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p53: A tale of complexity and context

Moshe Oren[1,*] and Carol Prives[2,*]
[1]Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel
[2]Department of Biological Sciences, Columbia University, New York, NY, USA
*Correspondence: moshe.oren@weizmann.ac.il (M.O.), clp3@columbia.edu (C.P.)
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The story of p53 is illuminating. Despite widespread attention, the tumor-suppressive functions of wild-type p53 or the oncogenic activities of its cancer-associated mutants are still not fully understood, and our discoveries have not yet led to major therapeutic breakthroughs. There is still much to learn about this fascinating protein.

With the looming 45th anniversary of the discovery of p53, we note that there are almost 120,000 papers that deal with research on this potent tumor suppressor. Indeed, just a few years ago, the *TP53* gene, encoding p53, was voted the most "popular" human gene.[1] Now, on the 50th anniversary of *Cell*, we reflect on what we have learned about p53 after all this attention. We describe here the paradox of how, depending on its mutational status in cancer, p53 can be both a tumor suppressor and an apparent oncogene. The major challenge of translating the vast store of p53 knowledge into clinical practice is discussed as well. We start by recounting the story of how p53 came to be so renowned.[2]

Early experiments suggest that *TP53* is an oncogene

In 1979, a new cellular protein was described by several teams in the USA and Europe. Initially given a different name by each team, the then very tiny community eventually converged on "p53" as a consensus. This name, avoiding the assignment of any specific function, purportedly reflected its molecular weight as deduced from its migration in polyacrylamide gels (in retrospect, it should have been dubbed "p44," according to its actual molecular mass, determined upon the cloning of p53). Regardless, the case for p53 seemed quite simple: a cellular protein, scarcely present in normal cells but often much more abundant in cancer cells (Figure 1) and in cells transformed by the oncogenic SV40 DNA virus. By then, numerous RNA tumor viruses had been shown to highjack cellular genes and drive their overexpression, thereby converting them from proto-oncogenes into oncogenes. It was thus natural to postulate that p53 is a similar sort of oncoprotein whose upregulation by tumor viruses or other mechanisms might contribute to cancer. But for any function to be rigorously deduced, the p53 gene needed first to be cloned. This was long before the sequencing of the human genome and even years before PCR was invented. Therefore, researchers had to resort to a cumbersome procedure involving painstaking identification of RNA (via hybridization to hundreds of different recombinant DNA clones) that could be translated into a protein reactive with p53-specific antibodies. Consequently, what nowadays can be achieved in a couple of weeks by a competent undergraduate student took over a couple of years of intensive teamwork. Eventually, the mission was accomplished, and p53 genes (mostly as cDNAs) were successfully cloned from several cancer cell lines. When tested experimentally, they exerted effects, including immortalization of primary cells, multilayered growth in culture, and increased tumorigenicity *in vivo*, that were indeed consistent with p53's being pro-oncogenic (Figure 1). Thus, all the signs pointed to p53's being yet another oncogene. Consequently, cancer research meetings often included a talk on p53 in their "Oncogenes" sessions. The notion that p53 is an oncoprotein, however, did not last for long.

Late 1980s: Wild-type p53 is revealed to be a *bona fide* tumor suppressor

In the early 1970s, Knudsen proposed that the childhood cancer retinoblastoma requires two separate "hits." This seminal hypothesis, which implied the existence of tumor-suppressor genes, eventually led to the cloning of the *RB1* gene in the mid-1980s, rendering tumor-suppressor genes a molecular reality. This was followed by the discovery in 1989 of human colorectal cancer tumors in which one *TP53* allele is deleted while the other allele is mutated, as expected of a tumor-suppressor gene rather than an oncogene. Similar mutations were soon also documented in many other cancer types. Of note, deletion of the p53 gene had already been observed earlier in some mouse cancer models and human cancer cell lines, but these observations were dismissed as exceptions that do not defy the "p53 oncogene" rule. However, when *bona fide* wild-type (WT) p53 was finally tested, it not only failed to elicit oncogenic transformation of cultured cells—it actually potently suppressed transformation driven by classical oncoproteins, including MYC and RAS. Hence, authentic WT p53 behaved as a tumor suppressor, not an oncoprotein (Figure 1).

