofink C, Ramharter J, Rinnenthal J, Savchenko A, Schnitzer R, Weinstabl H, Weyer-Czernilofsky U, Wunberg T and McConnell DB (2016) Discovery of Novel Spiro[3H-indole-3,2'-pyrrolidin]-2(1H)-one Compounds as Chemically Stable and Orally Active Inhibitors of the MDM2-p53 Interaction. *J Med Chem* **59**:10147-10162.
- Gollner A, Weinstabl H, Fuchs JE, Rudolph D, Garavel G, Hofbauer KS, Karolyi-Oezguer J, Gmaschitz G, Hela W, Kerres N, Grondal E, Werni P, Ramharter J, Broeker J and McConnell DB (2019) Targeted Synthesis of Complex Spiro[3H-indole-3,2'-pyrrolidin]-2(1H)-ones by Intramolecular Cyclization of Azomethine Ylides: Highly Potent MDM2-p53 Inhibitors. *ChemMedChem* **14**:88-93.
- Gounder MM, Bauer TM, Schwartz GK, Weise AM, LoRusso P, Kumar P, Tao B, Hong Y, Patel P, Lu Y, Lesegretain A, Tirunagaru VG, Xu F, Doebele RC and Hong DS (2023) A First-in-Human Phase I Study of Milademetan, an MDM2 Inhibitor, in Patients With Advanced Liposarcoma, Solid Tumors, or Lymphomas. *J Clin Oncol* **41**:1714-1724.
- Grasberger BL, Lu T, Schubert C, Parks DJ, Carver TE, Koblisch HK, Cummings MD, LaFrance LV, Milkiewicz KL, Calvo RR, Maguire D, Lattanze J, Franks CF, Zhao S, Ramachandren K, Bylebyl GR, Zhang M, Manthey CL, Petrella EC, Pantoliano MW, Deckman IC, Spurlino JC, Maroney AC, Tomczuk BE, Molloy CJ and Bone RF (2005) Discovery and cocrystal structure of benzodiazepinedione HDM2 antagonists that activate p53 in cells. *J Med Chem* **48**:909-912.
- Graves B, Thompson T, Xia M, Janson C, Lukacs C, Deo D, Di Lello P, Fry D, Garvie C, Huang KS, Gao L, Tovar C, Lovey A, Wanner J and Vassilev LT (2012) Activation of the p53 pathway by small-molecule-induced MDM2 and MDMX dimerization. *Proc Natl Acad Sci U S A* **109**:11788-11793.
- Grigoreva TA, Novikova DS, Petukhov AV, Gureev MA, Garabadzhiu AV, Melino G, Barlev NA and Tribulovich VG (2017) Proapoptotic modification of substituted isoindolinones as MDM2-p53 inhibitors. *Bioorg Med Chem Lett* **27**:5197-5202.
- Gu L, Findley HW and Zhou M (2002) MDM2 induces NF-kappaB/p65 expression transcriptionally through Sp1-binding sites: a novel, p53-independent role of MDM2 in doxorubicin resistance in acute lymphoblastic leukemia. *Blood* **99**:3367-3375.

- Guo L, Xu Y, Zhou A, Zhang L, Sun L, Gao Y, Chen J, Shan X, Zhang J, Ge J, An X, Liu X, Zhang Y and Ning X (2020) A stimuli-responsive combination therapy for recovering p53-inactivation associated drug resistance. *Mater Sci Eng C Mater Biol Appl* **108**:110403.
- Guo Z, Zhuang C, Zhu L, Zhang Y, Yao J, Dong G, Wang S, Liu Y, Chen H, Sheng C, Miao Z and Zhang W (2012) Structure-activity relationship and antitumor activity of thio-benzodiazepines as p53-MDM2 protein-protein interaction inhibitors. *Eur J Med Chem* **56**:10-16.
- Han Y, Song C, Wang J, Tang H, Peng Z and Lu S (2018) HOXA13 contributes to gastric carcinogenesis through DHRS2 interacting with MDM2 and confers 5-FU resistance by a p53-dependent pathway. *Mol Carcinog* **57**:722-734.
- Hao X, Bahia RK, Cseh O, Bozek DA, Blake S, Rinnenthal J, Weyer-Czernilofsky U, Rudolph D and Artee Luchman H (2023) BI-907828, a novel potent MDM2 inhibitor, inhibits glioblastoma brain tumor stem cells in vitro and prolongs survival in orthotopic xenograft mouse models. *Neuro Oncol* **25**:913-926.
- Hardcastle IR, Ahmed SU, Atkins H, Calvert AH, Curtin NJ, Farnie G, Golding BT, Griffin RJ, Guyenne S, Hutton C, Kallblad P, Kemp SJ, Kitching MS, Newell DR, Norbedo S, Northen JS, Reid RJ, Saravanan K, Willems HM and Lunec J (2005) Isoindolinone-based inhibitors of the MDM2-p53 protein-protein interaction. *Bioorg Med Chem Lett* **15**:1515-1520.
- Haronikova L, Bonczek O, Zatloukalova P, Kokas-Zavadil F, Kucerikova M, Coates PJ, Fahraeus R and Vojtesek B (2021) Resistance mechanisms to inhibitors of p53-MDM2 interactions in cancer therapy: can we overcome them? *Cell Mol Biol Lett* **26**:53.
- Hata AN, Rowley S, Archibald HL, Gomez-Caraballo M, Siddiqui FM, Ji F, Jung J, Light M, Lee JS, Debussche L, Sidhu S, Sadreyev RI, Watters J and Engelman JA (2017) Synergistic activity and heterogeneous acquired resistance of combined MDM2 and MEK inhibition in KRAS mutant cancers. *Oncogene* **36**:6581-6591.
- Hauck PM, Wolf ER, Olivos DJ, 3rd, Batuello CN, McElyea KC, McAtarsney CP, Cournoyer RM, Sandusky GE and Mayo LD (2017) Early-Stage Metastasis Requires Mdm2 and Not p53 Gain of Function. *Mol Cancer Res* **15**:1598-1607.
- Haupt Y, Maya R, Kazaz A and Oren M (1997) Mdm2 promotes the rapid degradation of p53. *Nature* **387**:296-299.
- Hayashi S, Ozaki T, Yoshida K, Hosoda M, Todo S, Akiyama S and Nakagawara A (2006) p73 and MDM2 confer the resistance of epidermoid carcinoma to cisplatin by blocking p53. *Biochem Biophys Res Commun* **347**:60-66.
- He Q, Zou X, Duan D, Liu Y and Xu Q (2016) Malignant transformation of bone marrow stromal cells induced by the brain glioma niche in rats. *Mol Cell Biochem* **412**:1-10.
- He S, Ma J, Fang Y, Liu Y, Wu S, Dong G, Wang W and Sheng C (2021) Homo-PROTAC mediated suicide of MDM2 to treat non-small cell lung cancer. *Acta Pharm Sin B* **11**:1617-1628.
- Her NG, Oh JW, Oh YJ, Han S, Cho HJ, Lee Y, Ryu GH and Nam DH (2018) Potent effect of the MDM2 inhibitor AMG232 on suppression of glioblastoma stem cells. *Cell Death Dis* **9**:792.
- Herman AG, Hayano M, Poyurovsky MV, Shimada K, Skouta R, Prives C and Stockwell BR (2011) Discovery of Mdm2-MdmX E3 ligase inhibitors using a cell-based ubiquitination assay. *Cancer Discov* **1**:312-325.

- Herting F, Friess T, Umana P, Middleton S and Klein C (2018) Chemotherapy-free, triple combination of obinutuzumab, venetoclax and idasanutlin: antitumor activity in xenograft models of non-Hodgkin lymphoma. *Leuk Lymphoma* **59**:1482-1485.
- Herting F, Herter S, Friess T, Muth G, Bacac M, Sulcova J, Umana P, Dangl M and Klein C (2016) Antitumour activity of the glycoengineered type II anti-CD20 antibody obinutuzumab (GA101) in combination with the MDM2-selective antagonist idasanutlin (RG7388). *Eur J Haematol* **97**:461-470.
- Higgins B, Glenn K, Walz A, Tovar C, Filipovic Z, Hussain S, Lee E, Kolinsky K, Tannu S, Adames V, Garrido R, Linn M, Meille C, Heimbrook D, Vassilev L and Packman K (2014) Preclinical optimization of MDM2 antagonist scheduling for cancer treatment by using a model-based approach. *Clin Cancer Res* **20**:3742-3752.
- Ho J, Schmidt D, Lowinus T, Ryoo J, Dopfer EP, Gonzalo Núñez N, Costa-Pereira S, Toffalori C, Punta M, Fetsch V, Wertheimer T, Rittmann MC, Braun LM, Follo M, Briere C, Vinnakota JM, Langenbach M, Koppers F, Shoumariyeh K, Engel H, Rückert T, Märklin M, Holzmayer S, Illert AL, Magon F, Andrieux G, Duquesne S, Pfeifer D, Staniek J, Rizzi M, Miething C, Köhler N, Duyster J, Menssen HD, Boerries M, Buescher JM, Cabezas-Wallscheid N, Blazar BR, Apostolova P, Vago L, Pearce EL, Becher B and Zeiser R (2022) Targeting MDM2 enhances antileukemia immunity after allogeneic transplantation via MHC-II and TRAIL-R1/2 upregulation. *Blood* **140**:1167-1181.
- Holzer P (2017) Discovery of Potent and Selective p53-MDM2 Protein-Protein Interaction Inhibitors as Anticancer Drugs. *Chimia (Aarau)* **71**:716-721.
- Holzer P, Masuya K, Furet P, Kallen J, Valat-Stachyra T, Ferretti S, Berghausen J, Bouisset-Leonard M, Buschmann N, Pissot-Soldermann C, Rynn C, Ruetz S, Stutz S, Chene P, Jeay S and Gessier F (2015) Discovery of a Dihydroisoquinolinone Derivative (NVP-CGM097): A Highly Potent and Selective MDM2 Inhibitor Undergoing Phase 1 Clinical Trials in p53wt Tumors. *J Med Chem* **58**:6348-6358.
- Honda R, Tanaka H and Yasuda H (1997) Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. *FEBS Lett* **420**:25-27.
- Hong Y, Ishizuka T, Watanabe A, Tachibana M, Lee M, Ishizuka H, LaCreta F and Abutarif M (2021) Model-based assessments of CYP3A-mediated drug-drug interaction risk of milademetan. *Clin Transl Sci* **14**:2220-2230.
- Hou H, Sun D and Zhang X (2019) The role of MDM2 amplification and overexpression in therapeutic resistance of malignant tumors. *Cancer Cell Int* **19**:216.
- Hu B, Zhong L, Weng Y, Peng L, Huang Y, Zhao Y and Liang XJ (2020) Therapeutic siRNA: state of the art. *Signal Transduct Target Ther* **5**:101.
- Ingelshed K, Spiegelberg D, Kannan P, Pâvénius L, Hacheney J, Jiang L, Eisinger S, Lianoudaki D, Lama D, Castillo F, Bosdotter C, Kretzschmar WW, Al-Radi O, Fritz N, Villablanca EJ, Karlsson MCI, Wermeling F, Nestor M, Lane DP and Sedimbi SK (2022) The MDM2 Inhibitor Navtemadlin Arrests Mouse Melanoma Growth In Vivo and Potentiates Radiotherapy. *Cancer Res Commun* **2**:1075-1088.
- Ishizawa J, Nakamaru K, Seki T, Tazaki K, Kojima K, Chachad D, Zhao R, Heese L, Ma W, Ma MCJ, DiNardo C, Pierce S, Patel KP, Tse A, Davis RE, Rao A and Andreeff M (2018) Predictive Gene Signatures Determine Tumor Sensitivity to MDM2 Inhibition. *Cancer Res* **78**:2721-2731.

- Issaeva N, Bozko P, Enge M, Protopopova M, Verhoef LG, Masucci M, Pramanik A and Selivanova G (2004) Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumors. *Nat Med* **10**:1321-1328.
- Italiano A, Miller WH, Jr., Blay JY, Gietema JA, Bang YJ, Mileschkin LR, Hirte HW, Higgins B, Blotner S, Nichols GL, Chen LC, Petry C, Yang QJ, Schmitt C, Jamois C and Siu LL (2021) Phase I study of daily and weekly regimens of the orally administered MDM2 antagonist idasanutlin in patients with advanced tumors. *Invest New Drugs* **39**:1587-1597.
- Jain AK and Barton MC (2016) Outside the p53 RING: Transcription Regulation by Chromatin-Bound MDM2. *Mol Cell* **62**:805-807.
- Jeay S, Ferretti S, Holzer P, Fuchs J, Chapeau EA, Wartmann M, Sterker D, Romanet V, Murakami M, Kerr G, Durand EY, Gaulis S, Cortes-Cros M, Ruetz S, Stachyra TM, Kallen J, Furet P, Wurthner J, Guerreiro N, Halilovic E, Jullion A, Kauffmann A, Kuriakose E, Wiesmann M, Jensen MR, Hofmann F and Sellers WR (2018) Dose and Schedule Determine Distinct Molecular Mechanisms Underlying the Efficacy of the p53-MDM2 Inhibitor HDM201. *Cancer Res* **78**:6257-6267.
- Jeay S, Gaulis S, Ferretti S, Bitter H, Ito M, Valat T, Murakami M, Ruetz S, Guthy DA, Rynn C, Jensen MR, Wiesmann M, Kallen J, Furet P, Gessier F, Holzer P, Masuya K, Wurthner J, Halilovic E, Hofmann F, Sellers WR and Graus Porta D (2015) A distinct p53 target gene set predicts for response to the selective p53-HDM2 inhibitor NVP-CGM097. *Elife* **4**.
- Jiang L, Malik N, Acedo P and Zawacka-Pankau J (2019) Protoporphyrin IX is a dual inhibitor of p53/MDM2 and p53/MDM4 interactions and induces apoptosis in B-cell chronic lymphocytic leukemia cells. *Cell Death Discov* **5**:77.
- Jones SN, Hancock AR, Vogel H, Donehower LA and Bradley A (1998) Overexpression of Mdm2 in mice reveals a p53-independent role for Mdm2 in tumorigenesis. *Proc Natl Acad Sci U S A* **95**:15608-15612.
- Kawata Y, Nagasaka K, Oda K, Makii C, Takeuchi M, Oki S, Honjo H, Kojima M, Miyagawa Y, Taguchi A, Tanikawa M, Sone K, Hiraike H, Matsumoto Y, Wada-Hiraike O, Ayabe T, Osuga Y and Fujii T (2020) Effect of murine double-minute 2 inhibitors in preclinical models of advanced clear cell carcinomas originating from ovaries and kidneys. *Cancer Sci* **111**:3824-3834.
- Kim M, Laramy JK, Gampa G, Parrish KE, Brundage R, Sarkaria JN and Elmquist WF (2019) Brain Distributional Kinetics of a Novel MDM2 Inhibitor SAR405838: Implications for Use in Brain Tumor Therapy. *Drug Metab Dispos* **47**:1403-1414.
- Klein AM, de Queiroz RM, Venkatesh D and Prives C (2021) The roles and regulation of MDM2 and MDMX: it is not just about p53. *Genes Dev* **35**:575-601.
- Koblish HK, Zhao S, Franks CF, Donatelli RR, Tominovich RM, LaFrance LV, Leonard KA, Gushue JM, Parks DJ, Calvo RR, Milkiewicz KL, Marugan JJ, Raboisson P, Cummings MD, Grasberger BL, Johnson DL, Lu T, Molloy CJ and Maroney AC (2006) Benzodiazepinedione inhibitors of the Hdm2:p53 complex suppress human tumor cell proliferation in vitro and sensitize tumors to doxorubicin in vivo. *Mol Cancer Ther* **5**:160-169.
- Kondo S, Barnett GH, Hara H, Morimura T and Takeuchi J (1995) MDM2 protein confers the resistance of a human glioblastoma cell line to cisplatin-induced apoptosis. *Oncogene* **10**:2001-2006.
- Kong LZ, Kim SM, Wang C, Lee SY, Oh SC, Lee S, Jo S and Kim TD (2023) Understanding nucleic acid sensing and its therapeutic applications. *Exp Mol Med*.

