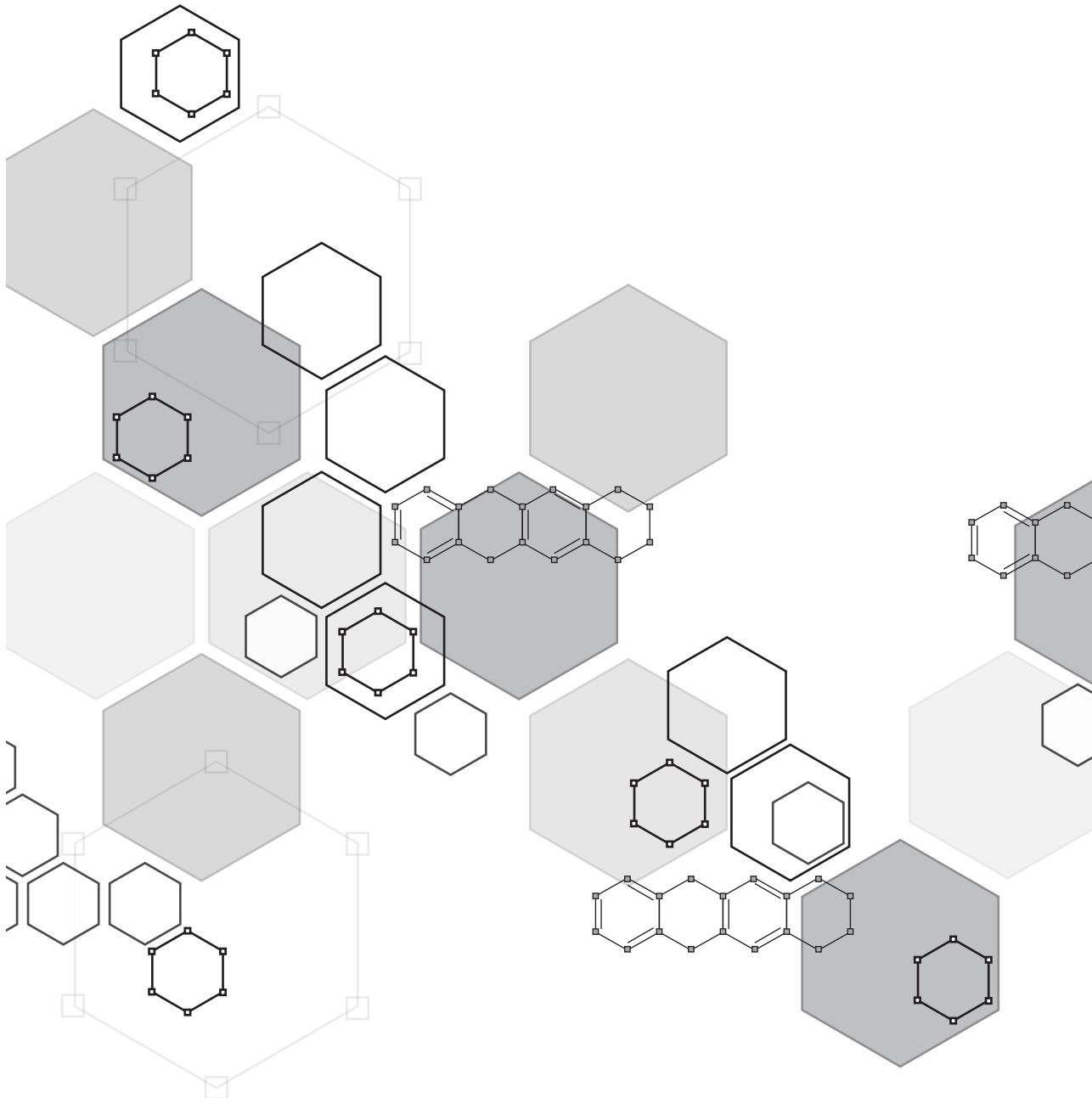
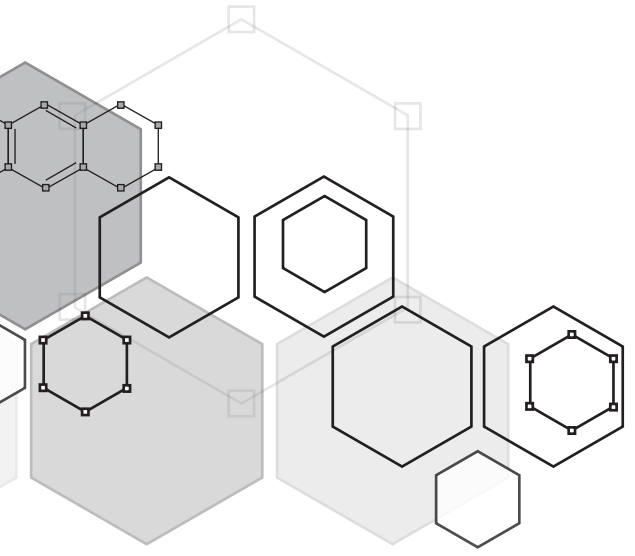
Novel insights into old anticancer drugs

