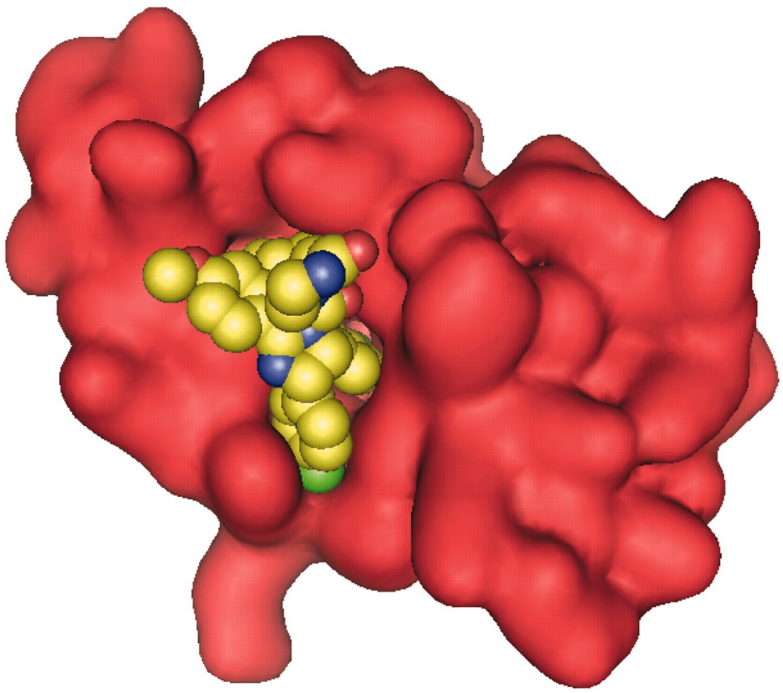
# Nutlin-3 as targeting drug

*The latest insights about the ins and outs of targeting therapy with Nutlin-3*



*Nutlin-3 bound at p53 pocket of MDM2*

Bachelor thesis

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## Abstract

The interaction of the tumorsupressor protein p53 with MDM2 is a potential target for small inhibitors like Nutlin-3, RITA and MI-219. MDM2 is the major negative regulator of p53 through a negative feedback loop. Inhibiting the MDM2-complex with for example Nutlin-3 leads to a higher expression of the p53 protein. The effects of Nutlin-3 are mostly seen in tumor cell lines with wild-type p53 whereas cell lines with mutated p53 often show less or less effect. In cell lines with mutated p53 other pathways need to be activated. Cell cycle arrest seems to be the main response of wild-type p53 cells to Nutlin-3 treatment but the exact mechanism is still unclear. This study further gives an overview of different cancers and the effect of Nutlin-3 treatment on these cancers including some other drugs interfering in the p53-MDM2 interaction. Overall Nutlin-3 is a promising drug which has a future in front of it.

The image on the cover page is published in PNAS volume 103 no. 6 pages 1657-1658 at introduction of an article of Tovar *et al.* (1)

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# Contents

## Introduction

The tumorsupressor protein p53 is one of the most studied proteins in the human body. P53 connects several cell functions and is also concerned in cell proliferation. Activated by different kinds of stress, p53 activates different pathways involved in cell proliferation. The cell can go into apoptosis, cell cycle arrest or senescence. Senescence is the process where cells do not undergo mitosis, but in stead of going in apoptosis these cells stay alive. These cells are characterized with a specific phenotype. It is thought that this process is a major contributor to aging. The pathways leading to the other two processes, apoptosis and cell cycle arrest are affected in nearly all cancers which lead to an unrestrained proliferation. In cancer p53 is mutated in 50% of all tumors, while the other 50% contain wild-type p53. (2) Most of the tumors containing wild-type p53 have a mutation elsewhere in the p53-pathway. (3) Understanding the signal transduction route of p53 leads to drugable targets and a good therapy against cancers.

The interaction of murine double minute 2 (MDM2) with p53 is one of those targets for targeting drugs. MDM2, also known as human double minute 2 (HDM2), is the major negative regulator of p53 and often different expressed in cancers with mutated and wild-type p53. The binding site of MDM2 partly overlaps with the transactivation domain of p53 en thereby inhibits the activity of p53. (Figure 1a) (4, as reviewed in 5) The amount of MDM2 is moreover controlled by p53 itself which makes it a negative-feedback loop. (as reviewed in 5) This loop can be interrupted by small-molecule inhibitors. Examples of the inhibitors are Nutlin-3, ML-219 and several others. (as reviewed in 5) This review focused on Nutlin-3 which is extensively studied the last few years. (Figure 2) But before concentrate at the interaction of Nutlin-3 with MDM2 some other pathways around the MDM2-p53 interaction are essential to be known. (Figure 3)