- Kono M, Kumai T, Hayashi R, Yamaki H, Komatsuda H, Wakisaka R, Nagato T, Ohkuri T, Kosaka A, Ohara K, Kishibe K, Takahara M, Katada A, Hayashi T, Celis E, Kobayashi H and Harabuchi Y (2021) Interruption of MDM2 signaling augments MDM2-targeted T cell-based antitumor immunotherapy through antigen-presenting machinery. *Cancer Immunol Immunother* **70**:3421-3434.
- Konopleva M, Martinelli G, Daver N, Papayannidis C, Wei A, Higgins B, Ott M, Mascarenhas J and Andreeff M (2020) MDM2 inhibition: an important step forward in cancer therapy. *Leukemia* **34**:2858-2874.
- Konopleva MY, Rollig C, Cavenagh J, Deeren D, Girshova L, Krauter J, Martinelli G, Montesinos P, Schafer JA, Ottmann O, Petrini M, Pigneux A, Rambaldi A, Recher C, Rodriguez-Veiga R, Taussig D, Vey N, Yoon SS, Ott M, Muehlbauer S, Beckermann BM, Catalani O, Genevray M, Mundt K, Jamois C, Fenaux P and Wei AH (2022) Idasanutlin plus cytarabine in relapsed or refractory acute myeloid leukemia: results of the MIRROS trial. *Blood Adv* **6**:4147-4156.
- Kubbutat MH, Jones SN and Vousden KH (1997) Regulation of p53 stability by Mdm2. *Nature* **387**:299-303.
- Kussie PH, Gorina S, Marechal V, Elenbaas B, Moreau J, Levine AJ and Pavletich NP (1996) Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. *Science* **274**:948-953.
- Lane N, Hsu B, Visich J, Xie B, Khan A and Dananberg J (APRIL 2021) A phase 2, randomized, double-blind, placebo-controlled study of senolytic molecule UBX0101 in the treatment of painful knee osteoarthritis. *Osteoarthritis and Cartilage* **29**:S52-S53.
- Langenbach M, Giesler S, Richtsfeld S, Costa-Pereira S, Rindlisbacher L, Wertheimer T, Braun LM, Andrieux G, Duquesne S, Pfeifer D, Woessner NM, Menssen HD, Taromi S, Duyster J, B rries M, Brummer T, Blazar BR, Minguet S, Turko P, Levesque MP, Becher B and Zeiser R (2023) MDM2 Inhibition Enhances Immune Checkpoint Inhibitor Efficacy by Increasing IL15 and MHC Class II Production. *Mol Cancer Res* **21**:849-864.
- Lehmann C, Friess T, Birzele F, Kiialainen A and Dangl M (2016) Superior anti-tumor activity of the MDM2 antagonist idasanutlin and the Bcl-2 inhibitor venetoclax in p53 wild-type acute myeloid leukemia models. *J Hematol Oncol* **9**:50.
- Leslie PL, Ke H and Zhang Y (2015) The MDM2 RING Domain and Central Acidic Domain Play Distinct Roles in MDM2 Protein Homodimerization and MDM2-MDMX Protein Heterodimerization*. *Journal of Biological Chemistry* **290**:12941-12950.
- Levine AJ (2020) p53: 800 million years of evolution and 40 years of discovery. *Nat Rev Cancer* **20**:471-480.
- Li F, Chen D, Sun Q, Wu J, Gan Y, Leong KW and Liang XJ (2023) MDM2-Targeting Reassembly Peptide (TRAP) Nanoparticles for p53-Based Cancer Therapy. *Adv Mater* **35**:e2305164.
- Li J, Zhang S, Gao L, Chen Y and Xie X (2011) A cell-based high-throughput assay for the screening of small-molecule inhibitors of p53-MDM2 interaction. *J Biomol Screen* **16**:450-456.
- Li R, Zatloukalova P, Muller P, Gil-Mir M, Kote S, Wilkinson S, Kemp AJ, Hernychova L, Wang Y, Ball KL, Tao K, Hupp T and Vojtesek B (2020a) The MDM2 ligand Nutlin-3 differentially alters expression of the immune blockade receptors PD-L1 and CD276. *Cell Mol Biol Lett* **25**:41.

- Li W, Peng X, Lang J and Xu C (2020b) Targeting Mouse Double Minute 2: Current Concepts in DNA Damage Repair and Therapeutic Approaches in Cancer. *Front Pharmacol* **11**:631.
- Li Y, Stockton ME, Bhuiyan I, Eisinger BE, Gao Y, Miller JL, Bhattacharyya A and Zhao X (2016) MDM2 inhibition rescues neurogenic and cognitive deficits in a mouse model of fragile X syndrome. *Sci Transl Med* **8**:336ra361.
- Li Y, Wang Z, Ajani JA and Song S (2021) Drug resistance and Cancer stem cells. *Cell Commun Signal* **19**:19.
- Li Y, Yang J, Aguilar A, McEachern D, Przybranowski S, Liu L, Yang CY, Wang M, Han X and Wang S (2019) Discovery of MD-224 as a First-in-Class, Highly Potent, and Efficacious Proteolysis Targeting Chimera Murine Double Minute 2 Degradable Capable of Achieving Complete and Durable Tumor Regression. *J Med Chem* **62**:448-466.
- Liu Y, Wang X, Wang G, Yang Y, Yuan Y and Ouyang L (2019) The past, present and future of potential small-molecule drugs targeting p53-MDM2/MDMX for cancer therapy. *Eur J Med Chem* **176**:92-104.
- LoRusso P, Yamamoto N, Patel MR, Laurie SA, Bauer TM, Geng J, Davenport T, Teufel M, Li J, Lahmar M and Gounder MM (2023) The MDM2–p53 Antagonist Brigimadlin (BI 907828) in Patients with Advanced or Metastatic Solid Tumors: Results of a Phase Ia, First-in-Human, Dose-Escalation Study. *Cancer Discovery* **13**:1802-1813.
- Lu J, Guan S, Zhao Y, Yu Y, Wang Y, Shi Y, Mao X, Yang KL, Sun W, Xu X, Yi JS, Yang T, Yang J and Nuchtern JG (2016a) Novel MDM2 inhibitor SAR405838 (MI-773) induces p53-mediated apoptosis in neuroblastoma. *Oncotarget* **7**:82757-82769.
- Lu J, McEachern D, Li S, Ellis MJ and Wang S (2016b) Reactivation of p53 by MDM2 Inhibitor MI-77301 for the Treatment of Endocrine-Resistant Breast Cancer. *Mol Cancer Ther* **15**:2887-2893.
- Lundgren K, Montes de Oca Luna R, McNeill YB, Emerick EP, Spencer B, Barfield CR, Lozano G, Rosenberg MP and Finlay CA (1997) Targeted expression of MDM2 uncouples S phase from mitosis and inhibits mammary gland development independent of p53. *Genes Dev* **11**:714-725.
- Luo H, Yount C, Lang H, Yang A, Riemer EC, Lyons K, Vanek KN, Silvestri GA, Schulte BA and Wang GY (2013) Activation of p53 with Nutlin-3a radiosensitizes lung cancer cells via enhancing radiation-induced premature senescence. *Lung Cancer* **81**:167-173.
- Ma B, Niu F, Qu X, He W, Feng C, Wang S, Ouyang Z, Yan J, Wen Y, Xu D, Shao Y, Ma PX and Lu W (2019) A tetrameric protein scaffold as a nano-carrier of antitumor peptides for cancer therapy. *Biomaterials* **204**:1-12.
- Ma Y, Lahue BR, Gibeau CR, Shipps GW, Jr., Bogen SL, Wang Y, Guo Z and Guzi TJ (2014a) Pivotal Role of an Aliphatic Side Chain in the Development of an HDM2 Inhibitor. *ACS Med Chem Lett* **5**:572-575.
- Ma Y, Lahue BR, Shipps GW, Jr., Brookes J and Wang Y (2014b) Substituted piperidines as HDM2 inhibitors. *Bioorg Med Chem Lett* **24**:1026-1030.
- Ma Y, Yuan R, Meng Q, Goldberg ID, Rosen EM and Fan S (2000) P53-independent down-regulation of Mdm2 in human cancer cells treated with adriamycin. *Mol Cell Biol Res Commun* **3**:122-128.
- Maganti HB, Jrade H, Cafariello C, Manias Rothberg JL, Porter CJ, Yockell-Lelievre J, Battaion HL, Khan ST, Howard JP, Li Y, Grzybowski AT, Sabri E, Ruthenburg AJ, Dilworth FJ, Perkins TJ, Sabloff M, Ito CY and Stanford WL (2018) Targeting the MTF2-MDM2

- Axis Sensitizes Refractory Acute Myeloid Leukemia to Chemotherapy. *Cancer Discov* **8**:1376-1389.
- Makii C, Ikeda Y, Oda K, Uehara Y, Nishijima A, Koso T, Kawata Y, Kashiyama T, Miyasaka A, Sone K, Tanikawa M, Tsuruga T, Mori-Uchino M, Nagasaka K, Matsumoto Y, Wada-Hiraike O, Kawana K, Hasegawa K, Fujiwara K, Aburatani H, Osuga Y and Fujii T (2019) Anti-tumor activity of dual inhibition of phosphatidylinositol 3-kinase and MDM2 against clear cell ovarian carcinoma. *Gynecol Oncol* **155**:331-339.
- Makii C, Oda K, Ikeda Y, Sone K, Hasegawa K, Uehara Y, Nishijima A, Asada K, Koso T, Fukuda T, Inaba K, Oki S, Machino H, Kojima M, Kashiyama T, Mori-Uchino M, Arimoto T, Wada-Hiraike O, Kawana K, Yano T, Fujiwara K, Aburatani H, Osuga Y and Fujii T (2016) MDM2 is a potential therapeutic target and prognostic factor for ovarian clear cell carcinomas with wild type TP53. *Oncotarget* **7**:75328-75338.
- Manfredi JJ (2021) Mdm2 and MdmX: Partners in p53 Destruction. *Cancer Res* **81**:1633-1634.
- Marcellino BK, Yang X, Ümit Kaniskan H, Brady C, Chen H, Chen K, Qiu X, Clementelli C, Herschbein L, Li Z, Elghaity-Beckley S, Arandela J, Kelly B, Hoffman R, Liu J, Xiong Y, Jin J and Shih AH (2023) An MDM2 degrader for treatment of acute leukemias. *Leukemia* **37**:370-378.
- Marine JC, Francoz S, Maetens M, Wahl G, Toledo F and Lozano G (2006) Keeping p53 in check: essential and synergistic functions of Mdm2 and Mdm4. *Cell Death & Differentiation* **13**:927-934.
- Mascarenhas J, Passamonti F, Burbury K, El-Galaly TC, Gerds A, Gupta V, Higgins B, Wonde K, Jamois C, Kovic B, Huw LY, Katakam S, Maffioli M, Mesa R, Palmer J, Bellini M, Ross DM, Vannucchi AM and Yacoub A (2022) The MDM2 antagonist idasanutlin in patients with polycythemia vera: results from a single-arm phase 2 study. *Blood Adv* **6**:1162-1174.
- Maser T, Zagorski J, Kelly S, Ostrander A, Goodyke A, Nagulapally A, Bond J, Park Y and Saulnier Sholler G (2020) The MDM2 inhibitor CGM097 combined with the BET inhibitor OTX015 induces cell death and inhibits tumor growth in models of neuroblastoma. *Cancer Med* **9**:8144-8158.
- Mayo M, Chutake Y, Karnik R, McDonald A, Cho PS, Filiatrault J, Chen D, Dixit V, Proctor W, Breitkopf S, Qi F, Hu K-N, Sharma K, Ewesuedo R, Weiss M, Growney JD, Williams J and Schalm SS (2022) Development of KT-253, a Highly Potent and Selective Heterobifunctional MDM2 Degradar for the Treatment of Acute Myeloid Leukemia. *Blood* **140**:6239-6240.
- Mayr C, Bund D, Schlee M, Bamberger M, Kofler DM, Hallek M and Wendtner CM (2006) MDM2 is recognized as a tumor-associated antigen in chronic lymphocytic leukemia by CD8+ autologous T lymphocytes. *Exp Hematol* **34**:44-53.
- Meek DW and Knippschild U (2003) Posttranslational modification of MDM2. *Mol Cancer Res* **1**:1017-1026.
- Minsky N and Oren M (2004) The RING domain of Mdm2 mediates histone ubiquitylation and transcriptional repression. *Mol Cell* **16**:631-639.
- Mladek AC GS, Kim M, Mohammad AS, Bakken KK, He H, Hu Z, Burgenske DM, Carlson BL, Elmquist WF, and Sarkaria J. (2019) MDM2 inhibitor KRT-232 extends survival in glioblastoma patient-derived xenograft models. *Mol Cancer Ther* **18**:C051.
- Momand J, Jung D, Wilczynski S and Niland J (1998) The MDM2 gene amplification database. *Nucleic Acids Res* **26**:3453-3459.