The end of the 1980s marked the start of the modern era of research into p53 as a central tumor suppressor. As noted then (and still true to the present) from myriad analyses of patient datasets, *TP53* is the most frequently mutated gene in human cancer. The *TP53* mutation landscape is somewhat unusual for a tumor suppressor. *TP53* mutations are mostly of the missense variety, i.e., point mutations, resulting in full-length, often highly expressed proteins. Among these, a handful "hotspot" mutations loom above the others with much higher frequency. Notably, DNA sequencing

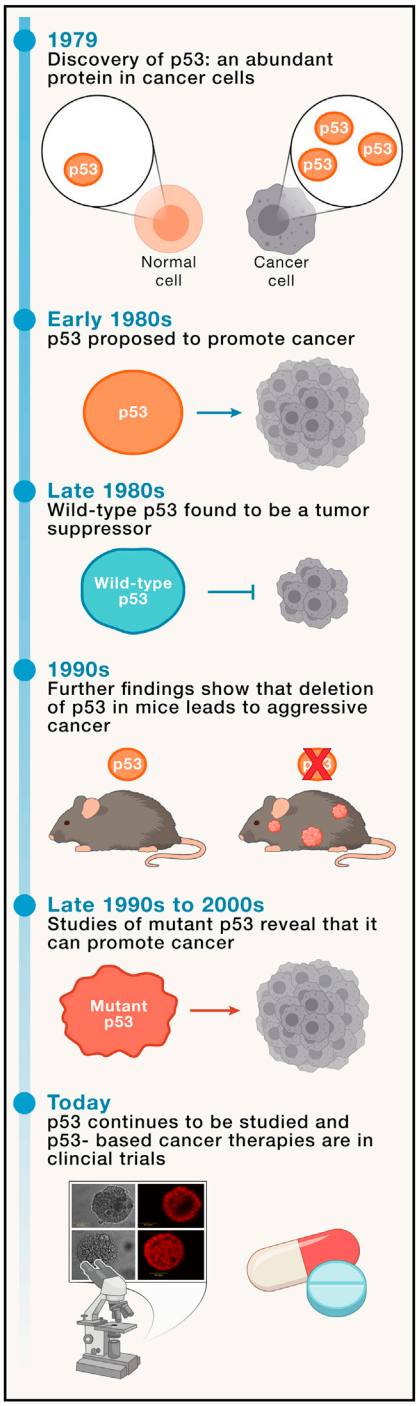

Figure 1. A very abridged timeline of p53 research
See text for details.

revealed that all the previously studied oncogenic p53 cDNA clones, isolated from cancer cells, also carried similar point mutations; as discussed below, such mutants can prevent WT p53 from functioning and may even acquire oncogenic activities. Moreover, in many tumors that retain WT *TP53*, the functionality of their p53 protein is compromised by other alterations, such as overexpression of p53's negative regulators MDM2 and MDM4 (aka MDMX), which can individually functionally repress p53 and also work together to target p53 for proteasomal degradation. The challenge that lay ahead was to decipher what WT p53 does, how it operates as a tumor suppressor, and how p53 mutations contribute to cancer.

1990s: The golden age of p53

Once p53 became a hot topic, the '90s produced a series of critical findings that greatly advanced our understanding.[3] The excitement of the research community about p53 culminated in its selection in 1993 as *Science* magazine's molecule of the year. p53 was also featured on the cover of *Newsweek* (1996) and was the subject of numerous newspaper articles, attesting that its fame had spread even to the general public.

New key findings seemed to completely clarify how p53 works. First came the discoveries that Li-Fraumeni syndrome (LFS) cancer-prone families harbor germline *TP53* mutations (they are born with one WT and one mutant allele) and that the vast majority of the LFS patients' tumors have lost the WT allele. This sealed the deal that p53 is a tumor suppressor in humans. But what about mice? They were key to being able to determine how p53 works in a physiological setting and an essential counterpart to studying p53 biochemically. Building on the excitement about p53 as a tumor suppressor, an International p53 Workshop was held at Princeton in June 1991. After a few days of talks relaying what p53 does and how it works, we learned that there was to be a "late-breaking" talk from a group who had apparently generated a p53 knockout mouse; it is worth mentioning here that, like gene cloning in the previous decade, engineering knockout mice was not quite as straightforward as it has now become. When the news was finally presented to the eagerly anticipating audience, it was revealed that yes, mice had been successfully generated that did not express any p53. But to everyone's bitter disappointment, the now-three-month-old mice appeared to be perfectly normal. Oh no! Why work on p53 if mice do not care whether or not they have it?