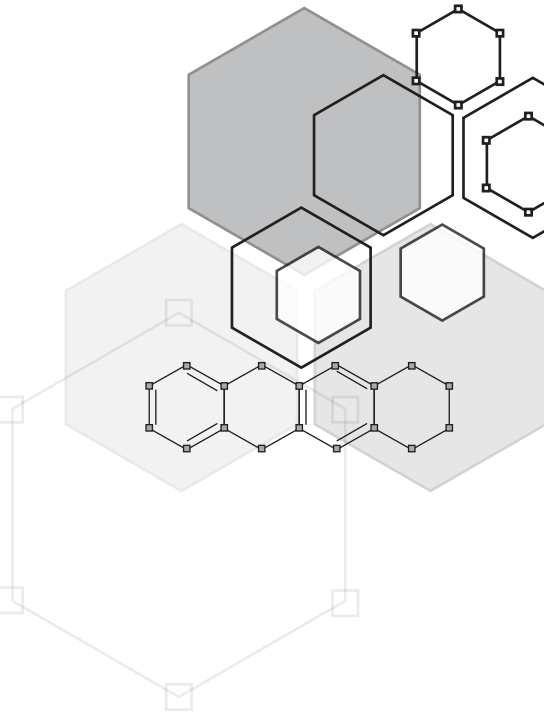
Zanden, S.Y. van der



Summary and future prospects

9





SUMMARY AND FUTURE PROSPECTS

After infectious diseases, cancer is the leading cause of death worldwide, with about 1 in 5 men and 1 in 6 women diagnosed with cancer during their lifetime [1]. A tumor results intrinsically from tissue growth deregulation and develops when normal cells start dividing in an uncontrolled manner. In order for a normal cell to transform into a cancer cell, genes that tightly control the cell cycle and cell growth get altered, often via mutations in or deletions of so-called oncogenes or tumor suppressor genes [2]. Many therapies exist to treat cancer, and multiple new treatment options are developed and implemented in the clinic as medical research keeps progressing. These treatments include targeted therapies and cancer-immunotherapy, which have shown high efficiency in certain tumor types. Nevertheless, the primary treatment options for most cancers remain surgery, irradiation, and/or chemotherapy. Anthracyclines are one of the most extensively used classes of chemotherapeutics to treat various solid and hematological tumors [3]. The first compound of this class of chemotherapeutic drugs, daunorubicin, was already reported in 1960, after being isolated from a soil bacterial sample in Italy [4, 5]. This compound is a product of the actinobacterium strain *Streptomyces peucetius*. While it was initially studied for its antibiotic properties, its anticancer properties were soon discovered [5]. Shortly after, daunorubicin's close structural homolog doxorubicin was isolated from a slightly different *S. peucetius* culture [6]. Daunorubicin and doxorubicin act by interfering with the catalytic cycle of topoisomerase IIα (Topo IIα), resulting in the formation of DNA double-strand breaks [7]. Cells then activate the DNA damage repair pathway to repair the breaks, or to initiate cell death when the damage is too severe [8]. Rapidly replicating cells, such as tumor cells, are in general more sensitive to the resulting DNA damage than normal cells, thus constituting a chemotherapeutic window [8]. A second mechanism by which the anthracycline drugs impose their antitumor effect is via eviction of histones [9, 10]. This has multiple consequences such as epigenetic and transcriptomic changes, which are together referred to as chromatin damage.
Nowadays, multiple anthracycline variants are used to treat over one million patients every year. However, the exact molecular mechanism by which these drug kill tumor cells remain unclear. In addition, treatment with anthracyclines coincides with severe adverse effects such as cardiotoxicity, secondary tumor formation and gonadotoxicity. Understanding how these highly effective anticancer drugs function and why they cause these severe toxicities would have tremendous impact on cancer treatment and the quality of life of cancer survivors. Therefore, even today, studying old anticancer drugs has high therapeutic potential and opens new exciting paths to improve currently available treatment options.