- Momand J, Zambetti GP, Olson DC, George D and Levine AJ (1992) The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* **69**:1237-1245.
- Moreira J, Almeida J, Saraiva L, Cidade H and Pinto M (2021) Chalcones as Promising Antitumor Agents by Targeting the p53 Pathway: An Overview and New Insights in Drug-Likeness. *Molecules* **26**.
- Moschos SJ, Sandhu S, Lewis KD, Sullivan RJ, Puzanov I, Johnson DB, Henary HA, Wong H, Upreti VV, Long GV and Flaherty KT (2022) Targeting wild-type TP53 using AMG 232 in combination with MAPK inhibition in Metastatic Melanoma; a phase 1 study. *Invest New Drugs* **40**:1051-1065.
- Mullard A (2021) Targeted protein degraders crowd into the clinic. *Nat Rev Drug Discov* **20**:247-250.
- Munisamy M, Mukherjee N, Thomas L, Pham AT, Shakeri A, Zhao Y, Kolesar J, Rao PPN, Rangnekar VM and Rao M (2021) Therapeutic opportunities in cancer therapy: targeting the p53-MDM2/MDMX interactions. *Am J Cancer Res* **11**:5762-5781.
- Nag S, Qin J, Srivenugopal KS, Wang M and Zhang R (2013) The MDM2-p53 pathway revisited. *J Biomed Res* **27**:254-271.
- Ng SY, Yoshida N, Christie AL, Ghandi M, Dharia NV, Dempster J, Murakami M, Shigemori K, Morrow SN, Van Scoyk A, Cordero NA, Stevenson KE, Puligandla M, Haas B, Lo C, Meyers R, Gao G, Cherniack A, Louissaint A, Jr., Nardi V, Thorner AR, Long H, Qiu X, Morgan EA, Dorfman DM, Fiore D, Jang J, Epstein AL, Dogan A, Zhang Y, Horwitz SM, Jacobsen ED, Santiago S, Ren JG, Guerlavais V, Annis DA, Aivado M, Saleh MN, Mehta A, Tsherniak A, Root D, Vazquez F, Hahn WC, Inghirami G, Aster JC, Weinstock DM and Koch R (2018) Targetable vulnerabilities in T- and NK-cell lymphomas identified through preclinical models. *Nat Commun* **9**:2024.
- Niemantsverdriet M, Schuurin E, Elst AT, van der Wekken AJ, van Kempen LC, van den Berg A and Groen HJM (2018) KRAS Mutation as a Resistance Mechanism to BRAF/MEK Inhibition in NSCLC. *J Thorac Oncol* **13**:e249-e251.
- Nishikawa S and Iwakuma T (2023) Drugs Targeting p53 Mutations with FDA Approval and in Clinical Trials. *Cancers (Basel)* **15**.
- Nor F, Warner KA, Zhang Z, Acasigua GA, Pearson AT, Kerk SA, Helman JI, Sant'Ana Filho M, Wang S and Nor JE (2017) Therapeutic Inhibition of the MDM2-p53 Interaction Prevents Recurrence of Adenoid Cystic Carcinomas. *Clin Cancer Res* **23**:1036-1048.
- Obrador-Hevia A, Martinez-Font E, Felipe-Abrio I, Calabuig-Farinas S, Serra-Sitjar M, Lopez-Guerrero JA, Ramos R, Alemany R and Martin-Broto J (2015) RG7112, a Small-Molecule Inhibitor of MDM2, Enhances Trabectedin Response in Soft Tissue Sarcomas. *Cancer Invest.*
- Okamoto H, Fujishima F, Nakamura Y, Zuguchi M, Miyata G, Kamei T, Nakano T, Katsura K, Abe S, Taniyama Y, Teshima J, Watanabe M, Sato A, Ohuchi N and Sasano H (2013) Murine double minute 2 and its association with chemoradioresistance of esophageal squamous cell carcinoma. *Anticancer Res* **33**:1463-1471.
- Okoro DR, Rosso M and Bargonetti J (2012) Splicing up mdm2 for cancer proteome diversity. *Genes Cancer* **3**:311-319.
- Oliner JD, Kinzler KW, Meltzer PS, George DL and Vogelstein B (1992) Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature* **358**:80-83.

- Oliner JD, Saiki AY and Caenepeel S (2016) The Role of MDM2 Amplification and Overexpression in Tumorigenesis. *Cold Spring Harb Perspect Med* **6**.
- Onel K and Cordon-Cardo C (2004) MDM2 and prognosis. *Mol Cancer Res* **2**:1-8.
- Ou M, Xu X, Chen Y, Li L, Zhang L, Liao Y, Sun W, Quach C, Feng J and Tang L (2021) MDM2 induces EMT via the BRAf signaling pathway through 1433. *Oncol Rep* **46**.
- Pairawan S, Zhao M, Yuca E, Annis A, Evans K, Sutton D, Carvajal L, Ren JG, Santiago S, Guerlavais V, Akcakanat A, Tapia C, Yang F, Bose PSC, Zheng X, Dumbrava EI, Aivado M and Meric-Bernstam F (2021) First in class dual MDM2/MDMX inhibitor ALRN-6924 enhances antitumor efficacy of chemotherapy in TP53 wild-type hormone receptor-positive breast cancer models. *Breast Cancer Res* **23**:29.
- Pan R, Ruvolo V, Mu H, Levenson JD, Nichols G, Reed JC, Konopleva M and Andreeff M (2017) Synthetic Lethality of Combined Bcl-2 Inhibition and p53 Activation in AML: Mechanisms and Superior Antileukemic Efficacy. *Cancer Cell* **32**:748-760 e746.
- Papai Z, Chen LC, Da Costa D, Blotner S, Vazvaei F, Gleave M, Jones R and Zhi J (2019) A single-center, open-label study investigating the excretion balance, pharmacokinetics, metabolism, and absolute bioavailability of a single oral dose of [(14)C]-labeled idasanutlin and an intravenous tracer dose of [(13)C]-labeled idasanutlin in a single cohort of patients with solid tumors. *Cancer Chemother Pharmacol* **84**:93-103.
- Patil SA, Addo JK, Deokar H, Sun S, Wang J, Li W, Suttle DP, Wang W, Zhang R and Buolamwini JK (2017) Synthesis, Biological Evaluation and Modeling Studies of New Pyrido[3,4-b]indole Derivatives as Broad-Spectrum Potent Anticancer Agents. *Drug Des* **6**.
- Patnaik A, Tolcher A, Beeram M, Nemunaitis J, Weiss GJ, Bhalla K, Agrawal M, Nichols G, Middleton S, Beryozkina A, Sarapa N, Peck R and Zhi J (2015) Clinical pharmacology characterization of RG7112, an MDM2 antagonist, in patients with advanced solid tumors. *Cancer Chemother Pharmacol* **76**:587-595.
- Perry ME (2004) Mdm2 in the response to radiation. *Mol Cancer Res* **2**:9-19.
- Peterson LF, Lo MC, Liu Y, Giannola D, Mitrikeska E, Donato NJ, Johnson CN, Wang S, Mercer J and Talpaz M (2017) Induction of p53 suppresses chronic myeloid leukemia. *Leuk Lymphoma* **58**:1-14.
- Peterson LF, Mitrikeska E, Giannola D, Lui Y, Sun H, Bixby D, Malek SN, Donato NJ, Wang S and Talpaz M (2011) p53 stabilization induces apoptosis in chronic myeloid leukemia blast crisis cells. *Leukemia* **25**:761-769.
- Popowicz GM, Czarna A, Wolf S, Wang K, Wang W, Domling A and Holak TA (2010) Structures of low molecular weight inhibitors bound to MDMX and MDM2 reveal new approaches for p53-MDMX/MDM2 antagonist drug discovery. *Cell Cycle* **9**:1104-1111.
- Portman N, Milioli HH, Alexandrou S, Coulson R, Yong A, Fernandez KJ, Chia KM, Halilovic E, Segara D, Parker A, Haupt S, Haupt Y, Tilley WD, Swarbrick A, Caldon CE and Lim E (2020) MDM2 inhibition in combination with endocrine therapy and CDK4/6 inhibition for the treatment of ER-positive breast cancer. *Breast Cancer Res* **22**:87.
- Prabakaran PJ, Javai AM, Swick AD, Werner LR, Nickel KP, Sampene E, Hu R, Ong IM, Bruce JY, Hartig GK, Wieland AM, Canon J, Harari PM and Kimple RJ (2017) Radiosensitization of Adenoid Cystic Carcinoma with MDM2 Inhibition. *Clin Cancer Res* **23**:6044-6053.

- Qin JJ, Wang W, Sarkar S and Zhang R (2016) Oral delivery of anti-MDM2 inhibitor SP141-loaded FcRn-targeted nanoparticles to treat breast cancer and metastasis. *J Control Release* **237**:101-114.
- Raboisson P, Marugan JJ, Schubert C, Koblish HK, Lu T, Zhao S, Player MR, Maroney AC, Reed RL, Huebert ND, Lattanze J, Parks DJ and Cummings MD (2005) Structure-based design, synthesis, and biological evaluation of novel 1,4-diazepines as HDM2 antagonists. *Bioorg Med Chem Lett* **15**:1857-1861.
- Ramsay RR, Popovic-Nikolic MR, Nikolic K, Uliassi E and Bolognesi ML (2018) A perspective on multi-target drug discovery and design for complex diseases. *Clin Transl Med* **7**:3.
- Ray-Coquard I, Blay JY, Italiano A, Le Cesne A, Penel N, Zhi J, Heil F, Rueger R, Graves B, Ding M, Geho D, Middleton SA, Vassilev LT, Nichols GL and Bui BN (2012) Effect of the MDM2 antagonist RG7112 on the P53 pathway in patients with MDM2-amplified, well-differentiated or dedifferentiated liposarcoma: an exploratory proof-of-mechanism study. *Lancet Oncol* **13**:1133-1140.
- Reutershan MH, Machacek MR, Altman MD, Bogen S, Cai M, Cammarano C, Chen D, Christopher M, Cryan J, Daublain P, Fradera X, Geda P, Goldenblatt P, Hill AD, Kemper RA, Kutilek V, Li C, Martinez M, McCoy M, Nair L, Pan W, Thompson CF, Scapin G, Shizuka M, Spatz ML, Steinhuebel D, Sun B, Voss ME, Wang X, Yang L, Yeh TC, Dussault I, Marshall CG and Trotter BW (2021) Discovery of MK-4688: an Efficient Inhibitor of the HDM2-p53 Protein-Protein Interaction. *J Med Chem* **64**:16213-16241.
- Rew Y and Sun D (2014) Discovery of a small molecule MDM2 inhibitor (AMG 232) for treating cancer. *J Med Chem* **57**:6332-6341.
- Rew Y, Sun D, Gonzalez-Lopez De Turiso F, Bartberger MD, Beck HP, Canon J, Chen A, Chow D, Deignan J, Fox BM, Gustin D, Huang X, Jiang M, Jiao X, Jin L, Kayser F, Kopecky DJ, Li Y, Lo MC, Long AM, Michelsen K, Oliner JD, Osgood T, Ragains M, Saiki AY, Schneider S, Toteva M, Yakowec P, Yan X, Ye Q, Yu D, Zhao X, Zhou J, Medina JC and Olson SH (2012) Structure-based design of novel inhibitors of the MDM2-p53 interaction. *J Med Chem* **55**:4936-4954.
- Richmond J, Carol H, Evans K, High L, Mendo A, Robbins A, Meyer C, Venn NC, Marschalek R, Henderson M, Sutton R, Kurmasheva RT, Kees UR, Houghton PJ, Smith MA and Lock RB (2015) Effective targeting of the P53-MDM2 axis in preclinical models of infant MLL-rearranged acute lymphoblastic leukemia. *Clin Cancer Res* **21**:1395-1405.
- Riedinger C, Noble ME, Wright DJ, Mulks F, Hardcastle IR, Endicott JA and McDonnell JM (2011) Understanding small-molecule binding to MDM2: insights into structural effects of isoindolinone inhibitors from NMR spectroscopy. *Chem Biol Drug Des* **77**:301-308.
- Ringshausen I, O'Shea CC, Finch AJ, Swigart LB and Evan GI (2006) Mdm2 is critically and continuously required to suppress lethal p53 activity in vivo. *Cancer Cell* **10**:501-514.
- Riscal R, Schrepfer E, Arena G, Cissé MY, Bellvert F, Heuillet M, Rambow F, Bonneil E, Sabourdy F, Vincent C, Ait-Arsa I, Levade T, Thibaut P, Marine JC, Portais JC, Sarry JE, Le Cam L and Linares LK (2016) Chromatin-Bound MDM2 Regulates Serine Metabolism and Redox Homeostasis Independently of p53. *Mol Cell* **62**:890-902.
- Rivera MI, Stinson SF, Vistica DT, Jorden JL, Kenney S and Sausville EA (1999) Selective toxicity of the tricyclic thiophene NSC 652287 in renal carcinoma cell lines: differential accumulation and metabolism. *Biochem Pharmacol* **57**:1283-1295.