Fortunately for the researchers (but not for the mice), those normal-looking mice all came down with aggressive lymphomas just a few months after the Princeton workshop (Figure 1). p53 was important after all and had finally fulfilled all the criteria for qualifying as a full-fledged tumor suppressor. Researchers eagerly sought to use cells from these mice to test their ideas about how p53 might work, and one of the first important observations was that cells from p53-null mice are genomically more unstable than their WT counterparts. Key findings emanating from p53-null mice did not stop there. Indeed, mouse models, generated with increasing sophistication, have been the underpinnings of many fundamental discoveries about p53.[4]

The '90s also saw the discovery of two p53 siblings, p63 and p73 (named as such for convenience). While each has been the subject of very interesting studies, it is safe to say that neither is yet fully appreciated as to whether, when, or how it functions to suppress (or promote) cancer.

Going hand in hand with mouse genetic experiments was research on p53 as a protein and its roles in human cells. We learned that p53 is a DNA sequence-dependent transcription factor whose protein sequence comprises N-terminal transactivation domains and a central DNA binding domain (DBD) followed by an oligomerization domain and, at its extreme C terminus, a complex regulatory domain. Notably, most of the missense mutant forms of p53, including the frequently recurring hotspot mutants, were clustered in the DBD. When the crystal structure of the DBD was reported, showing how p53 binds to a specific DNA sequence, it also indicated why the hotspot mutants fail to do so. p53 was also shown to undergo extensive posttranslational modifications in response to a wide variety of stress signals emanating from upstream damage sensors, such as ATM. These modifications foster p53 activation most critically by disrupting the interactions between p53 and MDM2/MDM4, which normally restrict p53 baseline levels and activity. Solidifying the importance of these interactions was the discovery that deletion of mouse

Mdm2 or Mdm4 causes early embryonic lethality, which is completely rescued by co-deletion of p53, a finding that was eventually extended to many tissues in living mice. Indeed, if it were not for MDM2/MDM4, the p53 in our cells would likely kill us!

The cellular outcomes of increased p53 activity, including cell-cycle arrest, cellular senescence, and apoptotic cell death, were nicely consistent with p53's function as a tumor suppressor. In parallel, the realization that p53 is a stress-dependent regulator of transcription instigated a search for its transcriptional targets. The earliest findings, showing that activated p53 induces expression of the cell-cycle-arresting cyclin-dependent kinase inhibitor p21 (aka Cip1, WAF1, or CDKN1A) and of the pro-apoptotic protein BAX, further seemed to support a simple picture wherein inducing only a few key genes that bring about growth arrest or (better still) cell death was all that was needed of p53 for it to be so important in cancer prevention. As p53 research moved into the 21st century, however, the story became more elaborate.

2000 and onward: p53 is complicated!

The intricacy of tumor suppression by WT p53

As researchers continued to enthusiastically probe the mysteries of this protein, p53 revealed its amazing complexity.[5] Many questions arose that as yet remain unanswered. The simple view that WT p53 has a few transcriptional targets, which account for its ability to either stop growth or kill cells and thereby protect myriad tissue types from diverse forms of cancer, was short lived. Indeed, p53 emerged as a protein of great versatility[6] that can regulate literally hundreds of genes whose products are greatly varied and are matched by a multitude of cellular outcomes that greatly exceed the trio of cell-cycle arrest, apoptosis, and senescence. Further, burgeoning evidence shows that target-gene regulation by p53 varies greatly between different cell types and tissues,[7] thereby demonstrating the remarkable context dependence of p53 as a transcription regulator. We know now that p53 can orchestrate a plethora of biological processes and cell states, including multiple key metabolic pathways, different DNA repair processes, stem cell modulation, aging, development, fertility, and still others. Likewise, p53's role in cell death is not limited only to apoptosis. For example, p53 turned out to be a critical regulator of ferroptosis, an exciting relative newcomer to the pantheon of different types of cell death. We also learned that different stress signals elicit different p53-dependent transcriptional responses, which in turn elicit diverse cellular outcomes. Thus, p53 manifests a great degree of "molecular intelligence." Moreover, it became apparent that p53 also has non-cell autonomous activities. Most notable is p53's crosstalk with the immune system, wherein WT p53 supports the expression of secreted factors that instruct an anti-tumoral immune microenvironment.