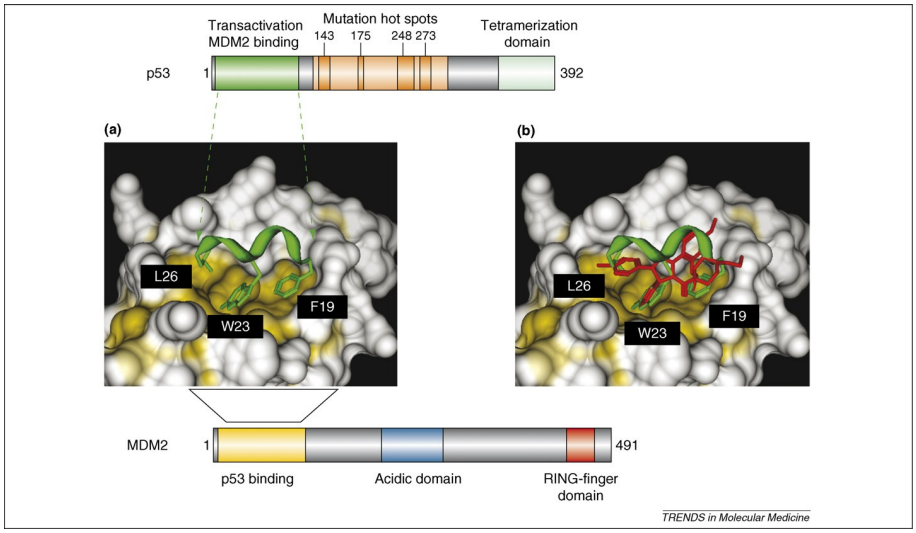


*Figure 1 MDM2-p53 interaction. (a) The binding site of MDM2 with p53 partly overlaps with the transactivation domain of p53. Thus MDM2 inhibits the expression of p53. (b) Nutlin binds to the p53 pocket and thereby blocks the p53-MDM2 interaction. (as reviewed in 5)*

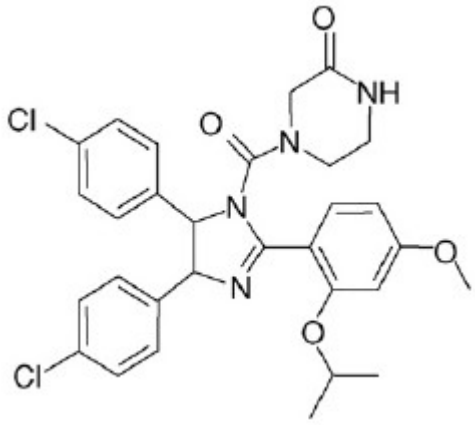


*Figure 2 Chemical structure Nutlin-3 [4-[4,5-Bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxy-phenyl)-4,5-dihydro-imidazole-1-carbonyl]-piperazin-2-one]. (5)*

p14$^{ARF}$, also known as ARF, is a tumor suppressor which inhibits MDM2 and thereby upregulates p53. Also p14$^{ARF}$ has a negative feedback loop because p53 inhibits the expression of p14$^{ARF}$. (6) A complete different pathway, but also interacting with MDM2, is the PI3K/Akt-pathway. Akt phosphorylates MDM2 on ser166 and ser186 after which MDM2 becomes active (7).The PI3K/Akt pathway is again regulated by PTEN, which inhibits the amount of p-Akt (phosphor-Akt) by blocking the interaction of PI3K with Akt. (see Figure 1) (as reviewed in 8)