- Roy S, Laroche-Clary A, Verbeke S, Derieppe MA and Italiano A (2020) MDM2 Antagonists Induce a Paradoxical Activation of Erk1/2 through a P53-Dependent Mechanism in Dedifferentiated Liposarcomas: Implications for Combinatorial Strategies. *Cancers (Basel)* **12**.
- Rusiecki R, Witkowski J and Jaszczewska-Adamczak J (2019) MDM2-p53 Interaction Inhibitors: The Current State-of-Art and Updated Patent Review (2010-Present). *Recent Pat Anticancer Drug Discov* **14**:324-369.
- Sahin I, Zhang S, Navaraj A, Zhou L, Dizon D, Safran H and El-Deiry WS (2020) AMG-232 sensitizes high MDM2-expressing tumor cells to T-cell-mediated killing. *Cell Death Discov* **6**:57.
- Sakamoto KM, Kim KB, Kumagai A, Mercurio F, Crews CM and Deshaies RJ (2001) Protacs: chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. *Proc Natl Acad Sci U S A* **98**:8554-8559.
- Sakurai K, Chung HS and Kahne D (2004) Use of a retroinverso p53 peptide as an inhibitor of MDM2. *J Am Chem Soc* **126**:16288-16289.
- Saleh MN, Patel MR, Bauer TM, Goel S, Falchook GS, Shapiro GI, Chung KY, Infante JR, Conry RM, Rabinowits G, Hong DS, Wang JS, Steidl U, Naik G, Guerlavais V, Vukovic V, Annis DA, Aivado M and Meric-Bernstam F (2021) Phase 1 Trial of ALRN-6924, a Dual Inhibitor of MDMX and MDM2, in Patients with Solid Tumors and Lymphomas Bearing Wild-Type TP53. *Clin Cancer Res*.
- Sant P, Chawla J-YB, Antoine Italiano, Martin Gutierrez, Axel Le Cesne, Carlos Alberto Gomez-Roca, Launce G. Gouw, Margaret von Mehren, Andrew Wagner, Robert G. Maki, Brian Higgins, Steven Middleton, Gwen L. Nichols, David Geho, Steven Blotner, Jianguo Zhi, Lin Chi Chen (2013) Phase Ib study of RG7112 with doxorubicin (D) in advanced soft tissue sarcoma (ASTS). *Journal of Clinical Oncology* **31**: 10514-10514.
- Sato A, Sunayama J, Matsuda K, Seino S, Suzuki K, Watanabe E, Tachibana K, Tomiyama A, Kayama T and Kitanaka C (2011) MEK-ERK signaling dictates DNA-repair gene MGMT expression and temozolomide resistance of stem-like glioblastoma cells via the MDM2-p53 axis. *Stem Cells* **29**:1942-1951.
- Sawyer TK, Partridge AW, Kaan HYK, Juang YC, Lim S, Johannes C, Yuen TY, Verma C, Kannan S, Aronica P, Tan YS, Sherborne B, Ha S, Hochman J, Chen S, Surdi L, Peier A, Sauvagnat B, Dandliker PJ, Brown CJ, Ng S, Ferrer F and Lane DP (2018) Macrocyclic alpha helical peptide therapeutic modality: A perspective of learnings and challenges. *Bioorg Med Chem* **26**:2807-2815.
- Schoffski P, Lahmar M, Lucarelli A and Maki RG (2023) Brightline-1: phase II/III trial of the MDM2-p53 antagonist BI 907828 versus doxorubicin in patients with advanced DDLPS. *Future Oncol* **19**:621-629.
- Seipel K, Marques MAT, Sidler C, Mueller BU and Pabst T (2018) MDM2- and FLT3-inhibitors in the treatment of FLT3-ITD acute myeloid leukemia, specificity and efficacy of NVP-HDM201 and midostaurin. *Haematologica* **103**:1862-1872.
- Sekiguchi N, Kasahara S, Miyamoto T, Kiguchi T, Ohno H, Takagi T, Tachibana M, Sumi H, Kakurai Y, Yamashita T and Usuki K (2023) Phase I dose-escalation study of milademetan in patients with relapsed or refractory acute myeloid leukemia. *Int J Hematol* **117**:68-77.

- Senapati J, Muftuoglu M, Ishizawa J, Abbas HA, Loghavi S, Borthakur G, Yilmaz M, Issa GC, Dara SI, Basyal M, Li L, Naqvi K, Pourebrahim R, Jabbour EJ, Kornblau SM, Short NJ, Pemmaraju N, Garcia-Manero G, Ravandi F, Khoury J, Daver N, Kantarjian HM, Andreeff M and DiNardo CD (2023) A Phase I study of Milademetan (DS3032b) in combination with low dose cytarabine with or without venetoclax in acute myeloid leukemia: Clinical safety, efficacy, and correlative analysis. *Blood Cancer J* **13**:101.
- Shaikh MF, Morano WF, Lee J, Gleeson E, Babcock BD, Michl J, Sarafraz-Yazdi E, Pincus MR and Bowne WB (2016) Emerging Role of MDM2 as Target for Anti-Cancer Therapy: A Review. *Ann Clin Lab Sci* **46**:627-634.
- Shangary S and Wang S (2009) Small-molecule inhibitors of the MDM2-p53 protein-protein interaction to reactivate p53 function: a novel approach for cancer therapy. *Annu Rev Pharmacol Toxicol* **49**:223-241.
- Shattuck-Brandt RL, Chen SC, Murray E, Johnson CA, Crandall H, O'Neal JF, Al-Rohil RN, Nebhan CA, Bharti V, Dahlman KB, Ayers GD, Yan C, Kelley MC, Kauffmann RM, Hooks M, Grau A, Johnson DB, Vilgelm AE and Richmond A (2020) Metastatic Melanoma Patient-Derived Xenografts Respond to MDM2 Inhibition as a Single Agent or in Combination with BRAF/MEK Inhibition. *Clin Cancer Res* **26**:3803-3818.
- Sheng W, Dong M, Chen C, Wang Z, Li Y, Wang K, Li Y and Zhou J (2017) Cooperation of Musashi-2, Numb, MDM2, and P53 in drug resistance and malignant biology of pancreatic cancer. *FASEB J* **31**:2429-2438.
- Shi Y, Sang P and Cai J (2021) Discovery of alpha-helix-mimicking sulfono-gamma-AApeptides as p53-MDM2 inhibitors. *Future Med Chem* **13**:1021-1023.
- Shigdar S, Schrand B, Giangrande PH and de Franciscis V (2021) Aptamers: Cutting edge of cancer therapies. *Mol Ther* **29**:2396-2411.
- Shojaei Baghini S, Gardanova ZR, Abadi SAH, Zaman BA, İlhan A, Shomali N, Adili A, Moghaddar R and Yaseri AF (2022) CRISPR/Cas9 application in cancer therapy: a pioneering genome editing tool. *Cell Mol Biol Lett* **27**:35.
- Shvarts A, Steegenga WT, Riteco N, van Laar T, Dekker P, Bazuine M, van Ham RC, van der Houven van Oordt W, Hateboer G, van der Eb AJ and Jochemsen AG (1996) MDMX: a novel p53-binding protein with some functional properties of MDM2. *The EMBO Journal* **15**:5349-5357.
- Singh AK, Chauhan SS, Singh SK, Verma VV, Singh A, Arya RK, Maheshwari S, Akhtar MS, Sarkar J, Rangnekar VM, Chauhan PMS and Datta D (2016) Dual targeting of MDM2 with a novel small-molecule inhibitor overcomes TRAIL resistance in cancer. *Carcinogenesis* **37**:1027-1040.
- Singh S, Ramamoorthy M, Vaughan C, Yeudall WA, Deb S and Palit Deb S (2013) Human oncoprotein MDM2 activates the Akt signaling pathway through an interaction with the repressor element-1 silencing transcription factor conferring a survival advantage to cancer cells. *Cell Death Differ* **20**:558-566.
- Soares J, Espadinha M, Raimundo L, Ramos H, Gomes AS, Gomes S, Loureiro JB, Inga A, Reis F, Gomes C, Santos MMM and Saraiva L (2017) DIMP53-1: a novel small-molecule dual inhibitor of p53-MDM2/X interactions with multifunctional p53-dependent anticancer properties. *Mol Oncol* **11**:612-627.
- Song H, Sun B, Liao Y, Xu D, Guo W, Wang T, Jing B, Hu M, Li K, Yao F and Deng J (2019) GPRC5A deficiency leads to dysregulated MDM2 via activated EGFR signaling for lung tumor development. *Int J Cancer* **144**:777-787.

- Sonkin D (2015) Expression signature based on TP53 target genes doesn't predict response to TP53-MDM2 inhibitor in wild type TP53 tumors. *Elife* **4**.
- Sosin AM, Burger AM, Siddiqi A, Abrams J, Mohammad RM and Al-Katib AM (2012) HDM2 antagonist MI-219 (spiro-oxindole), but not Nutlin-3 (cis-imidazoline), regulates p53 through enhanced HDM2 autoubiquitination and degradation in human malignant B-cell lymphomas. *J Hematol Oncol* **5**:57.
- Stein EM, DeAngelo DJ, Chromik J, Chatterjee M, Bauer S, Lin CC, Suarez C, De Vos F, Steeghs N, Cassier PA, Tai D, Kiladjian JJ, Yamamoto N, Mous R, Esteve J, Minami H, Ferretti S, Guerreiro N, Meille C, Radhakrishnan R, Pereira B, Mariconti L, Halilovic E, Fabre C and Carpio C (2021) Results from a First-in-Human Phase I Study of Siremadlin (HDM201) in Patients with Advanced Wild-Type TP53 Solid Tumors and Acute Leukemia. *Clin Cancer Res*.
- Stein EM, DeAngelo DJ, Chromik J, Chatterjee M, Bauer S, Lin CC, Suarez C, de Vos F, Steeghs N, Cassier PA, Tai D, Kiladjian JJ, Yamamoto N, Mous R, Esteve J, Minami H, Ferretti S, Guerreiro N, Meille C, Radhakrishnan R, Pereira B, Mariconti L, Halilovic E, Fabre C and Carpio C (2022) Results from a First-in-Human Phase I Study of Siremadlin (HDM201) in Patients with Advanced Wild-Type TP53 Solid Tumors and Acute Leukemia. *Clin Cancer Res* **28**:870-881.
- Stoll R, Renner C, Hansen S, Palme S, Klein C, Belling A, Zeslawski W, Kamionka M, Rehm T, Mühlhahn P, Schumacher R, Hesse F, Kaluza B, Voelter W, Engh RA and Holak TA (2001) Chalcone derivatives antagonize interactions between the human oncoprotein MDM2 and p53. *Biochemistry* **40**:336-344.
- Sun D, Li Z, Rew Y, Gribble M, Bartberger MD, Beck HP, Canon J, Chen A, Chen X, Chow D, Deignan J, Duquette J, Eksterowicz J, Fisher B, Fox BM, Fu J, Gonzalez AZ, Gonzalez-Lopez De Turiso F, Houze JB, Huang X, Jiang M, Jin L, Kayser F, Liu JJ, Lo MC, Long AM, Lucas B, McGee LR, McIntosh J, Mihalic J, Oliner JD, Osgood T, Peterson ML, Roveto P, Saiki AY, Shaffer P, Toteva M, Wang Y, Wang YC, Wortman S, Yakowec P, Yan X, Ye Q, Yu D, Yu M, Zhao X, Zhou J, Zhu J, Olson SH and Medina JC (2014) Discovery of AMG 232, a potent, selective, and orally bioavailable MDM2-p53 inhibitor in clinical development. *J Med Chem* **57**:1454-1472.
- Sun D, Zhu Y, Zhu J, Tao J, Wei X, Wo Y and Hou H (2020) Primary resistance to first-generation EGFR-TKIs induced by MDM2 amplification in NSCLC. *Mol Med* **26**:66.
- Sun SH, Zheng M, Ding K, Wang S and Sun Y (2008) A small molecule that disrupts Mdm2-p53 binding activates p53, induces apoptosis and sensitizes lung cancer cells to chemotherapy. *Cancer Biol Ther* **7**:845-852.
- Sun W and Tang L (2016) MDM2 Increases Drug Resistance in Cancer Cells by Inducing EMT Independent of p53. *Curr Med Chem* **23**:4529-4539.
- Sun X, Gao H, Yang Y, He M, Wu Y, Song Y, Tong Y and Rao Y (2019) PROTACs: great opportunities for academia and industry. *Signal Transduct Target Ther* **4**:64.
- Suzuki A, Toi M, Yamamoto Y, Saji S, Muta M and Tominaga T (1998) Role of MDM2 overexpression in doxorubicin resistance of breast carcinoma. *Jpn J Cancer Res* **89**:221-227.
- Takahashi S, Fujiwara Y, Nakano K, Shimizu T, Tomomatsu J, Koyama T, Ogura M, Tachibana M, Kakurai Y, Yamashita T, Sakajiri S and Yamamoto N (2021) Safety and pharmacokinetics of milademetan, a MDM2 inhibitor, in Japanese patients with solid tumors: A phase I study. *Cancer Sci* **112**:2361-2370.

- Tang Y, Xuan Y, Qiao G, Ou Z, He Z, Zhu Q, Liao M and Yin G (2019) MDM2 promotes epithelial-mesenchymal transition through activation of Smad2/3 signaling pathway in lung adenocarcinoma. *Onco Targets Ther* **12**:2247-2258.
- Tonsing-Carter E, Bailey BJ, Saadatzadeh MR, Ding J, Wang H, Sinn AL, Peterman KM, Spragins TK, Silver JM, Sprouse AA, Georgiadis TM, Gunter TZ, Long EC, Minto RE, Marchal CC, Batuello CN, Safa AR, Hanenberg H, Territo PR, Sandusky GE, Mayo LD, Eischen CM, Shannon HE and Pollok KE (2015) Potentiation of Carboplatin-Mediated DNA Damage by the Mdm2 Modulator Nutlin-3a in a Humanized Orthotopic Breast-to-Lung Metastatic Model. *Mol Cancer Ther* **14**:2850-2863.
- Tovar C, Graves B, Packman K, Filipovic Z, Higgins B, Xia M, Tardell C, Garrido R, Lee E, Kolinsky K, To KH, Linn M, Podlaski F, Wovkulich P, Vu B and Vassilev LT (2013) MDM2 small-molecule antagonist RG7112 activates p53 signaling and regresses human tumors in preclinical cancer models. *Cancer Res* **73**:2587-2597.
- Tovar C, Rosinski J, Filipovic Z, Higgins B, Kolinsky K, Hilton H, Zhao X, Vu BT, Qing W, Packman K, Myklebost O, Heimbrook DC and Vassilev LT (2006) Small-molecule MDM2 antagonists reveal aberrant p53 signaling in cancer: implications for therapy. *Proc Natl Acad Sci U S A* **103**:1888-1893.
- Uy GL, Assouline S, Young AM, Blotner S, Higgins B, Chen LC and Yee K (2020) Phase 1 study of the MDM2 antagonist RO6839921 in patients with acute myeloid leukemia. *Invest New Drugs* **38**:1430-1441.
- Van Goethem A, Yigit N, Moreno-Smith M, Vasudevan SA, Barbieri E, Speleman F, Shohet J, Vandesompele J and Van Maerken T (2017) Dual targeting of MDM2 and BCL2 as a therapeutic strategy in neuroblastoma. *Oncotarget* **8**:57047-57057.
- Vassilev LT (2005) p53 Activation by small molecules: application in oncology. *J Med Chem* **48**:4491-4499.
- Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, Fotouhi N and Liu EA (2004) In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* **303**:844-848.
- Veneziani I, Infante P, Ferretti E, Melaiu O, Battistelli C, Lucarini V, Compagnone M, Nicoletti C, Castellano A, Petrini S, Ognibene M, Pezzolo A, Di Marcotullio L, Bei R, Moretta L, Pistoia V, Fruci D, Barnaba V, Locatelli F and Cifaldi L (2021) Nutlin-3a Enhances Natural Killer Cell-Mediated Killing of Neuroblastoma by Restoring p53-Dependent Expression of Ligands for NKG2D and DNAM-1 Receptors. *Cancer Immunol Res* **9**:170-183.
- Verreault M, Schmitt C, Goldwirt L, Pelton K, Haidar S, Levasseur C, Guehenne J, Knoff D, Labussiere M, Marie Y, Ligon AH, Mokhtari K, Hoang-Xuan K, Sanson M, Alexander BM, Wen PY, Delattre JY, Ligon KL and Idbaih A (2016) Preclinical Efficacy of the MDM2 Inhibitor RG7112 in MDM2-Amplified and TP53 Wild-type Glioblastomas. *Clin Cancer Res* **22**:1185-1196.
- Verstovsek S, Al-Ali HK, Mascarenhas J, Perkins A, Vannucchi AM, Mohan SR, Scott BL, Woszczyk D, Koschmieder S, García-Delgado R, László R, McGreivy JS, Rothbaum WP and Kiladjan J-J (2022) BOREAS: a global, phase III study of the MDM2 inhibitor navtemadlin (KRT-232) in relapsed/refractory myelofibrosis. *Future Oncology* **18**:4059-4069.