Treatment-limiting side effects of doxorubicin

Although doxorubicin is a very effective anticancer therapeutic, treatment is limited by various adverse effects. These side effects can be categorized into two groups: *(i.)* acute and generally reversible side effects, such as nausea, vomiting and diarrhea and *(ii.)* long-term and generally irreversible side effects, including gonadotoxicity, therapy-related tumor formation, and cumulative cardiotoxicity. These long-term side effects are especially treatment limiting and devastating for cancer survivors [11, 12]. To overcome these limitations, extensive research is done to identify the underlying mechanisms by which these drugs induce their anticancer function as well as their side effects. Additionally, hundreds of doxorubicin analogs have been isolated or synthesized in order to find effective treatment with less toxicity, however,

this did not result in new effective anthracyclines variants that entered the clinic. In **Chapter 1**, we describe the various mechanisms proposed by which doxorubicin functions and induces these side effects. Further, we provide several suggestions to overcome these toxicities, and we discuss perspectives on how to improve doxorubicin.

Novel factors controlling doxorubicin resistance

Besides the occurrence of side effects, doxorubicin treatment is hampered by drug resistance. To overcome the lack of knowledge on the molecular basis of doxorubicin resistance, we performed a genome-wide gene knockout screen. In **Chapter 2**, we describe the identification of three novel factors independently controlling doxorubicin resistance by interfering with the DNA double-strand break and repair pathway, namely Keap1, the SWI/SNF complex, and C9orf82 (also known as CAAP1). Both Keap1 and the SWI/SNF complex affect the promotion of DNA damage via Topo IIα. While the loss of the SWI/SNF complex limits the activity of Topo IIα by preventing the loading of the protein onto chromatin, Keap1 controls the expression of Topo IIα. On the other hand, C9orf82, controls the DNA repair pathway. Loss of C9orf82 accelerates γH2AX resolution and thereby promotes resistance to DNA double-strand break inducers such as the Topo IIα poisons doxorubicin and etoposide. Clinically, we showed that the expression of Keap1 and the SWI/SNF complex subunits SMARCB1 and SMARCA4, correlate with the response of triple-negative breast cancer patients to doxorubicin-containing regimes. Collectively, our work provides a molecular basis for doxorubicin resistance. Since mutations in various SWI/SNF complex subunits, as well as in Keap1 and C9orf82, have been found in different tumor types [13-19], profiling patients for mutations in these specific genes would help to predict their response towards doxorubicin-based treatment. This knowledge would prevent people from undergoing ineffective treatment and supports the selection of alternative regimes with drugs that do not target Topo IIα, such as the Topo I poison topotecan, or anthracycline analogues that only have chromatin damage activity (such as aclarubicin or diMe-Doxo, see below).

Chromatin damage is the main mechanism for the anticancer activity of anthracyclines

The anthracycline drug doxorubicin and its analogs daunorubicin, epirubicin, and idarubicin are Topo IIα poisons [7]. They induce DNA double-strand breaks by interfering with the catalytic cycle of topoisomerase forming DNA-Topo IIα-drug tertiary complexes [7]. As a consequence, DNA repair pathways are activated, the cell cycle is arrested, and apoptosis is initiated [20]. For a long time, it was thought that induction of DNA breaks via Topo IIα was the main mechanism by which these drugs function. But recently, a second mechanism of action was uncovered: chromatin damage via eviction of histones [9, 21]. **Chapter 3** describe our findings that indicates that histone eviction might be the major anticancer activity of these drugs. We studied the biological activity of doxorubicin (inducing both DNA- and chromatin damage), the structurally unrelated Topo IIα poison etoposide (inducing DNA damage only), the anthracycline family member aclarubicin (inducing chromatin damage only) and amrubicin (inducing DNA damage only), and the newly synthesized doxorubicin analog diMe-Doxo (inducing chromatin damage only). Our results demonstrate that drugs abstained from the classical DNA damaging capacity (i.e. aclarubicin and diMe-Doxo) are effective anticancer drugs *in vitro* and *in vivo*. Furthermore, we report that the doxorubicin-induced cardiotoxicity is caused by the combination of these two ac-