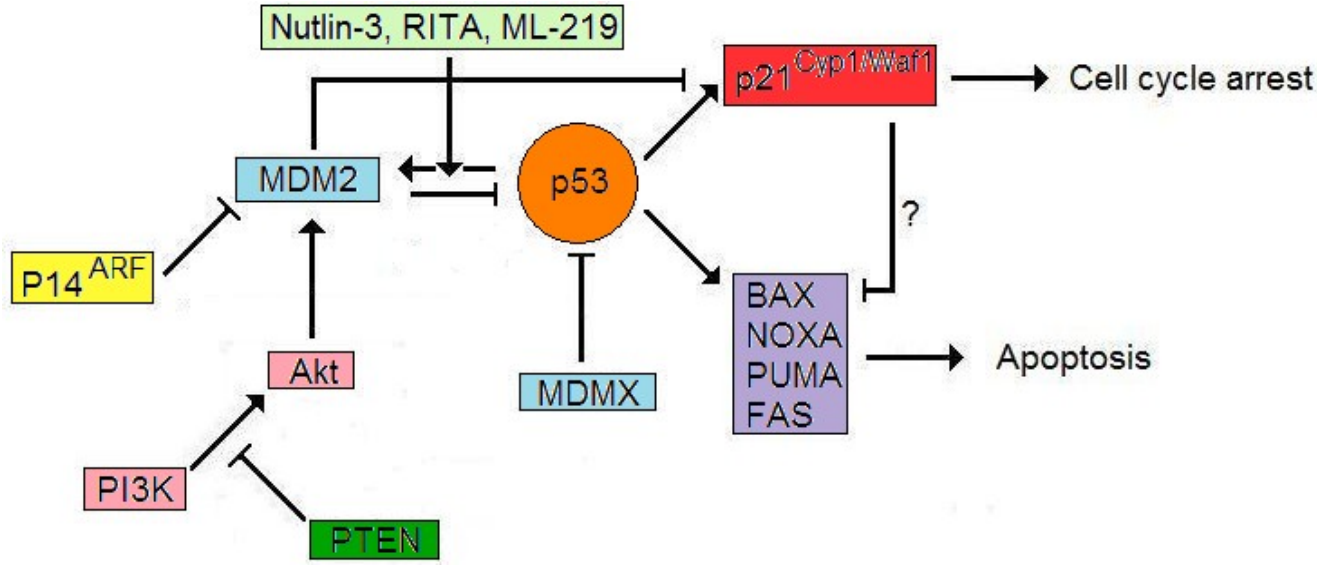


*Figure 3 An overview of interactions that may concern Nutlin-3 treatment.*

A slightly different negative regulator of p53 is murine double minute X (MDMX), also known as MDM4. MDMX has a high similarity with MDM2. MDMX can form hetero complexes with MDM2 and contribute to the degradation of p53 by ubiquitination and stabilize p53. (as reviewed in 6 and 9)

Pathways, which are downstream the p53-MDM2 interaction, are involved in the processes of p53-dependent cell cycle arrest or apoptosis. However, upstream pathways seem to interact with these factors. The main protein responsible for the induction of cell cycle arrest is p21$^{Cip1/Waf1}$. Upregulation of this protein is thought to be needed for undergoing the cell cycle arrest. (as reviewed in 10). P21$^{Cip1/Waf1}$ does interact again with other proteins involved in apoptosis but the discussion is still going. Furthermore MDM2 inhibits the expression of p21$^{Cip1/Waf1}$ On the other hand there is the process of apoptosis, which is

regulated by a dozen of factors like NOXA, PUMA, BAX, FAS and so on. (see Figure 1) More details are reviewed by Vassilev (5) and Brown *et al.* (6)

As described earlier, Nutlin-3 binds to the negative regulator of p53 called MDM2. (Figure 1b) By binding the MDM2-p53 interaction is disrupted and no negative feedback will take place. Logically, lacking its major negative regulator the expression of p53 will increase. Higher expression of p53 leads to more apoptosis and cell cycle arrest, which is the current idea now. However, several questions remain with respect to Nutlin-3 efficacy: Does this principle work with mutated p53? Is there more induction of apoptosis than cell cycle arrest? Do recent clinical results give more information about Nultin-3 therapy? All questions are still under discussion and not fully answered. Therefore the aim of this review is to study if Nutlin-3 is the right choice for treating cancers.

## Chapter 1. Nutlin-3 is not the only targeting drug of MDM2-p53 interaction

Nutlin-3 is one of the many targeting drugs used in pre-clinical studies, and it is even in clinical study already. But there are more drugs targeting the MDM2-p53 interaction. This part will discuss the advantages and disadvantages of two of these drugs, RITA and ML-219.

*RITA*

A drug targeting the interaction between p53 and MDM2 is RITA (reactivation of p53 and induction of tumor cell apoptosis). (Figure 4a) In stead of binding to the MDM2, RITA binds at the N-terminal domain of p53. (Figure 4b) RITA can also bind other faces of the p53 protein but is highly specific for the hydrophobic MDM2 p53 transactivation domain-binding cleft. (11) Zhao *et al.* found induction of apoptosis in mutated p53 cancer cell lines after treatment with RITA. (12) In further research they suggested that the increased phosphorylation of ser-46 in p53 is the cause of increased transcriptional activity after treatment with RITA. Surprisingly no induction of p53-dependent apoptosis was seen in wild-type p53 JHU-028 cells. (13) Complete opposite results were found by Zhang *et al*, where only induction of apoptosis was found in the wild-type 53 and not in the mutated p53. (14)