As if that were not sufficiently daunting, the story became even more complicated when it was reported that p53 mutants lacking the ability to induce growth arrest, senescence, or apoptosis could still suppress cancer in mice.[8] Thus, the seemingly solved question of how p53 suppresses tumor formation has again become open. Clearly, no single cellular outcome can be ascribed to how p53 does the job. Just as cancer can be viewed as many diseases, varying with the tissue of origin and the particular microenvironment, so p53 may have evolved to protect different key processes that are unique to the tissue contexts in which the tumors may arise.

The enigma of mutant p53: Counting gains and losses

We circle back to the early experiments done with mutant versions of p53 (mutp53). Why did they augment transformation and tumor growth? And why do cancer cells often display excessive amounts of mutp53, to the extent that intense p53 staining in histology slides is considered a proxy for the existence of a *TP53* mutation in a tumor? To answer this, it was proposed that some mutp53 variants might have acquired novel gain-of-function (GOF) oncogenic activities (Figure 1), resurrecting the "p53 oncogene" concept, albeit in altered form.[9] This was also based on evidence that genetically engineered mutp53 knockin mice develop different and more metastatic forms of cancer relative to p53 knockout mice.

Further support for mutp53 GOF emerged gradually through studies showing that common cancer-associated mutp53 variants inversely mirror the activities of WT p53 in their abilities to exert a plethora of molecular and biochemical effects, both cell autonomous and non-cell autonomous (Figure 2), altering the transcriptional landscape; deregulating metabolism; inhibiting DNA repair; inducing aneuploidy; augmenting cell proliferation, survival, migration, and invasion; attenuating anti-tumor immunity; and promoting metastasis, all possibly contributing to oncogenesis. Remarkably, the WT and tumor-derived mutant forms of p53 appear to function in direct opposition to each other to regulate virtually all of the iconic "hallmarks of cancer" proposed by Hanahan and Weinberg.[10] mutp53 GOF is largely exerted through interactions with other cellular proteins, including sequence-specific transcription factors. Particularly interesting targets are p63 and p73, transcription factors and members of the extended p53 family.

Like the confusion surrounding the tumor-suppressor action of WT p53, the existence of mutp53 GOF has also been recently challenged experimentally.[11,12] How can one resolve such apparent controversy? The almost inevitable conclusion is that mutp53 GOF is context dependent: the same p53 mutant may possess GOF in a particular context while completely lacking it in different contexts. This is epitomized by the observation that the impact of p53 hotspot mutants can shift all the way from tumor suppressive to cancer promoting, depending on metabolites secreted by gut microbes.[13]

Whether mutp53 exerts GOF effects is not just of academic interest. The answer will dictate, for example, the utility of drugs that promote degradation of mutp53 proteins. Where there is no mutp53 GOF, there is no point in getting rid of mutp53. Obviously, the context dependency of mutp53 action will be fertile soil for further investigation.

Where are the drugs?

Ever since the importance of p53 was appreciated, extensive efforts have been undertaken in both pharma and academia to develop p53-based therapies, primarily aiming to reconstitute p53 functionality in