- Vu B, Wovkulich P, Pizzolato G, Lovey A, Ding Q, Jiang N, Liu JJ, Zhao C, Glenn K, Wen Y, Tovar C, Packman K, Vassilev L and Graves B (2013) Discovery of RG7112: A Small-Molecule MDM2 Inhibitor in Clinical Development. *ACS Med Chem Lett* **4**:466-469.
- Vu TT, Stolzel F, Wang KW, Rollic C, Tursky ML, Molloy TJ and Ma DD (2021) miR-10a as a therapeutic target and predictive biomarker for MDM2 inhibition in acute myeloid leukemia. *Leukemia* **35**:1933-1948.
- Vummididi Giridhar P, Williams K, VonHandorf AP, Deford PL and Kasper S (2019) Constant Degradation of the Androgen Receptor by MDM2 Conserves Prostate Cancer Stem Cell Integrity. *Cancer Res* **79**:1124-1137.
- Wade M, Li YC and Wahl GM (2013) MDM2, MDMX and p53 in oncogenesis and cancer therapy. *Nat Rev Cancer* **13**:83-96.
- Wagner AJ, Banerji U, Mahipal A, Somaiah N, Hirsch H, Fancourt C, Johnson-Levonas AO, Lam R, Meister AK, Russo G, Knox CD, Rose S and Hong DS (2017) Phase I Trial of the Human Double Minute 2 Inhibitor MK-8242 in Patients With Advanced Solid Tumors. *J Clin Oncol* **35**:1304-1311.
- Wang B, Liu J, Tandon I, Wu S, Teng P, Liao J and Tang W (2021a) Development of MDM2 degraders based on ligands derived from Ugi reactions: Lessons and discoveries. *European Journal of Medicinal Chemistry* **219**:113425.
- Wang B, Wu S, Liu J, Yang K, Xie H and Tang W (2019a) Development of selective small molecule MDM2 degraders based on nutlin. *Eur J Med Chem* **176**:476-491.
- Wang H, Nan L, Yu D, Agrawal S and Zhang R (2001) Antisense anti-MDM2 oligonucleotides as a novel therapeutic approach to human breast cancer: in vitro and in vivo activities and mechanisms. *Clin Cancer Res* **7**:3613-3624.
- Wang H, Zeng X, Oliver P, Le LP, Chen J, Chen L, Zhou W, Agrawal S and Zhang R (1999) MDM2 oncogene as a target for cancer therapy: An antisense approach. *Int J Oncol* **15**:653-660.
- Wang HQ, Halilovic E, Li X, Liang J, Cao Y, Rakiec DP, Ruddy DA, Jeay S, Wuerthner JU, Timple N, Kasibhatla S, Li N, Williams JA, Sellers WR, Huang A and Li F (2017) Combined ALK and MDM2 inhibition increases antitumor activity and overcomes resistance in human ALK mutant neuroblastoma cell lines and xenograft models. *Elife* **6**.
- Wang HQ, Mulford IJ, Sharp F, Liang J, Kurtulus S, Trabucco G, Quinn DS, Longmire TA, Patel N, Patil R, Shirley MD, Chen Y, Wang H, Ruddy DA, Fabre C, Williams JA, Hammerman PS, Mataraza J, Platzer B and Halilovic E (2021b) Inhibition of MDM2 Promotes Antitumor Responses in p53 Wild-Type Cancer Cells through Their Interaction with the Immune and Stromal Microenvironment. *Cancer Res* **81**:3079-3091.
- Wang P, Lushnikova T, Odvody J, Greiner TC, Jones SN and Eischen CM (2008) Elevated Mdm2 expression induces chromosomal instability and confers a survival and growth advantage to B cells. *Oncogene* **27**:1590-1598.
- Wang S, Sun W, Zhao Y, McEachern D, Meaux I, Barriere C, Stuckey JA, Meagher JL, Bai L, Liu L, Hoffman-Luca CG, Lu J, Shangary S, Yu S, Bernard D, Aguilar A, Dos-Santos O, Besret L, Guerif S, Pannier P, Gorge-Bernat D and Debussche L (2014a) SAR405838: an optimized inhibitor of MDM2-p53 interaction that induces complete and durable tumor regression. *Cancer Res* **74**:5855-5865.
- Wang W, Cheng JW, Qin JJ, Hu B, Li X, Nijampatnam B, Velu SE, Fan J, Yang XR and Zhang R (2019b) MDM2-NFAT1 dual inhibitor, MA242: Effective against hepatocellular carcinoma, independent of p53. *Cancer Lett* **459**:156-167.

- Wang W, Hu B, Qin JJ, Cheng JW, Li X, Rajaei M, Fan J, Yang XR and Zhang R (2019c) A novel inhibitor of MDM2 oncogene blocks metastasis of hepatocellular carcinoma and overcomes chemoresistance. *Genes Dis* **6**:419-430.
- Wang W, Qin JJ, Rajaei M, Li X, Yu X, Hunt C and Zhang R (2020) Targeting MDM2 for novel molecular therapy: Beyond oncology. *Med Res Rev* **40**:856-880.
- Wang W, Qin JJ, Voruganti S, Nijampatnam B, Velu SE, Ruan KH, Hu M, Zhou J and Zhang R (2018) Discovery and Characterization of Dual Inhibitors of MDM2 and NFAT1 for Pancreatic Cancer Therapy. *Cancer Res* **78**:5656-5667.
- Wang W, Qin JJ, Voruganti S, Srivenugopal KS, Nag S, Patil S, Sharma H, Wang MH, Wang H, Buolamwini JK and Zhang R (2014b) The pyrido[b]indole MDM2 inhibitor SP-141 exerts potent therapeutic effects in breast cancer models. *Nat Commun* **5**:5086.
- Wang W, Qin JJ, Voruganti S, Wang MH, Sharma H, Patil S, Zhou J, Wang H, Mukhopadhyay D, Buolamwini JK and Zhang R (2014c) Identification of a new class of MDM2 inhibitor that inhibits growth of orthotopic pancreatic tumors in mice. *Gastroenterology* **147**:893-902 e892.
- Wang W, Rayburn ER, Velu SE, Nadkarni DH, Murugesan S and Zhang R (2009) In vitro and in vivo anticancer activity of novel synthetic makaluvamine analogues. *Clin Cancer Res* **15**:3511-3518.
- Wang Z, Song T, Feng Y, Guo Z, Fan Y, Xu W, Liu L, Wang A and Zhang Z (2016) Bcl-2/MDM2 Dual Inhibitors Based on Universal Pyramid-Like alpha-Helical Mimetics. *J Med Chem* **59**:3152-3162.
- Ware PL, Snow AN, Gvalani M, Pettenati MJ and Qasem SA (2014) MDM2 copy numbers in well-differentiated and dedifferentiated liposarcoma: characterizing progression to high-grade tumors. *Am J Clin Pathol* **141**:334-341.
- Warner KA, Nor F, Acasigua GA, Martins MD, Zhang Z, McLean SA, Spector ME, Chepeha DB, Helman J, Wick MJ, Moskaluk CA, Castilho RM, Pearson AT, Wang S and Nor JE (2016) Targeting MDM2 for Treatment of Adenoid Cystic Carcinoma. *Clin Cancer Res* **22**:3550-3559.
- Weilbacher A, Gutekunst M, Oren M, Aulitzky WE and van der Kuip H (2014) RITA can induce cell death in p53-defective cells independently of p53 function via activation of JNK/SAPK and p38. *Cell Death Dis* **5**:e1318.
- Wen W, Peng C, Kim MO, Ho Jeong C, Zhu F, Yao K, Zykova T, Ma W, Carper A, Langfald A, Bode AM and Dong Z (2014) Knockdown of RNF2 induces apoptosis by regulating MDM2 and p53 stability. *Oncogene* **33**:421-428.
- Werner LR, Huang S, Francis DM, Armstrong EA, Ma F, Li C, Iyer G, Canon J and Harari PM (2015) Small Molecule Inhibition of MDM2-p53 Interaction Augments Radiation Response in Human Tumors. *Mol Cancer Ther* **14**:1994-2003.
- Wienken M, Dickmanns A, Nemajerova A, Kramer D, Najafova Z, Weiss M, Karpiuk O, Kassem M, Zhang Y, Lozano G, Johnsen SA, Moll UM, Zhang X and Dobbelstein M (2016) MDM2 Associates with Polycomb Repressor Complex 2 and Enhances Stemness-Promoting Chromatin Modifications Independent of p53. *Mol Cell* **61**:68-83.
- Wienken M, Moll UM and Dobbelstein M (2017) Mdm2 as a chromatin modifier. *J Mol Cell Biol* **9**:74-80.
- Wu CC, Yu CT, Chang GC, Lai JM and Hsu SL (2011) Aurora-A promotes gefitinib resistance via a NF-kappaB signaling pathway in p53 knockdown lung cancer cells. *Biochem Biophys Res Commun* **405**:168-172.

- Xie X, He G and Siddik ZH (2020) Cisplatin in Combination with MDM2 Inhibition Downregulates Rad51 Recombinase in a Bimodal Manner to Inhibit Homologous Recombination and Augment Tumor Cell Kill. *Mol Pharmacol* **97**:237-249.
- Xu J, Han M, Shen J, Guan Q, Bai Z, Lang B, Zhang H, Li Z, Zuo D, Zhang W and Wu Y (2016) 2-Methoxy-5-((3,4,5-trimethoxyphenyl)seleninyl) phenol inhibits MDM2 and induces apoptosis in breast cancer cells through a p53-independent pathway. *Cancer Lett* **383**:9-17.
- Xu Z, Wu W, Yan H, Hu Y, He Q and Luo P (2021) Regulation of p53 stability as a therapeutic strategy for cancer. *Biochemical Pharmacology* **185**:114407.
- Yamaura T, Muto S, Mine H, Takagi H, Watanabe M, Ozaki Y, Inoue T, Fukuhara M, Okabe N, Matsumura Y, Hasegawa T, Osugi J, Hoshino M, Higuchi M, Shio Y and Suzuki H (2020) Genetic alterations in epidermal growth factor receptor-tyrosine kinase inhibitor-naïve non-small cell lung carcinoma. *Oncol Lett* **19**:4169-4176.
- Yang J, Li Y, Aguilar A, Liu Z, Yang C-Y and Wang S (2019) Simple Structural Modifications Converting a Bona fide MDM2 PROTAC Degradator into a Molecular Glue Molecule: A Cautionary Tale in the Design of PROTAC Degradators. *Journal of Medicinal Chemistry* **62**:9471-9487.
- Ye Q, Jiang M, Huang WT, Ling Y, Olson SH, Sun D, Xu G, Yan X, Wong BK and Jin L (2015) Pharmacokinetics and metabolism of AMG 232, a novel orally bioavailable inhibitor of the MDM2-p53 interaction, in rats, dogs and monkeys: in vitro-in vivo correlation. *Xenobiotica* **45**:681-692.
- Yee K, Papayannidis C, Vey N, Dickinson MJ, Kelly KR, Assouline S, Kasner M, Seiter K, Drummond MW, Yoon SS, Lee JH, Blotner S, Jukofsky L, Pierceall WE, Zhi J, Simon S, Higgins B, Nichols G, Monnet A, Muehlbauer S, Ott M, Chen LC and Martinelli G (2021) Murine double minute 2 inhibition alone or with cytarabine in acute myeloid leukemia: Results from an idasanutlin phase 1/1b study small star, filled. *Leuk Res* **100**:106489.
- Yi H, Yan X, Luo Q, Yuan L, Li B, Pan W, Zhang L, Chen H, Wang J, Zhang Y, Zhai Y, Qiu MZ and Yang DJ (2018) A novel small molecule inhibitor of MDM2-p53 (APG-115) enhances radiosensitivity of gastric adenocarcinoma. *J Exp Clin Cancer Res* **37**:97.
- Yu S, Qin D, Shangary S, Chen J, Wang G, Ding K, McEachern D, Qiu S, Nikolovska-Coleska Z, Miller R, Kang S, Yang D and Wang S (2009) Potent and orally active small-molecule inhibitors of the MDM2-p53 interaction. *J Med Chem* **52**:7970-7973.
- Yu Z, Zhuang C, Wu Y, Guo Z, Li J, Dong G, Yao J, Sheng C, Miao Z and Zhang W (2014) Design, synthesis and biological evaluation of sulfamide and triazole benzodiazepines as novel p53-MDM2 inhibitors. *Int J Mol Sci* **15**:15741-15753.
- Yurlova L, Derks M, Buchfellner A, Hickson I, Janssen M, Morrison D, Stansfield I, Brown CJ, Ghadessy FJ, Lane DP, Rothbauer U, Zolghadr K and Krausz E (2014) The fluorescent two-hybrid assay to screen for protein-protein interaction inhibitors in live cells: targeting the interaction of p53 with Mdm2 and Mdm4. *J Biomol Screen* **19**:516-525.
- Zafar A, Khan MJ and Naeem A (2023) MDM2- an indispensable player in tumorigenesis. *Mol Biol Rep* **50**:6871-6883.
- Zafar A, Wang W, Liu G, Xian W, McKeon F, Zhou J and Zhang R (2021) Targeting the p53-MDM2 pathway for neuroblastoma therapy: Rays of hope. *Cancer Lett* **496**:16-29.
- Zanjirband M, Curtin N, Edmondson RJ and Lunec J (2017) Combination treatment with rucaparib (Rubraca) and MDM2 inhibitors, Nutlin-3 and RG7388, has synergistic and dose reduction potential in ovarian cancer. *Oncotarget* **8**:69779-69796.