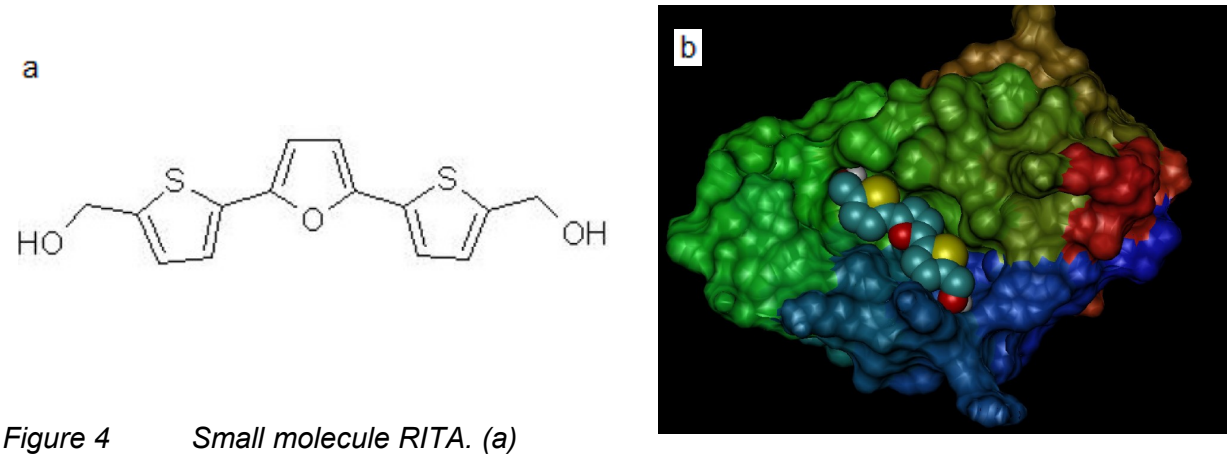


*Figure 4 Small molecule RITA. (a) Chemical structure RITA [2,5-bis(5-hydroxymethyl-2-thienyl)furan] (b) RITA bound to the p53 transactivation domain-binding cleft. (11)*

To find the exact mechanism of RITA-dependent interruption of the MDM2-p53 interaction a study in yeast is performed. It was proposed that RITA at least partially has a direct interaction with the protein p53 as seen in Figure 3b. (15) But, from the moment RITA was discovered the specificity of RITA is discussed. In 2005 a letter was send to the editor about the article previously mentioned (14), where they counter the fact of RITA inducing p53-dependent apoptosis using nuclear magnetic resonance (NMR). With this technique they can show binding of MDM2 to p53. Even with relative high concentrations of RITA, a MDM2-p53 complex was formed. (16) This was again disproved where RITA induced a conformational change of p53 and thereby had its effects. (16) This discussion indicates the precise mechanism is still unclear and the specificity of RITA is not sure. More recently, p53-independent induction of apoptosis was found in testicular cancer cells. (17) These results indicate a different pathway of how RITA induces apoptosis in cancer cells and maybe this explains why mutated p53 cancer cell lines do react on RITA treatment. The exact pathway of induction of apoptosis using RITA is still not clear and therefore it is till now hard to conclude things about the specificity of RITA to the p53-MDM2 interaction.

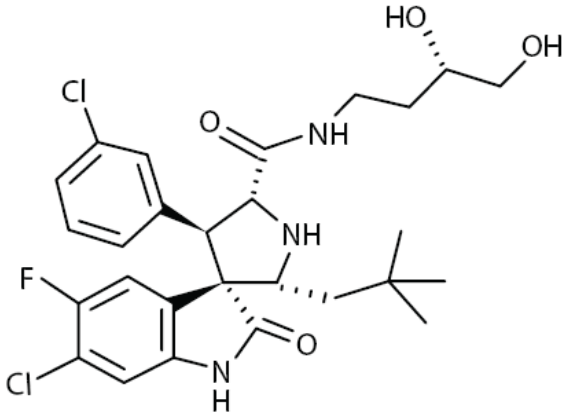


*Figure 5 Chemical structure MI-219 (18)*

*MI-219*

Recently the spiro-oxindole MI-219 drug is discovered also targeting MDM2-p53 interactions by binding the MDM2 protein. (Figure 5) ML-219 mimics the four essential binding points of p53 on MDM2 and binds with MDM2 with a much lower $K_i$ value, 5 nM, compared to Nutlin-3 which has a $K_i$ value of 36 nM. (as reviewed in 19, 20), meaning that the affinity to MDM2 is very high compared to Nutlin-3. (Figure 6) Also the oral bioavailability of ML-219 is high in mouse (55%). Just as Nutlin-3, wild-type p53 seems to be essential for a p53-dependent reaction of MI-219. Different to Nutlin-3 and other targeting drugs involved in the p53-MDM2 interactions, MI-219 is a nonpeptidic drug. (as reviewed in 19). All concluding that MI-219 is a very selective and potent targeting drug for the p53-MDM2 interaction.