tivities, since compounds that either induce DNA damage (etoposide) or chromatin damage (aclarubicin and diMe-Doxo) fail to induce cardiotoxicity in mice and human cardiac microtissues. While treatment of pluripotent stem cell derived human cardiac microtissues with amrubicin (DNA damage only) and aclarubicin (chromatin damage only) had no effect on the contraction amplitude and velocity of the microtissues, the combination treatment resulted in impaired contraction, similar to treatment with doxorubicin where these two activities are combined in one molecule. Also, therapy-related secondary tumors and infertility are absent or reduced for drugs possessing only one of these two activities. We showed that detoxification of doxorubicin is possible by separating the DNA- and chromatin damage activities by introducing a small modification on the 3' amine of the amino sugar moiety. In the different murine models tested, both aclarubicin and diMe-Doxo remain effective anticancer drugs with limited toxicity, suggesting that these drugs could be used for patients which are currently excluded from effective treatment, such as old patients or patients with a recurrent tumor with a history of anthracycline-based therapy.

Chemical features defining the biological activity of anthracycline drugs

Based on our findings described in **Chapter 3**, we anticipated that finding structural features responsible for chromatin damage activity without inducing DNA damage will advance the discovery of novel anthracycline analogs with limited toxicity. Therefore, we decided to synthesize and test three coherent sets of anthracycline analogs. In **Chapter 4**, we evaluated 10 doxorubicin/aclarubicin hybrid structures for their ability to induce DNA double strand breaks, eviction of histones, and cytotoxicity. These structures diverged by the anthraquinone aglycon, the nature of the carbohydrate portion, and the alkylation pattern of the amine on the first sugar moiety. Comparing these analogs, we observed a clear correlation between the efficiency of histone eviction and cytotoxicity *in vitro*, which is in line with our results described in Chapter 3. We observed that *N,N*-dimethylation of the carbohydrate considerably improved the histone eviction capacity of these compounds and thereby cytotoxicity. Furthermore, the doxorubicin anthraquinone aglycon appeared slightly more efficient in killing tumor cells *in vitro* than the aclarubicin aglycon, and the aclarubicin trisaccharide showed higher cytotoxicity than the doxorubicin monosaccharide. Hence, we yielded three structures, named compound 3, 8 and 11 (**Chapter 4**), that were more cytotoxic than doxorubicin. Remarkably, compound 11 that combines the structural features described above, was the most cytotoxic variant in this focused library. Besides, these three hybrid compounds were unable to produce DNA double-strand breaks but induced cell death via chromatin damage, strengthening our scenario of histone eviction as the main mechanism of action of this class of anticancer drugs.

Our findings raised the following broader question: 'Is there a structure-activity relationship for the stereoisomeric analogs of doxorubicin?" To test this, we synthesized and evaluated a targeted library of epimeric doxorubicin analogs (**Chapter 5**). We showed that both the *N*-substitution state and the stereochemistry of the 3' amine were critical for the biological activity of the drugs. While the orientation of the hydroxyl group at the 4' position did not affect cytotoxicity, compounds featuring an *N,N*-dimethylamine in the equatorial position showed improved cellular uptake, histone eviction effectivity, and cytotoxicity.

In **Chapter 6**, we described a third set of doxorubicin analogs designed to further investigate the mode of action of the 3' amine moiety. Next to diMe-Doxo, we synthesized and tested three non-basic 3' variants and four cyclic-doxorubicin analogs and compared their biological activity with doxorubicin. Where all the non-basic

doxorubicin induced effective DNA damage without histone eviction activity, the four cyclic-doxorubicin analogues, on the opposite, evicted histone without inducing DNA damage. Despite this divergence, they were all able to re-locate Topo IIα. Using ChIP-sequencing of endogenously tagged Topo IIα, we found that Topo IIα targeting occurred at distinct genomic locations. This difference appears to be determined by their structure (modification at the amino sugar) and subsequent biologic activity. A similar observations is made for the genomic selectivity of the chromatin damage activity of these drugs. Analogs that are able to induce DNA double-strand breaks affect distinct regions from the variant that are effective histone evicting drugs because of their tertiary amine at the 3' position.
In summary, the side group at the 3' position of the amino sugar determines the biological activity and genomic selectivity of the various analogues. Studying the effectivity and toxicity of the most potent analogs described above in an *in vivo* model, could lead to the development of anthracycline analogs for novel therapeutics. Besides, since these analogs selectivity target genomic locations for Topo IIα relocation and/or chromatin damage activity, identifying which exact genomic locations are targeted would shed new light on anthracycline molecular mode of action. Eventually, such information could be used to refine the selection of specific analogs over others for the treatment of different tumor types.