- Zhang F, Tagen M, Throm S, Mallari J, Miller L, Guy RK, Dyer MA, Williams RT, Roussel MF, Nemeth K, Zhu F, Zhang J, Lu M, Panetta JC, Boulos N and Stewart CF (2011a) Whole-body physiologically based pharmacokinetic model for nutlin-3a in mice after intravenous and oral administration. *Drug Metab Dispos* **39**:15-21.
- Zhang F, Throm SL, Murley LL, Miller LA, Steven Zatechka D, Jr., Kiplin Guy R, Kennedy R and Stewart CF (2011b) MDM2 antagonist nutlin-3a reverses mitoxantrone resistance by inhibiting breast cancer resistance protein mediated drug transport. *Biochem Pharmacol* **82**:24-34.
- Zhang L, Zhu D, Jiang J, Min Z and Fa Z (2023) The ubiquitin E3 ligase MDM2 induces chemoresistance in colorectal cancer by degradation of ING3. *Carcinogenesis* **44**:562-575.
- Zhang Q, Zeng SX and Lu H (2014) Targeting p53-MDM2-MDMX loop for cancer therapy. *Subcell Biochem* **85**:281-319.
- Zhang X, Zhang R, Chen H, Wang L, Ren C, Pataer A, Wu S, Meng QH, Ha MJ, Morris J, Xi Y, Wang J, Zhang J, Gibbons DL, Heymach JV, Meric-Bernstam F, Minna J, Swisher SG, Roth JA and Fang B (2020) KRT-232 and navitoclax enhance trametinib's anti-Cancer activity in non-small cell lung cancer patient-derived xenografts with KRAS mutations. *Am J Cancer Res* **10**:4464-4475.
- Zhang X, Zhang Z, Cheng J, Li M, Wang W, Xu W, Wang H and Zhang R (2012) Transcription factor NFAT1 activates the mdm2 oncogene independent of p53. *J Biol Chem* **287**:30468-30476.
- Zhang Z, Wang H, Li M, Rayburn ER, Agrawal S and Zhang R (2005) Stabilization of E2F1 protein by MDM2 through the E2F1 ubiquitination pathway. *Oncogene* **24**:7238-7247.
- Zhang Z, Wang H, Prasad G, Li M, Yu D, Bonner JA, Agrawal S and Zhang R (2004) Radiosensitization by antisense anti-MDM2 mixed-backbone oligonucleotide in in vitro and in vivo human cancer models. *Clin Cancer Res* **10**:1263-1273.
- Zhao CY, Grinkevich VV, Nikulenkov F, Bao W and Selivanova G (2010) Rescue of the apoptotic-inducing function of mutant p53 by small molecule RITA. *Cell Cycle* **9**:1847-1855.
- Zhao Y, Liu L, Sun W, Lu J, McEachern D, Li X, Yu S, Bernard D, Ochsenbein P, Ferey V, Carry JC, Deschamps JR, Sun D and Wang S (2013) Diastereomeric spirooxindoles as highly potent and efficacious MDM2 inhibitors. *J Am Chem Soc* **135**:7223-7234.
- Zhou J, Kryczek I, Li S, Li X, Aguilar A, Wei S, Grove S, Vatan L, Yu J, Yan Y, Liao P, Lin H, Li J, Li G, Du W, Wang W, Lang X, Wang W, Wang S and Zou W (2021a) The ubiquitin ligase MDM2 sustains STAT5 stability to control T cell-mediated antitumor immunity. *Nat Immunol* **22**:460-470.
- Zhou X, Singh M, Sanz Santos G, Guerlavais V, Carvajal LA, Aivado M, Zhan Y, Oliveira MMS, Westerberg LS, Annis DA, Johnsen JI and Selivanova G (2021b) Pharmacological activation of p53 triggers viral mimicry response thereby abolishing tumor immune evasion and promoting anti-tumor immunity. *Cancer Discov*.
- Zhu H, Gao H, Ji Y, Zhou Q, Du Z, Tian L, Jiang Y, Yao K and Zhou Z (2022) Targeting p53-MDM2 interaction by small-molecule inhibitors: learning from MDM2 inhibitors in clinical trials. *J Hematol Oncol* **15**:91.
- Zou P, Zheng N, Yu Y, Yu S, Sun W, McEachern D, Yang Y, Yu LX, Wang S and Sun D (2012) Preclinical pharmacokinetics of MI-219, a novel human double minute 2 (HDM2) inhibitor and prediction of human pharmacokinetics. *J Pharm Pharm Sci* **15**:265-280.

Zou Q, Jin J, Hu H, Li HS, Romano S, Xiao Y, Nakaya M, Zhou X, Cheng X, Yang P, Lozano G, Zhu C, Watowich SS, Ullrich SE and Sun SC (2014) USP15 stabilizes MDM2 to mediate cancer-cell survival and inhibit antitumor T cell responses. *Nat Immunol* **15**:562-570.

Footnotes

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Figure Legends

Figure 1. Simplified timeline of the milestone discoveries of MDM2 and its inhibitors

Figure 2. Representative examples highlighting the role of MDM2 in drug resistance.

MDM2-p53 feedback loop regulates MGMT expression to promote temozolomide resistance. MDM2/AKT/AR signaling enhances the EMT to increase the resistance to chemotherapy. MDM2 associates with stem cell markers CD133 and CD34 in maintaining the stemness properties of cancer cells, contributing to the chemotherapy resistance. Increased NF- κ B transcriptional activity is involved in Aurora-A promoted gefitinib resistance. MDM2 negatively regulates NFAT1. The combination of MDM2 inhibitors and ICIs may overcome the resistance of patients to immunotherapy by activating cytotoxic T cells and blocking the immune checkpoint. Abbreviations: AKT, Protein kinase B; AR, androgen receptor; MDM2, mouse double minute 2, MGMT, O-6-methylguanine-DNA methyltransferase; NFAT1, nuclear factor of activated T cells 1; NF- κ B, nuclear factor kappa B

Figure 3. Structures of representative MDM2 inhibitors. A. Peptide inhibitors. B. Representative single ring inhibitors. C. Representative bicyclic inhibitors. D. Others.

Figure 4. Structures of representative PROTAC MDM2 inhibitors.

Tables

Table 1. Selected examples of completed clinical trials of MDM2 inhibitors

Compounds	Core or Category	Sponsors	Combination	Conditions	Phase	NCT Number	Results
ALRN-6924	Peptide	Aileron		Solid tumors and lymphomas with WT TP53	Phase I/IIa	NCT02264613	3.1 mg/kg on days 1, 8, and 15 in a 28-day cycle. The disease control rate was 59% (Saleh et al., 2021).
ALRN-6924	Peptide	Aileron	Cytarabine	Acute myeloid leukemia and advanced myelodysplastic syndrome	Phase I	NCT02909972	Among the enrolled 12 patients, the combination was generally well tolerated with transient, self-resolving Grade 3 and 4 neutropenia, pulmonary embolism, thrombocytopenia, leukopenia, increased ALT, and fall (F. Meric-Bernier et al., 2019).
ALRN-6924	Peptide	Aileron	Cytarabine	Resistant (refractory) pediatric solid tumor, brain tumor, lymphoma, or leukemia.	Phase I	NCT03654716	NA
RG7112 (RO5045337)	Cis-imidazoline	Roche		Advanced solid tumors	Phase I	NCT00559533	N/A
RG7112 (RO5045337)	Cis-imidazoline	Roche		Liposarcoma patients who are eligible for debulking surgery	Phase I	NCT01143740	N/A
RG7112 (RO5045337)	Cis-imidazoline	Roche	Doxorubicin	Soft tissue sarcoma	Phase I	NCT01605526	Among the enrolled 20 patients with ASTS, high rate of grade 3/4 neutropenia (60%) or thrombocytopenia (45%) were observed (Sant P. Chawla 2013).
RG7112 (RO5045337)	Cis-imidazoline	Roche	Cytarabine	Acute Myelogenous Leukemia	Phase I	NCT01635296	N/A
RG7112 (RO5045337)	Cis-imidazoline	Roche		Hematologic neoplasms	Phase I	NCT00623870	Gastrointestinal toxicity
RG7112 (RO5045337)	Cis-imidazoline	Roche		Advanced solid tumors	Phase I	NCT01164033	High-dose daily treatment for 3 to 5 days was better than weekly and low-dose

							daily treatment for a longer duration (Patnaik et al., 2015).
RG7112 (RO5045337)	Cis-imidazoline	Roche		Participants who have completed parent studies NCT00623870, NCT00559533, NCT01164033, NCT01605526, or NCT01635296.	Phase I	NCT01677780	N/A
Idasanutlin (RO5503781, RG7388)	Pyrrolidine	Roche		Solid tumors	Phase I	NCT03362723	N/A
Idasanutlin (RO5503781, RG7388)	Pyrrolidine	Roche	Cytarabine	Wild-type p53 patients	Phase I	NCT01773408	There was an 18.9% remission rate for monotherapy compared with a 35.6% rate for combination therapy (Yee et al., 2021).
Idasanutlin (RO5503781, RG7388)	Pyrrolidine	Roche		Polycythemia vera	Phase I	NCT02407080	N/A
Idasanutlin (RO5503781, RG7388)	Pyrrolidine	Roche	Venetoclax	Relapsed or refractory acute myeloid leukemia	Phase I	NCT02670044	N/A
Idasanutlin (RO5503781, RG7388)	Pyrrolidine	Roche		Neoplasms	Phase I	NCT01462175	The agent was administered at 500 mg daily for 5 days in a 28-day cycle and showed hematological toxicity (Italiano et al., 2021).
Idasanutlin (RO5503781, RG7388)	Pyrrolidine	Roche		Solid tumors	Phase I	NCT02828930	The absolute bioavailability of Idasanutlin was 40.1%. Excretion of Idasanutlin was primarily via the fecal route (Papai et al., 2019).
RO6839921 (RG7775)	Pyrrolidine pegylated	Roche		Solid tumors and acute myeloid leukemia	Phase I	NCT02098967	At the 110 mg dose for solid tumor patients, 8% had DLTs. The MTD was 200 mg for AML patients. Stable disease was noted in 34% of the 14 patients with solid tumors and the disease control rate was 42% in AML patients (Abdul Razak et al., 2020; Jy et al., 2020).
Milademetan (RAIN-32,	Spirooxindole	Rain Therapeutics		Healthy participants	Early Phase I	NCT03647202	The AUC of Milademetan was reduced by 24% when administered with a high-

DS-3032b)

Milademetan (RAIN-32, DS-3032b)	Spirooxindole	Rain Therapeutics	Itraconazole Posaconazole	Healthy participants	Early Phase I	NCT03614455	fat and high-calorie diet compared with the fasting state (Hong et al., 2021). Milademetan and itraconazole or posaconazole combination increased milademetan AUC _{inf} by 2.15-fold and 2.49-fold, respectively; the PBPK model predicted that the milademetan AUCR after concomitant administration with fluconazole, erythromycin, and verapamil were 1.72-, 1.91-, and 2.02-fold, respectively; suggesting that the milademetan dose should be reduced when combined with strong CYP3A inhibitors (Hong et al., 2021).
Milademetan (RAIN-32, DS-3032b)	Spirooxindole	Rain Therapeutics		Relapsed or refractory acute myeloid leukemia	Phase I	NCT03671564 JapicCTI-184054	N/A
Milademetan (RAIN-32, DS-3032b)	Spirooxindole	Rain Therapeutics		Advanced solid tumors and lymphomas	Phase I	NCT01877382	The MTD was 160 mg in the once-daily (q.d.) 21/28 schedule and 260 mg in the q.d. 3/14 × 2 schedule (1 cycle was 28 days) (Hong et al., 2021).
Milademetan (RAIN-32, DS-3032b)	Spirooxindole	Rain Therapeutics		Solid tumors	Phase I	JapicCTI-142693	The agent was given at 90 mg daily for 21 days in a 28-day cycle (Takahashi et al., 2021).
Milademetan (DS-3032b, Rain-32)	Spirooxindole	Rain Therapeutics	Cytarabine, Venetoclax	Acute myeloid leukemia, refractory acute myeloid leukemia, and refractory acute myeloid leukemia	Phase I/II	NCT03634228	the MTD was determined to be 260 mg/day of milademetan, 600 mg of Venetoclax, and 20 mg of low dose Cytarabine were administered twice daily. The combination treatment resulted in modest response rates, thus the phase II expansion portion of the study was not conducted. The combination treatment was associated with significant and dose-limiting gastrointestinal toxicity (Senapati et al., 2023).
APG-115 (AA-115,	Spirooxindole	Ascentage Pharma		Advanced solid tumors or	Phase I	NCT02935907	The MTD RP2D of APG-115 (every other day QOD) for 21 days of a 28-

alrizomadlin)				lymphoma			day cycle) was determined as 100 mg (Drew W. Rasco, 2019).
SAR405838 (MI-773)	Spirooxindole	Sanofi	Pimasertib	Locally advanced or metastatic solid tumors	Phase I	NCT01985191	The drug was given at 200 mg daily plus 45 mg pimasertib two times a day. One of 24 patients had a partial response and 63% of patients had stable when the drug was combined with pimasertib (de Weger et al., 2019a).
SAR405838 (MI-773)	Spirooxindole	Sanofi		Advanced solid tumors	Phase I	NCT01636479	The treatment was given at 300 mg once daily. SAR405838 treatment was associated with an increased plasma MIC-1. The best response rate was 56% of patients with stable disease and 32% progression-free disease at 3 months (de Jonge et al., 2017).
Navtemadlin KRT-232 (AMG 232)	Piperidinones	Kartos	Trametinib	Relapsed/refractory acute myeloid leukemia	Phase I	NCT02016729	The drug was administered at 360 mg for single treatment or 60 mg when it was combined with trametinib. Four of 13 (38%) patients responded to treatment (Erba et al., 2019).
Navtemadlin KRT-232 (AMG 232)	Piperidinones	Kartos		Advanced p53 wild-type solid tumors or multiple myeloma	Phase I	NCT01723020	Navtemadlin was given at a dose of 240 mg, every 3 weeks (Gluck et al., 2020)
NVP-CGM097	Dihydro-isoquinolinones	Novartis		Advanced solid tumors	Phase I	NCT01760525	The drug was given at 10-400 mg every week for 3 weeks or 300-700 mg every week for 2 weeks with 1 week off. The MTD was not reached. Some (39%) patients responded to the treatment, including one partial response and 19 patients with stable disease (Bauer et al., 2021)
Siremadlin (HDM201)	Pyrrolidono-imidazole	Novartis		Advanced solid and hematological TP53wt tumors	Phase I	NCT02143635	Siremadlin was given at 250 mg on day 1 of a 21-day cycle (1A regiment); 120 mg on days 1 and 8 of a 28-day cycle (1B regiment); and 45 mg on days 1 to day 7 with a 28-day cycle (2C regiment). There was a 10.3% response rate in solid tumor patients and a rate of 4.2% with 1B, 20% with 1A, and

Siremadlin (HDM201)	Pyrrolidono- imidazole	Novartis	LEE011	Liposarcoma	Phase I	NCT02343172	22.2% with the 2C regimen in AML patients (Stein et al., 2021). N/A
UBX0101	N/A	Unity		Knee osteoarthritis	Phase I	NCT04229225 NCT03513016	N/A
MK-8242					Phase I	NCT01463696	At the RP2D of 400 mg BID (7 days- on/14 days-off dosing schedule), MK- 8242 activates the p53 pathway with an acceptable tolerability profile (Wagner et al., 2017).