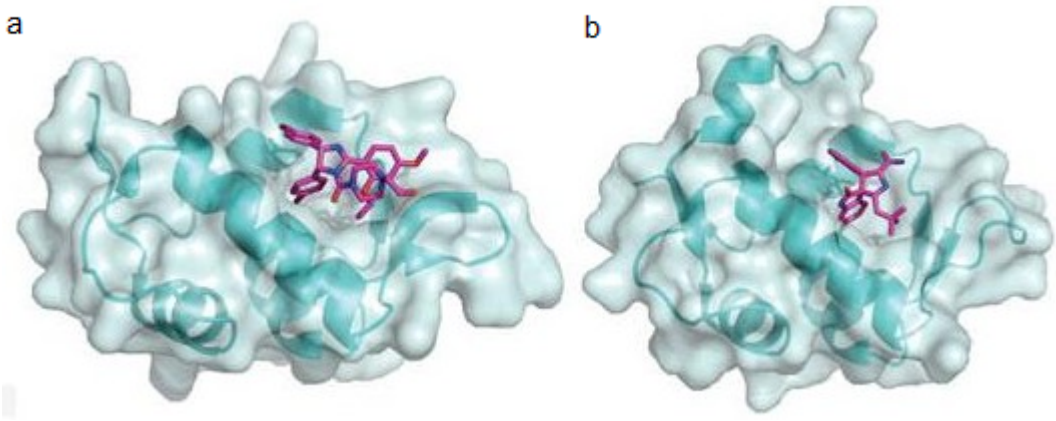


*Figure 6 Interaction of small molecule inhibitors with MDM2. (a) Nutlin-3 bound to the N-terminal domain of MDM2. (b) ML-219 bound to the N-terminal domain of MDM2. (21)*

Nowadays the mechanisms on which ML-219 works are still under investigation. Azmi *et al.* recently added new insights in the pathways affected through MDM2 inhibition by MI-219 (22) Knowing these exact mechanisms is needed to use ML-219 as a therapeutic drug against several cancers containing wild-type p53. Till now MI-219 is a very promising drug which may be even more effective than Nutlin-3 treatment but further research should reveal the chances of MI-219 becoming a drug used in targeted cancer therapy.

## Chapter 2. Effects of Nutlin-3 in wild-type p53 and mutant p53

A lot of discussion is going on about the Nutlin-3 treatment. A part of the discussion is about the differences between wild-type p53 and mutant p53 in reaction to Nutlin-3 treatment. In this chapter is explained, whether Nutlin-3 is an effective treatment for cancers with wild-type p53 as well as cancers with mutant p53. An overview of the some studies at mutated or wild-type p53 cell lines is found in table 1.

The interaction between MDM2 and p53 is blocked by the targeting drug Nutlin-3 as mentioned before. Nutlin-3 binds a domain of MDM2 and thereby blocks the inhibition of p53. Half of all tumors have a mutation in de p53 and the other half has often mutations up or downstream the p53 pathway. (2, 3) Maerken *et al.* studied 34 human neuroblastoma cell lines. In 9 cell lines with a mutated p53, resistance to Nutlin-3 was observed. The other 25 cell lines with wild-type p53 were nearly all sensitive to Nutlin-3 treatment resulting in cytotoxic and antiproliferating effects. Only 2 of 25 cell lines with wild-type p53 were resistant to Nutlin-3 treatment, due to a downstream mutation in the p53 pathway. (23)
Maybe a wild-type p53 and no mutations in the downstream pathway of p53 is essential for an effect of Nutlin-3. Nowadays it is well established that wild-type p53 is needed to induce a p53-depended apoptosis or cell cycle arrest. There are several targeting drugs like Pas and PRIMA-1 which restores the wild-type functions in mutated p53 and then treatment with Nutlin-3 or RITA was done. (reviewed in 24) Still some studies found effects of Nutlin-3 in cell lines with mutated p53 without using targeting drugs like Pas and PRIMA-1 (25) Probably repressing the MDM2 does not only lead to a higher expression of p53 but also induces other pathways independent of p53. Some of those p53 independent pathways can also induce cell cycle arrest and cell death. It seems there are around 40 other proteins found that interact with MDM2, some with nearly no consequences and some that can be very important in cancer progression. (reviewed in 26) One of the proteins interacting with MDM2 is $p21^{Waf1}$. Binding with specific domains of MDM2 leads to degradation of $p21^{Waf1}$ en therefore to less cell cycle arrest. (ref 27) This may be a mechanism of cancer cells avoiding cell cycle arrest by upregulation of MDM2. But, Koster *et al.* showed a pathway where p21 is cytosolic localized thereby inhibiting Fas-induced apoptosis in cisplatin-resistant TC cells. (17, 28) Degradation of $p21^{Waf1}$ by MDM2 may thus lead to more Fas-induced apoptosis. Contradictory results were found in some other cancers, p21 would not be inhibiting the apoptosis, induced by non-genotoxic p53 activation. (29) The difference probably lays in the localization of p21, nuclear localized p21 does not prevent the apoptosis and cytosolic p21 does prevent apoptosis.