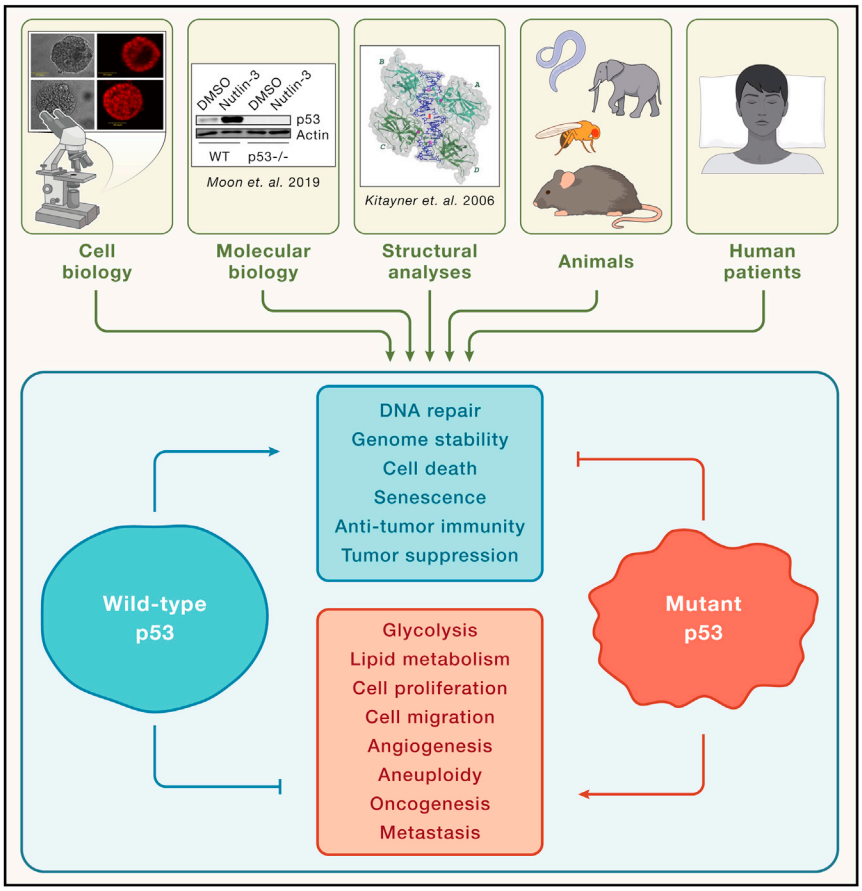

Figure 2. Different approaches reveal opposing outcomes of wild-type and mutant p53
Top: p53 has been studied using cell and molecular biology approaches; high-resolution structural analyses; and numerous animal model systems, including worms, flies, and mice (and, to some extent, even elephants), combined with extensive mining of human cancer patients' datasets.
Bottom: wild-type (WT) p53 helps maintain metabolic homeostasis and genome integrity and restrains cancer-associated processes, thereby suppressing tumorigenesis. Cancer-associated *TP53* mutations abrogate WT p53's tumor-suppressive activities and, in the case of missense mutations, may endow the resultant mutant p53 (mutp53) proteins with oncogenic gain-of-function activities, which further facilitate cancer progression.

cancer cells that have lost it, thereby hopefully triggering their demise.[14] As early as in the 1990s, at least two p53-based strategies had already made it into phase 3 clinical trials, raising great hopes that they would soon join oncologists' armamentarium. Both employed modified adenoviruses, one delivering a WT p53 gene into the tumor, thus representing *bona fide* gene therapy, and the other selectively killing cancer cells lacking functional p53 (oncolytic virus). Unfortunately, lethal complications encountered in virus-mediated gene therapy clinical studies around the turn of the century triggered an almost complete moratorium on further gene therapy work. Nevertheless, p53-based gene therapy was subsequently revisited in China, leading in 2005 to approval for cancer treatment of Gendicine, a p53-expressing recombinant adenovirus. Definitive conclusions on the efficacy of Gendicine are still being awaited.

With gene therapy in disfavor, subsequent work on p53-based therapy took two main directions, instructed by the *TP53* status of the treated cancer. About half of all human tumors harbor no *TP53* mutations, but their WT p53 apparently fails to perform its tumor-suppressive job. Since p53 is largely restricted by MDM2/MDM4 through direct binding, compounds that disrupt this binding are prime candidates for p53 potentiation. Alternatively, in tumors that express mutp53, it would seem cogent to design compounds that "correct" the defective mutp53, for example, by promoting its proper refolding, thus turning the excessive mutp53 into excessive WT p53. This would also eliminate putative mutp53 GOF, delivering two hits in one drug. In the same vein, compounds that target mutp53 for degradation should also abrogate its GOF. Notably, targeting mutp53 proteins is especially attractive because such proteins are unique to cancer cells. Indeed, many MDM2/MDM4 binding inhibitors and mutp53-targeting compounds have been developed over the years and have demonstrated impressive anti-cancer activities *in vitro* and in animal models.

So why are there still no approved p53-based drugs in the clinic? There are multiple answers to this question. Notably, MDM2 inhibitors would potentiate p53 not only in cancer cells but also in normal cells in which p53 is kept in check by MDM2. Some normal cells, particularly in the bone marrow, are particularly affected, as was sadly realized when promising MDM2 inhibitors entered clinical trials. On the other hand, while the tested mutp53-reactivating molecules proved to be more tolerable, they have so far not demonstrated sufficient efficacy in clinical trials.

Attempts to develop p53-based cancer therapy are actively continuing. A promising avenue may be compounds that are selective for individual p53 mutants, exemplified by PC14586 that specifically targets the Y220C mutant.[15] Moreover, inspired by the remarkable success of cancer immunotherapy, increasing efforts are being put into harnessing p53 knowledge toward tumor immune eradication, e.g., by modulating p53 activity in order to alter the immune tumor microenvironment or by directing the immune system to attack cancer cells that display mutp53-derived neoantigens. p53 is too obvious to be disregarded. We believe that despite the many hurdles, successful p53-based cancer therapy will eventually become a reality.