Table 2. Selected clinical trials ongoing or currently recruiting patients

Compounds	Sponsors	Title	Conditions	Combination	Phase	NCT Number
ALRN-6924	Aileron	ALRN-6924 and Paclitaxel in treating patients with advanced, metastatic, or unresectable solid tumors	Advanced malignant solid neoplasm	Paclitaxel	Phase I	NCT03725436
Siremadlin (HDM201)	Novartis	HDM201 and Pazopanib in patients with p53 wild-type advanced/metastatic soft tissue sarcomas	Advanced/metastatic soft tissue sarcoma	Pazopanib	Phase I/II	NCT05180695
Siremadlin (HDM201)	Novartis	Trametinib + HDM201 in CRC patients with RAS/RAF mutant and TP53 wild-type advanced/metastatic colorectal cancer mutant and TP53 wild-type	RAS/RAF mutant advanced/metastatic colorectal cancer	Trametinib	Phase I	NCT03714958
Siremadlin (HDM201)	Novartis	A study of Siremadlin in combination with Venetoclax plus Azacytidine in adult participants with AML who are ineligible for chemotherapy.	Acute myeloid leukemia who responded sub-optimally to venetoclax and azacitidine treatment and newly diagnosed unfit acute myeloid leukemia	Venetoclax azacitidine	Phase Ib/II	NCT05155709
Siremadlin (HDM201)	Novartis	A Phase 1, open-label, multi-center, single-dose, parallel group study to evaluate the pharmacokinetics of Siremadlin (HDM201) in participants with mild, moderate and severe hepatic impairment compared to matched healthy control participants	Mild, moderate, and severe hepatic impairment		Phase I	NCT05599932
Siremadlin (HDM201)	Novartis	A Randomized, open-label, Phase I/II open platform study evaluating safety and efficacy of novel Ruxolitinib combinations in myelofibrosis patients	Primary myelofibrosis, post polycythemia vera myelofibrosis, and post-essential thrombocythemia myelofibrosis	Ruxolitinib	Phase I/II	NCT04097821
Siremadlin (HDM201)	Centre Leon Berard	MegaMOST - a multicenter, open-label, biology driven, Phase II study evaluating the activity of anti-cancer treatments targeting tumor molecular alterations /characteristics in advanced / metastatic tumors	p53WT advanced/ metastatic tumors with cyclin-dependent kinase 6 and/or cyclin-dependent kinase 4, and/or cyclin dependent kinase inhibitor 2A homozygous deletion, and/or amplification of cyclin D1 and/or cyclin D3 with no deletion/losses more than single copy of retinoblastoma 1	Ribociclib	Phase II	NCT04116541

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Siremadlin (HDM201)	Novartis	A Phase Ib, multi-arm, open-label, study of HDM201 in combination with MBG453 or Venetoclax in adult subjects with acute myeloid leukemia (AML) or high-risk myelodysplastic syndrome (MDS)	by copy number Acute myeloid leukemia or high-risk myelodysplastic syndromes	MBG453, venetoclax	Phase Ib	NCT03940352
Siremadlin (HDM201)	Novartis	A Phase Ib/II, open label study of Siremadlin monotherapy and in combination with donor lymphocyte infusion as a treatment for patients with acute myeloid leukemia post-allogeneic stem cell transplantation who are in complete remission but at high risk for relapse.	Acute myeloid leukemia post-allogeneic stem cell transplant	Donor lymphocyte infusion	Phase Ib/II	NCT05447663
APG-115 (AA-115, alrizomadlin)	Ascentage Pharma	A phase Ib study of APG-115 single agent or in combination with Azacytidine or Cytarabine in patients with AML and MDS.	Acute myeloid leukemia, myelodysplastic syndromes	Cytarabine, Azacytidine	Phase Ib	NCT04275518
APG-115 (AA-115, alrizomadlin)	Ascentage Pharma	A study of APG-115 in combination with Pembrolizumab in patients with metastatic melanomas or advanced solid tumors	Unresectable or metastatic melanoma or advanced solid tumor	Pembrolizumab	Phase Ib/II	NCT03611868
APG-115 (AA-115, alrizomadlin)	Ascentage Pharma	A Phase Ib/II study of APG-115 in combination with PD-1 inhibitor in patients with advanced liposarcoma or other advanced solid tumors	Advanced liposarcoma or advanced solid tumors	Toripalimab	Phase Ib/II	NCT04785196
APG-115 (AA-115, alrizomadlin)	Ascentage Pharma	APG-115 in salivary gland cancer trial	Malignant salivary gland cancer, salivary gland cancer	Carboplatin	Phase I/II	NCT03781986
APG-115 (AA-115, alrizomadlin)	Ascentage Pharma	A Phase I clinical study of APG-115 alone or in combination with APG-2575 in children with recurrent or refractory neuroblastoma or solid tumors	Relapsed or refractory neuroblastoma or solid tumors	APG-2575	Phase I	NCT05701306
APG-115 (AA-115, alrizomadlin)	Ascentage Pharma	A Phase Ib/II study of APG-115 alone or in combination with Azacitidine in patients with relapse/refractory AML, CMML or MDS	Relapsed or refractory acute myeloid leukemia, chronic myelomonocytic leukemia (CMML), or myelodysplastic syndromes	5-azacitidine	Phase Ib/II	NCT04358393
APG-115	Ascentage	A Phase IIa study evaluating the	relapsed/refractory T-cell	APG-2575	Phase	NCT04496349

(AA-115, alrizomadlin)	Pharma	pharmacokinetics, safety, and efficacy of APG-115 as a single agent or in combination with APG-2575 in subjects with relapsed/refractory T-cell prolymphocytic leukemia (R/R T-PLL)	prolymphocytic leukemia (R/R T-PLL)		Ila	
Navtemadlin (KRT232, AMG232)	Kartos	Testing the addition of KRT-232 (AMG 232) to usual chemotherapy for relapsed multiple myeloma	Plasmacytoma, recurrent or refractory plasma cell myeloma	Dexamethasone , Carfilzomib, lenalidomide	Phase I	NCT03031730
Navtemadlin (KRT232, AMG232)	Kartos	KRT-232 with or without anti-PD-1/anti-PD-L1 for the treatment of patients with Merkel cell carcinoma	Merkel cell carcinoma	Avelumab	Phase Ib/II	NCT03787602
Navtemadlin (KRT-232, AMG 232)	Kartos	A Two-part, Phase 1b/2, multicenter, open-label, dose escalation and double-blind, randomized, placebo-controlled, dose expansion study of the safety, efficacy and pharmacokinetics of Navtemadlin plus Pembrolizumab as maintenance therapy in subjects with locally advanced and metastatic non-small cell lung cancer	Locally advanced and metastatic non-small cell lung cancer	Pembrolizumab	Phase Ib/II	NCT05705466
Navtemadlin (KRT232, AMG232)	Kartos	Safety and efficacy of KRT-232 in combination with Acalabrutinib in subjects with R/R DLBCL or R/R CLL	Diffuse large B cell lymphoma, chronic lymphocytic leukemia, non-Hodgkin's lymphoma	Acalabrutinib	Phase Ib/II	NCT04502394
Navtemadlin (KRT232, AMG232)	Kartos	TL-895 and KRT-232 study in acute myeloid leukemia	Relapsed/refractory fms-like tyrosine kinase 3 (FLT3) mutated acute myeloid leukemia	TL-895	Phase Ib/II	NCT04669067
Navtemadlin (KRT232, AMG232)	Kartos	KRT-232 and TKI study in chronic myeloid leukemia	Relapsed/refractory Philadelphia chromosome-positive chronic myeloid leukemia (Ph ⁺ CML)	Dasatinib, Nilotinib	Phase Ib/II	NCT04835584
Navtemadlin (KRT232, AMG232)	Kartos	An open-label, multicenter, phase 1b/2 study of the safety and efficacy of KRT-232 when administered alone and in combination with low-dose Cytarabine (LDAC) or Decitabine in patients with AML	Relapsed/refractory acute myeloid leukemia, acute myeloid leukemia secondary to myeloproliferative neoplasia	Decitabine, Cytarabine	Phase Ib/II	NCT04113616
Navtemadlin (KRT232, AMG232)	Kartos	KRT-232 versus best available therapy for the treatment of subjects with myelofibrosis who are relapsed or refractory to JAK inhibitor treatment	Relapsed/refractory to Janus kinase inhibitor (JAKi) primary myelofibrosis, post polycythemia vera myelofibrosis, or post-essential thrombocythemia	Best available therapy	Phase Ib/III	NCT03662126

Navtemadlin (KRT232, AMG232)	Kartos	KRT-232 compared to Ruxolitinib in patients with phlebotomy-dependent polycythemia vera	myelofibrosis Phlebotomy-dependent Polycythemia vera resistant or intolerant to hydroxyurea	Ruxolitinib	Phase IIa/IIb	NCT03669965
Navtemadlin (KRT232, AMG232)	Kartos	An Open-label, multicenter, phase 1b/2 study of the safety and efficacy of KRT-232 combined with Ruxolitinib in patients with primary myelofibrosis (PMF), post-polycythemia vera MF (Post-PV-MF), or post-essential thrombocythemia MF (Post ET-MF) who have a suboptimal response to Ruxolitinib	Primary myelofibrosis, post polycythemia vera myelofibrosis, and post-essential thrombocythemia myelofibrosis who have a suboptimal response to Ruxolitinib	Ruxolitinib	Phase Ib/II	NCT04485260
Navtemadlin (KRT232, AMG232)	Kartos	KRT-232 in subjects with relapsed or refractory small cell lung cancer	Relapsed or refractory small-cell lung cancer		Phase II	NCT05027867
Navtemadlin (KRT232, AMG232)	Kartos	KRT-232 in combination with TL-895 for the treatment of R/R MF and KRT-232 for the treatment of JAKi intolerant MF	Relapsed/refractory to Janus kinase inhibitor (JAKi) myelofibrosis, post polycythemia vera myelofibrosis, post-essential thrombocythemia myelofibrosis, and primary myelofibrosis	TL-895	Phase I/II	NCT04640532
Navtemadlin (KRT232, AMG232)	Kartos	An Open-label, multicenter, Phase 2 study assessing the safety and efficacy of KRT-232 or TL-895 in Janus kinase inhibitor treatment-naïve myelofibrosis	JAKi treatment-naïve myelofibrosis, post polycythemia vera myelofibrosis, post-essential thrombocythemia myelofibrosis, and primary myelofibrosis	TL-895	Phase II	NCT04878003
Navtemadlin (KRT232, AMG232)	Telios Pharma	Phase I/II, first in human, dose escalation trial of TL 895 monotherapy in subjects with relapsed/refractory B cell malignancies and expansion of TL-895 monotherapy and combination therapy with Navtemadlin in treatment-naïve chronic lymphocytic leukemia or small lymphocytic lymphoma subjects and subjects with relapsed/refractory chronic lymphocytic leukemia or relapsed/refractory small lymphocytic lymphoma	Treatment-naïve and relapsed/refractory chronic lymphocytic leukemia or small lymphocytic lymphoma (SLL)	TL-895	Phase I/II	NCT02825836

Navtemadlin (KRT232, AMG232)	Kartos	Navtemadlin and radiation therapy in treating patients with soft tissue sarcoma	Leukemia and resectable soft tissue sarcoma	Radiation therapy	Phase Ib	NCT03217266
Navtemadlin (KRT232, AMG232)	Kartos	A Phase 1b study with expansion cohort of escalating doses of KRT-232 (AMG 232) administered in combination with standard induction chemotherapy (Cytarabine and Idarubicin) in newly diagnosed acute myelogenous leukemia (AML)	Acute myeloid leukemia	Cytarabine, Idarubicin	Phase Ib	NCT04190550
Navtemadlin (KRT232, AMG232)	Kartos	A Phase 2/3 study of Navtemadlin as maintenance therapy in subjects with TP53WT advanced or recurrent endometrial cancer who responded to chemotherapy	Advanced or recurrent endometrial cancer		Phase II/III	NCT05797831
Idasanutlin (RO5503781, RG7388)	Mayo Clinic	Idasanutlin, Ixazomib Citrate, and Dexamethasone in treating patients with relapsed multiple myeloma	Recurrent plasma cell myeloma with loss of chromosome 17p	Dexamethasone, Ixazomib Citrate	Phase I/II	NCT02633059
Idasanutlin (RO5503781, RG7388)	Roche	A Study evaluating the safety, tolerability, pharmacokinetics and preliminary activity of Idasanutlin in combination with either chemotherapy or Venetoclax in treatment of pediatric and young adult participants with relapsed/refractory acute leukemias or solid tumors	Relapsed or refractory acute myeloid leukemia, acute lymphoblastic leukemia, and Neuroblastoma	Venetoclax, Cyclophosphamide, Topotecan, Fludarabine, Cytarabine	Phase I/II	NCT04029688
Idasanutlin (RO5503781, RG7388)	Roche	NCT Neuro Master Match - N ² M ² (NOA-20) (N ² M ²)	Newly diagnosed isocitrate dehydrogenase (IDH) wildtype glioblastoma bearing an unmethylated MGMT promoter		Phase I/IIa	NCT03158389
Idasanutlin (RO5503781, RG7388)	Roche	iSTAR: Phase 1b Trial of Idasanutlin and Selinexor Therapy For Children With Progressive/Relapsed Atypical Teratoid Rhabdoid Tumors, Extra-CNS Malignant Rhabdoid Tumors Or Synchronous/Metachronous Rhabdoid Tumors	Children with progressive or recurrent atypical teratoid/rhabdoid tumors (AT/RT), malignant rhabdoid tumors (MRT) and synchronous/metachronous rhabdoid tumors	Selinexor	Phase Ib	NCT05952687
Milademetan (DS-3032b, Rain-32)	RAIN	A Randomized multicenter Phase 3 study of Milademetan versus Trabectedin in patients with	Dedifferentiated liposarcoma	Trabectedin	Phase III	NCT04979442