In contradiction with many other studies Zhao *et al.* found a restoration of transcriptional activity and p53 mediated apoptosis in mutated p53 cells after treatment of RITA. This was found in lung, breast, skin and colon carcinoma's and also in Burkitt lymphoma. They restored the transcriptional activity of p53 adding RITA for 24 hours. Cells died in approximately 7 to 9 days after treatment. (12) Further research is needed to find out why in this case, and in many more studies inductions of apoptosis was found in mutant cells. However, most of the times this is observed with another targeting drug then Nutlin-3. (13)

Insights in recent years performed with a lot of different cancer cell lines showed that wild-type p53 is mostly essential for a p53 dependent apoptosis or cell cycle arrest after treatment with Nutlin-3. But this does not exclude the involvement of other pathways that are activated or downregulated by treatment of Nutlin-3. Insights of p53-dependent apoptosis in mutated cells in reaction to RITA is also surprising and needs more studying of the exact mechanisms. Therefore more research is needed in the pathways around the p53-MDM2 interaction.

| Cancer | Studied by | Targeting drugs used | Reaction |
|---|---|---|---|
| Retinoblastoma | Maerken *et al.* (23) | Nutlin | + |
| Ewing's sarcoma | Sonnemann *et al.* (42) | Nutlin | + |
| Chronic Lymphocytic Leukemia | Zauli *et al.* (25) | Nutlin | + |
| acute lymphoblastic leukemia | Zhu *et al.* (40) | Nutlin | + |
| Testicular cancer | Koster *et al.* (22) | Nutlin | + |

| Cancer | Studied by | Targeting drugs used | Reaction |
|---|---|---|---|
| Retinoblastoma | Maerken *et al.* (23) | Nutlin | - |
| Ewing's sarcoma | Sonnemann *et al.*(42) | Nutlin | - |
| Lung-, breast-, skin-, colon carcinomas and Burkitt lymphoma | Zhao *et al.* (12) | RITA | + |
| Chronic Lymphocytic Leukemias | Zauli *et al.* (25) | Nutlin | + |

Table 1. Targeting drugs used in wild-type or mutated p53 cancer cell lines. (a) Wild-type p53 cancer cell lines (b) Mutated p53 cancer cell lines. In the column reaction the “+” is used if there was a reaction seen and the “-“ means no reaction observed.

## Chapter 3. Cell cycle arrest or apoptosis

The two major pathways by which p53 inhibit cancer growth are cell cycle arrest, a process where $p21^{Cyp1/Waf1}$ is activated, and apoptosis. The induction of p21, a cyclin dependent kinase inhibitor, causes an arrest in the cell cycle at G1 to S or G2 to M-phase. The apoptotic pathway is activated when p53 induces transcription of several pro-apoptotic factors such as NOXA, PUMA and BAX all leading to caspase activation and in the end, regulated cell death. This pathway is also known as the intrinsic apoptotic pathway. Relating this to this review, which pathway is induced when treating cells with Nutlin-3.

Nutlin-3 induces apoptosis in most hematologic malignancies like different types of leukemia. (5, 30) Apoptosis is mainly mediated by the transcription-independent pathway where p53 directly bind to mitochondria but also by the transcription dependent pathway, activating genes like NOXA and PUMA, is shown. (as reviewed in 30) Contrary, most of the solid tumors undergo reversible cell cycle arrest in stead of definitive apoptosis after treatment with Nutlin-3 (1), making it harder to eliminate the tumor with Nutlin-3 therapy combined with chemotherapeutics. One of the mechanisms thought to be responsible for the anti-apoptotic reaction to Nutlin-3 in solid tumors is the induction of p21. This difference between hematologic malignancies and solid tumors may be due to the differences in mutations downstream p53. The pathway inducing apoptosis is more complicated compared to the pathway leading to cell cycle arrest. (as reviewed in 30) Perhaps more mutations in de apoptotic pathway are found in solid tumors compared to hematologic malignancies.

A process that may be responsible for the anti-apoptotic response to Nutlin-3 treatment in solid tumors can be the MDMX-p53 interaction. The inhibition of p53 on a MDMX dependent manner blocks the transcriptional activity of p53. (as reviewed in 9) Recently is discovered that the Hsp90 chaperone complex, highly upregulated in cancers, is essential for their survival. By treating the cells with Nutlin-3 and with an Hsp90 chaperone inhibitor called 17AAG, which interferes with the p53-MDMX complex, the transcriptional activity of p53, and especially the apoptotic pathway, increases. Summarizing, 17AAG destabilizes MDMX, induces pro-apoptotic genes like PUMA, reduces p21 levels and in the end also inhibits the PI3K/Akt pathway. (31) All leading to an apoptotic response in stead of cell cycle arrest and thereby maybe a good drug for therapy.