A role for nuclear DNA sensors in chromatin damage-induced cell death
Chromatin damage, via eviction of histones, is the main anticancer activity of the different anthracycline drugs [22]. Yet, the exact mechanism by which histone eviction induces cell death remains unclear. Under physiological circumstances, DNA is closely packed in nucleosomes and the chromatin compaction state tightly controls the regulation of gene expression [23]. Large stretches of histone-free DNA are therefore uncommon in eukaryotic cells, and we predicted that this unnatural situation would induce a cellular response. Hence, we hypothesized that anthracycline-induced histone-free DNA can be sensed by proteins that can restore histone-DNA association, that can initiate an immune response as they 'think' that the cell is infected by a DNA virus and/or that initiate cell death. In **Chapter 7**, we described three nuclear DNA sensors from the PYHIN protein family (IFIX, IFI16 and MNDA), which are known for their role during viral infection [24-26]. These three sensors specifically re-locate to DNA upon treatment with histone evicting anthracycline drugs. Furthermore, we showed that DNA binding of IFI16 is enhanced upon treatment with doxorubicin and aclarubicin, but not with etoposide. These results indicated that the 'naked DNA' resulting from the chromatin damage activity of these drugs can be sensed and bound by the DNA sensors. Mass spectrometry analyses to identify novel interaction partners for these three nuclear DNA sensors yielded two proteins from the ubiquitin machinery (the de-ubiquitinating enzyme USP7, and the E3-ligase TRIM26) and a DNA helicase (XRCC6), all known to play a role in viral infection and cell death [27-29]. Yet, if and how the interactions of these proteins with the DNA sensors could lead to cell death upon chromatin damage is as of yet unclear. Work by Johnstone and colleagues showed that the DNA sensors IFI16 can interact with p53 to regulate the cell cycle and apoptosis [30]. This interaction enhances the p53-mediated transcription of its target gene p21 in U2Os cells, allowing cell cycle regulation [31]. Since both IFI16 and USP7 are known to interact with p53 [30, 32], we hypothesized that USP7 is recruited to the histone-free DNA via IFI16 to de-ubiquitinate p53 and initiate a stress response. On the other hand, sensing histone-free DNA could activate the innate immune system via the recruitment

of TRIM26. While not much is known about the function of TRIM26, this protein is described to interact and activate TBK1 and thereby regulate IRF3 and NF-κB activation and IFN-β induction upon detection of RNA virus infection [28]. Activation of an innate immune response upon TRIM26 recruitment to the DNA might play a role in the anti-tumor response *in vivo*. The molecular mechanism by which the sequential binding of 'naked-DNA', by the DNA sensors, and USP7 and/or TRIM26 leads to anthracycline-induced cell death remains to be explored. In particular, p53-mediated apoptosis upon the complex formation of the DNA sensors with USP7, TRIM26, or other interactors would be a promising lead for further investigations.
Besides, expression of the PYHIN protein family in hematopoietic cells is positively regulated by type I and/or type II interferons [33]. We showed that stimulation with IFNβ or IFNγ upregulates the expression of IFI16 in MelJuSo cells. Consequently, IFNβ stimulation makes these cells more sensitive to treatment with anthracycline drugs. This observation is supported by a study from Fujiuchi and colleagues, who showed that enhanced expression of IFI16 in MCF-7 cells increased the cellular susceptibility for apoptosis induced by p53-dependent ionizing radiation [34]. Together, this indicates that IFI16 might play a role in DNA/genotoxic stress-induced cell death in general. More experiments needs to be done to reveal the consequences of the PYHIN family protein activation in response to anthracycline treatment.