Milademetan (DS-3032b, Rain-32)	RAIN	dedifferentiated liposarcoma A Phase 2 basket study of Milademetan in advanced/metastatic solid tumors (MANTRA-2)	Advanced/metastatic solid tumor refractory or intolerant to standard-of-care therapy		Phase II	NCT05012397
BI 907828 (Brigimadlin)	Boehringer Ingelheim	This Study aims to find the best dose of BI 907828 in patients with different types of advanced cancer (solid tumors)	Neoplasm		Phase Ia/Ib	NCT03449381
BI 907828 (Brigimadlin)	Boehringer Ingelheim	Brightline-1: a study to compare BI 907828 with Doxorubicin in people with a type of cancer called dedifferentiated liposarcoma	Advanced dedifferentiated liposarcoma	Doxorubicin	Phase II/III	NCT05218499
BI 907828 (Brigimadlin)	Boehringer Ingelheim	A Phase Ia/Ib, open label, dose-escalation study of the combination of BI 907828 with BI 754091 (Ezabenlimab) and BI 754111 and the Combination of BI 907828 with BI 754091 (Ezabenlimab) followed by expansion cohorts, in patients with advanced solid tumors)	Neoplasm	BI 754091 (Ezabenlimab), BI 754111	Phase Ia/Ib	NCT03964233
BI 907828 (Brigimadlin)	Boehringer Ingelheim	An Open-label, non-randomized Phase I investigation of human ADME (absorption, distribution, metabolism and excretion) and absolute oral bioavailability of BI 907828 in patients with advanced solid tumors	Advanced solid tumors		Phase I	NCT05613036
BI 907828 (Brigimadlin)	Boehringer Ingelheim	An Open-label fixed sequence trial to investigate the potential drug-drug interaction when BI 907828 is co-administered with an OATP1B1 and/or OATP1B3 transporter inhibitor or with a CYP3A4 inhibitor in patients with various solid tumors	Solid tumors	Rifampicin, Itraconazole	Phase I	NCT05372367
BI 907828 (Brigimadlin)	Boehringer Ingelheim	Brightline-2: a Phase IIa/IIb, open-label, single-arm, multi-center trial of Brigimadlin (BI 907828) for treatment of patients with locally advanced / metastatic, MDM2 amplified, TP53 wild-type biliary tract adenocarcinoma, pancreatic ductal adenocarcinoma, or other selected solid tumors	Locally advanced/metastatic, biliary tract adenocarcinoma, pancreatic ductal adenocarcinoma, urothelial bladder cancer, and lung adenocarcinoma		Phase IIa/IIb	NCT05512377

BI 907828 (Brigimadlin)	Boehringer Ingelheim	A Phase 0/Ia study of BI 907828 concentrations in brain tissue and a non-randomized open-label, dose Escalation study of BI 907828 in combination with radiotherapy in patients with newly diagnosed glioblastoma	Newly diagnosed glioblastoma	Radiation therapy	Phase 0/Ia	NCT05376800
KT-253	Kymera Therapeutics	Safety and clinical activity of kt-253 in adult patients with high grade myeloid malignancies, acute lymphocytic leukemia, lymphoma, solid tumors	Relapsed or refractory (R/R) high grade myeloid malignancies, acute lymphocytic leukemia (ALL), R/R lymphoma, and R/R solid tumors		Phase I	NCT05775406

Figure 1

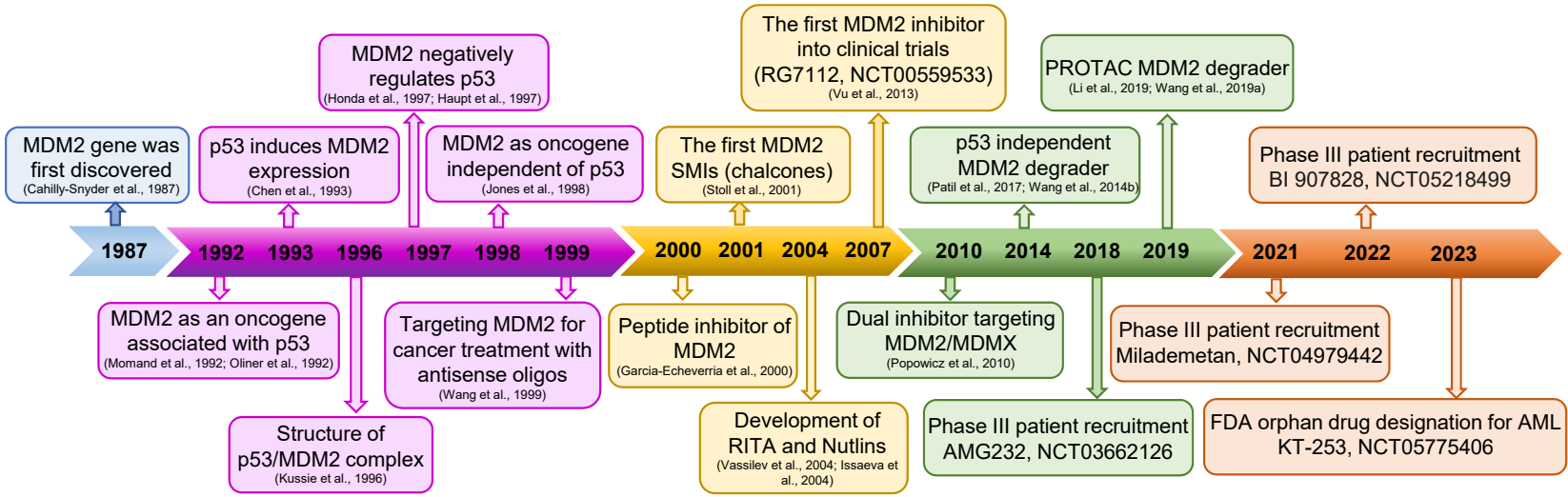


Figure 2

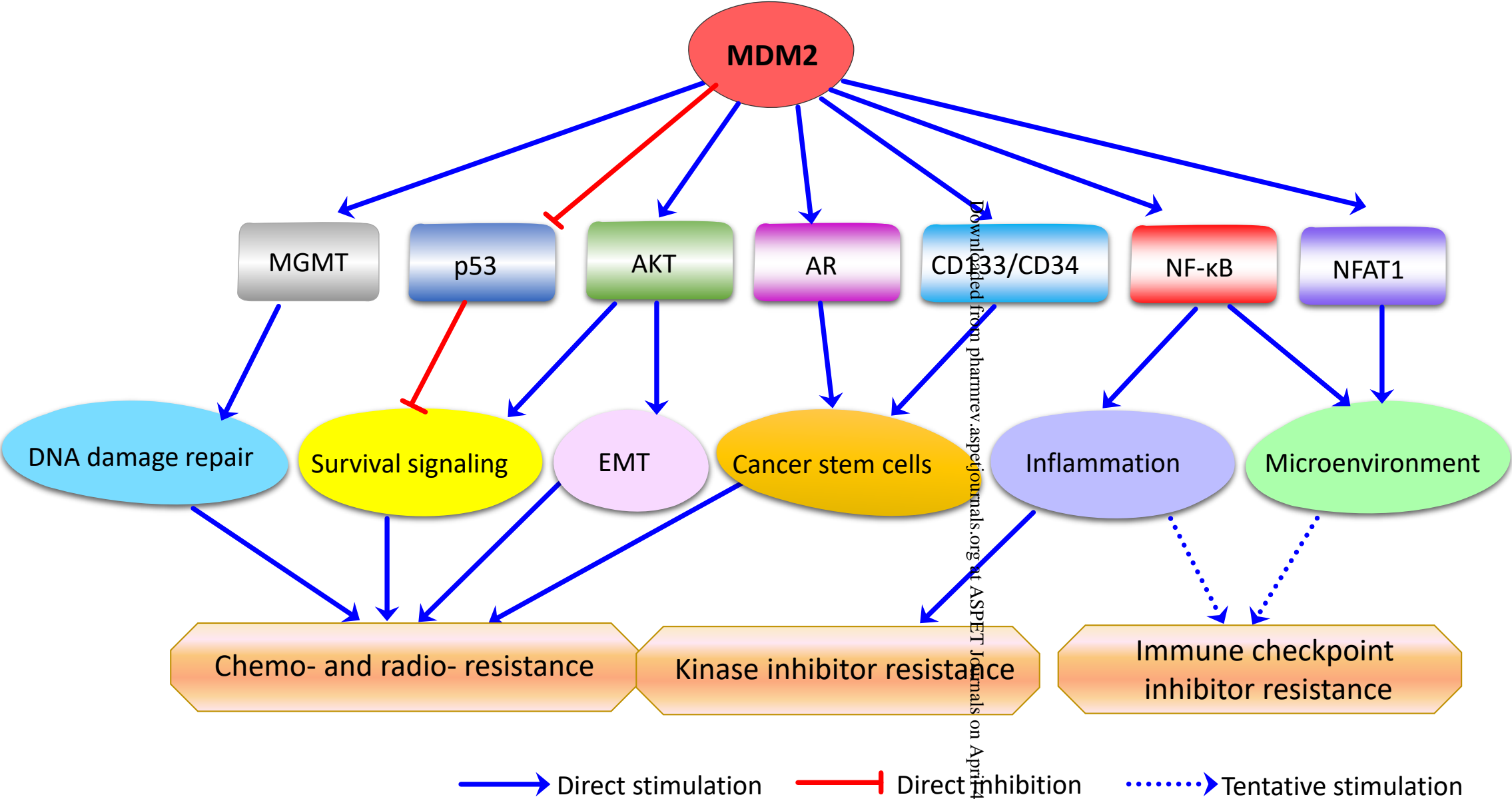


Figure 3

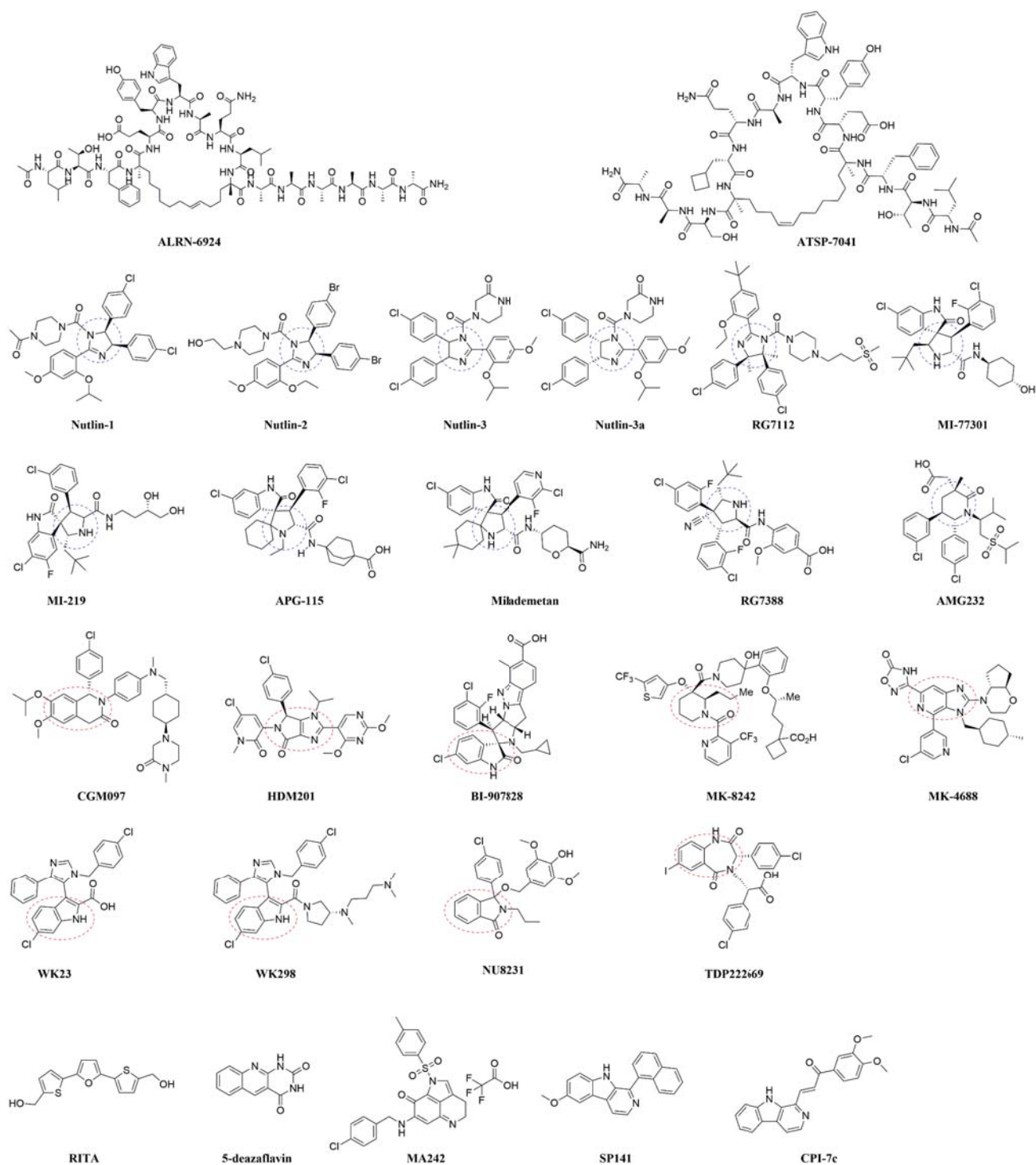


Figure 4