Lam *et al.* also researched the prospective of MDMX in treatment with Nutlin-3. By using PARP as an indicator of caspase mediated apoptosis they found that MDMX indeed can protect cells against a p53-dependent apoptosis after Nutlin-3 treatment. (32) These results correspond with earlier results where MDMX induces anti-apoptotic proteins. In the same study they think that going into apoptosis after Nutlin-3 treatment depends on the amount of pro- and anti-apoptotic genes and this amount differs in each cancer. (33)

To go further into those anti-apoptotic genes, they can be seen as pro-survival genes. Mcl-1, Bcl-2, IGF-1R, survivin and lot of other genes are regulated by p53 and responsible for a viable cell. (34) To activate apoptosis the pro-apoptotic genes like Bax and Puma need to be activated but also repression of pro-survival genes is necessary for starting the process of apoptosis. It is assumed that if there is no suppression of survival genes it is more likely the cells go into a growth arrest, or in different words, cell cycle arrest. (8) It seems that those two responses of p53, activation of pro-apoptotic genes and suppression of pro-survival genes, are regulated by two different branches, a pro-apoptotic branch and a pro-survival branch which are independently regulated. (34)

Taking all these studies together it suggests that in general cancer cells treated with Nutlin-3 undergo cell cycle arrest. It seems that MDMX plays an important role in the preventing apoptosis because in cancers with high MDMX expression like retinoblastoma, breast carcinomas and leukemia nearly no apoptosis is seen. (33) A different contributor that is proposed to the anti-apoptotic reaction of Nutlin-3 treatment is p21. It seems that the localization of p21 is essential for the further progress but this discussion is very recent and still going on. And the third reason why maybe more cell cycle arrest is seen is because

there might be no suppression of the pro-survival genes. Understanding the exact mechanism is still the main project to proceed in the targeting therapies.

## Chapter 4. An overview of Nutlin-3 treatment in different cancer cell lines

All over the world experiments are done in different cell lines using Nutlin-3. Every cancer has its own characteristics, and therefore different responses to Nutlin-3 treatment. How does Nutlin-3 induce its effects in different cancers and which cancers are the most potential starting a Nutlin-3 therapy? Pediatric tumors and hematological malignancies are more sensitive to Nutlin-3 treatment just because of a high percentage of wild-type p53 state at diagnosis and Nutlin-3 is more effective to cancer with wild-type p53. (35). On top of that more apoptosis was seen in hematological malignancies. A lot of preclinical research in different cancer cell lines is now going on. In the next part a little overview of some cancer cell lines is given.

The eye cancer, retinoblastoma, are initiated with mutations in the gene retinoblastoma 1 (RB1). It is long thought that retinoblastomas bypass the p53 pathways and induce resistance in a more direct way. (36) But since the published study of Laurie *et al.* this theory is not holding completely anymore. After loss of the retinoblastoma protein RB1 during retinogenesis a tumor surveillance pathway is activated. This pathway is regulated by ARF, MDM2, MDMX and p53. Retinoblasts without RB1 expression undergo p53 dependent apoptosis. (36) Therefore also Nutlin-3 treatment could be a therapy used against retinoblastomas. Nutlin-3 affects RB1 expression by a MDM2 dependent pathway. More apoptosis was seen after treatment, where cell cycle arrest was observed in healthy human cell lines. (37) Indicating that the RB1 protein plays an important role in the Nutlin-3 induced apoptosis. (38)

Leukemia is one of those hematological malignancies which are more sensitive to Nutlin-3 treatment. B chronic lymphocytic leukemia (B-CLL) is in 80% the diagnosis of all leukemia's. (39) The effects of Nutlin-3 treatment in B-CLL are studied in combination with Dasatinib, a drug that has entered the clinic. Dasatinib is a tyrosine kinase inhibitor and inhibits the Bcr-Abl and Src family. Results showed a downregulation of MDM2 in a Nutlin-3 dependent matter but also in a Dasatinib dependent matter by the Akt-pathway. In conclusion therapy with Dasatinib + Nutlin-3 offers a good targeting strategy because it also affects mutated p53 cell lines of B-CLL. (25) The same Akt-pathway was involved in acute lymphoblastic leukemia cells (ALL). Also in this study targeting of the Akt-pathway in combination with Nutlin-3 treatment is suggested. (40)

Ewing's sarcoma often occurs in childhood and adolescence. Nowadays a survival around 70% is found after diagnosis. (41) The high amount of wild-type 53 and the fact that no studies were done yet using Ewing sarcoma cell lines and treat them with Nutlin-3, provoked Sonneman *et al.* to research the capabilities of Nutlin-3 treatment. As expected from earlier studies in retinoblastoma and leukemia's, Nutlin-3 induced p53-dependent apoptosis. In contrast also induction of cell senescence was found. (42) Treatment of Ewing's sarcoma with less genotoxic drugs is needed to increase the survival of patients with this cancer.