Finale

The story of p53 provides a vivid example of how what initially seems clear and simple turns out to be everything but that. Many questions that once were thought

to have been fully answered are still in need of better answers. Moreover, p53 is now recognized not only as a tumor suppressor but also as playing other physiological roles, revealed through mouse models and human disease.[6] Can such studies point the way to new treatments for conditions other than cancer? Future studies will certainly enlarge our understanding of this amazing protein and may even provide the long-awaited therapeutic breakthroughs that are being so fervently sought.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Dolgin, E. (2017). The most popular genes in the human genome. Nature *551*, 427–431. https://doi.org/10.1038/d41586-017-07291-9.
2. Levine, A.J., and Oren, M. (2009). The first 30 years of p53: growing ever more complex. Nat. Rev. Cancer *9*, 749–758. https://doi.org/10.1038/nrc2723.
3. Vousden, K.H., and Prives, C. (2009). Blinded by the Light: The Growing Complexity of p53. Cell *137*, 413–431. https://doi.org/10.1016/j.cell.2009.04.037.
4. Donehower, L.A. (2014). Insights into wild-type and mutant p53 functions provided by genetically engineered mice. Hum. Mutat. *35*, 715–727. https://doi.org/10.1002/humu.22507.
5. Kastenhuber, E.R., and Lowe, S.W. (2017). Putting p53 in Context. Cell *170*, 1062–1078. https://doi.org/10.1016/j.cell.2017.08.028.
6. Boutelle, A.M., and Attardi, L.D. (2021). p53 and Tumor Suppression: It Takes a Network. Trends Cell Biol. *31*, 298–310. https://doi.org/10.1016/j.tcb.2020.12.011.
7. Pant, V., Sun, C., and Lozano, G. (2023). Tissue specificity and spatio-temporal dynamics of the p53 transcriptional program. Cell Death Differ. *30*, 897–905. https://doi.org/10.1038/s41418-023-01123-2.
8. Li, T., Kon, N., Jiang, L., Tan, M., Ludwig, T., Zhao, Y., Baer, R., and Gu, W. (2012). Tumor suppression in the absence of p53-mediated cell-cycle arrest, apoptosis, and senescence. Cell *149*, 1269–1283. https://doi.org/10.1016/j.cell.2012.04.026.
9. Freed-Pastor, W.A., and Prives, C. (2012). Mutant p53: one name, many proteins. Genes Dev. *26*, 1268–1286. https://doi.org/10.1101/gad.190678.112.
10. Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. Cell *144*, 646–674. https://doi.org/10.1016/j.cell.2011.02.013.
11. Boettcher, S., Miller, P.G., Sharma, R., McConkey, M., Leventhal, M., Krivtsov, A.V., Giacomelli, A.O., Wong, W., Kim, J., Chao, S., et al. (2019). A dominant-negative effect drives selection of *TP53* missense mutations in myeloid malignancies. Science *365*, 599–604. https://doi.org/10.1126/science.aax3649.
12. Wang, Z., Burigotto, M., Ghetti, S., Vaillant, F., Tan, T., Capaldo, B.D., Palmieri, M., Hirokawa, Y., Tai, L., Simpson, D.S., et al. (2024). Loss-of-Function but Not Gain-of-Function Properties of Mutant TP53 Are Critical for the Proliferation, Survival, and Metastasis of a Broad Range of Cancer Cells. Cancer Discov. *14*, 362–379. https://doi.org/10.1158/2159-8290.CD-23-0402.
13. Kadosh, E., Snir-Alkalay, I., Venkatachalam, A., May, S., Lasry, A., Elyada, E., Zinger, A., Shaham, M., Vaalani, G., Mernberger, M., et al. (2020). The gut microbiome switches mutant p53 from tumour-suppressive to oncogenic. Nature *586*, 133–138. https://doi.org/10.1038/s41586-020-2541-0.
14. Hassin, O., and Oren, M. (2023). Drugging p53 in cancer: one protein, many targets. Nat. Rev. Drug Discov. *22*, 127–144. https://doi.org/10.1038/s41573-022-00571-8.
15. Poh, A. (2022). Glimmers of Hope for Targeting p53. Cancer Discov. *12*, OF5. https://doi.org/10.1158/2159-8290.CD-ND2022-0009.