In melanomas it is again slightly different. Mutations of the p53 gene are very rarely in melanomas so there must be other components of the p53 pathway inactive to evade regulated cell death. The answer lies in the expression of the secreted protein acidic and rich in cysteine (SPARC), which activates MDM2 through the PI3K/Akt pathway. SPARC enhances the interaction between PI3K and Akt which lead to a higher phosphorylation of MDM2 by Akt and thereby higher expression of MDM2. This leads to silencing of p53 in melanomas. (7, as reviewed in 8) Blocking the high amount of MDM2 using Nutlin-3 and thereby increase the expression of p53 could be a way to stop melanoma growth. And indeed, it is proposed that melanoma cells undergo cell cycle arrest, rather then apoptosis, after Nutlin-3 treatment. (43, 44) It is not yet know why these cells undergo more cell cycle arrest instead of apoptosis.

In testicular cancer also nearly no mutated p53 is found and most of the patients are sensitive to cisplatin treatment. (45) Only approximately 10% of the patients are resistant to

cisplatin treatment. In contrast to melanomas is treatment of testicular cancer cells with Nutlin-3 and cisplatin leading to Fas/FasL system induced-apoptosis and not to cell cycle arrest. (17) The same results were found in Hodgkin cell lines and acute myeloid leukemia. This may be due to the localization of p21, because cytosolic p21 inhibits the cisplatin induced cell cycle arrest and apoptosis. (28) So maybe p21 does not protect cells against apoptosis. Overall Nutlin-3 in combination with cisplatin is proposed to be a good treatment against resistant testicular cancer cells. (17)

Overall, these results indicate that Nutlin-3 is effective in many cancers and thereby a promising targeting drug. The mechanism by which Nutlin-3 triggers these effects is moreover the same but the mechanisms by which apoptosis or cell cycle arrest is enriched differs between the cancers.

## Conclusion

Improving cancer treatment is important because the current treatment does not always work properly. Identification of the main problem, which is characteristic for every cancer, is a theory that does not hold anymore. As described before, every cancer has its own Achilles heel and needs a special therapeutic approach. The question if Nutlin-3 is the key drug in lots of cancers is hard to answer but it seems that treatment with only Nutlin-3 is in most cases not enough to cure the cancer. Many studies have looked for combinations of targeted therapy to induce maximum effect. (25,43,44) At first, Nutlin-3 awaits clinical trials and when this leads to a new targeting drug the first use will probably will be in combination with chemotherapeutics. Hopefully combinations of different targeting drugs can be used in future preventing the sometimes bad side effects of chemotherapeutics.

First Nutlin-3 will probably be used in tumors containing wild-type p53. Studying the mutated p53 is still going on and the induction of apoptosis or cell cycle arrest in mutated p53 tumors by Nutlin-3 needs more prove. Maybe here also a combination drug is needed to induce apoptosis in mutated p53 tumors.

Lately disturbing results where found. Nutlin-3 interacts with MDM2, the negative regulator of p53. Therefore it was thought that Nutlin-3 treatment did not affect p53. However, Aziz *et al.* showed that Nutlin-3 does affect p53. After exposing SJSA-1, containing wild type p53, multiple times to Nutlin-3 and then select individual clones, two groups where formed. Group 1 which was apoptosis resistant but did undergo cell cycle arrest and group 2 was resistant to apoptosis as well as cell cycle arrest. They found some mutated p53 clones in group 1 and p53 was mutated in all clones of group 2. In group 2 clones were unable to bind the promoters of p21 and PUMA. (46) These results are just published so more research is needed to confirm disturbing results because this might be important for an eventually clinical use of Nutlin-3.

Beside the fact that Nutlin-3 may induce resistance it could also affect the healthy cells. Most of the times p53 and MDM2 are low expressed in cells and therefore effects of Nutlin-3 on these cells is not expected. But organs that undergo fast renewal may be more susceptible to Nutlin-3. Results of the clinical trials which are now in progress will tell more about the side effects of Nutlin-3 treatment.

Awaiting the results from the clinical trials more research is needed to get an exact idea of the pathways around the p53-MDM2 interaction. If Nutlin-3 does work well there is always a next problem to solve.

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