Small molecules to improve cancer-immunotherapy
While chemotherapy has a long history in cancer treatment, cancer-immunotherapy is a relatively new treatment option used in the clinic [35, 36]. Its operating principle is to direct a systemic cytotoxic ($CD8^+$) T lymphocyte response toward tumor cells, which ideally also eradicates secondary lesions [37]. Various immunotherapy strategies are known, but especially checkpoint blockade therapies, using monoclonal antibodies against CTLA-4, PD-1, or PD-L1, have led to prominent breakthroughs in the cancer-immunotherapy field. However, despite its impressive achievements, checkpoint blockade therapy is only successful in a fraction of the patients [36]. This limited effectivity generally results from inhibitory immune cells or other mechanisms that counteract the cytotoxic T cell response towards the tumor. To overcome these immunosuppressive mechanisms, various combination treatments have been suggested and tested. Especially the use of small-molecule based combination therapies are promising. They offer valuable opportunities to increase the efficacy of cancer immunotherapy, either by targeting immunosuppressive cells in the tumor microenvironment in a rationale mechanism-guided fashion, or by stimulating tumor immunogenicity. An overview of these novel small-molecule based combination therapies is provided in **Chapter 8**.

In summary, we demonstrated that the combination of DNA- and chromatin damages coinciding with doxorubicin treatment is the underlying mechanism responsible for its severe long-term side effects. Furthermore, we showed that chromatin damage is most likely the major anticancer activity of several anthracycline drugs. We identified specific structural features that are responsible for the biological activity of anthracycline drugs and determined novel analogs with improved cytotoxicity. The exact mechanism by which histone eviction leads to cell death remains unknown, but we anticipate that nuclear DNA sensors may play a role in the detection of histone-free DNA. Together, the findings described in this thesis illustrate that studying an old anticancer drug with novel concepts and techniques can open uncharted paths to improve current cancer treatment.

REFERENCES

1. (2018) Latest global cancer data: Cancer burden rises to 18.1 million new cases and 9.6 million cancer deaths in 2018. www.iarc.fr.
2. Lee, E. Y. H. P. & Muller, W. J. (2010) Oncogenes and Tumor Suppressor Genes, Cold Spring Harbor Perspectives in Biology. 2.
3. Hortobagyi, G. N. (1997) Anthracyclines in the treatment of cancer - An overview, Drugs. 54, 1-7.
4. Camerino B, P. G. (1960) Derivati della parazina II. Sulfonamdopir (in Italian). , Gazz Chim Ital 90: 1802–1815.
5. Di Marco, A., Cassinelli, G. & Arcamone, F. (1981) The discovery of daunorubicin, Cancer Treat Rep. 65 Suppl 4, 3-8.
6. Arcamone, F., Cassinelli, G., Fantini, G., Grein, A., Orezzi, P., Pol, C. & Spalla, C. (1969) Adriamycin, 14-hydroxydaunomycin, a new antitumor antibiotic from S. peucetius var. caesius, Biotechnol Bioeng. 11, 1101-10.
7. Nitiss, J. L. (2009) Targeting DNA topoisomerase II in cancer chemotherapy, Nat Rev Cancer. 9, 338-50.
8. Misteli, T. & Soutoglou, E. (2009) The emerging role of nuclear architecture in DNA repair and genome maintenance, Nature Reviews Molecular Cell Biology. 10, 243-254.
9. Pang, B., Qiao, X., Janssen, L., Velds, A., Groothuis, T., Kerkhoven, R., Nieuwland, M., Ovaa, H., Rottenberg, S., van Tellingen, O., Janssen, J., Huijgens, P., Zwart, W. & Neefjes, J. (2013) Drug-induced histone eviction from open chromatin contributes to the chemotherapeutic effects of doxorubicin, Nature communications. 4, 1908.
10. Pang, B., de Jong, J., Qiao, X., Wessels, L. F. & Neefjes, J. (2015) Chemical profiling of the genome with anti-cancer drugs defines target specificities, Nat Chem Biol. 11, 472-80.
11. Lotrionte, M., Biondi-Zoccai, G., Abbate, A., Lanzetta, G., D'Ascenzo, F., Malavasi, V., Peruzzi, M., Frati, G. & Palazzoni, G. (2013) Review and meta-analysis of incidence and clinical predictors of anthracycline cardiotoxicity, Am J Cardiol. 112, 1980-4.
12. Mistry, A. R., Felix, C. A., Whitmarsh, R. J., Mason, A., Reiter, A., Cassinat, B., Parry, A., Walz, C., Wiemels, J. L., Segal, M. R., Ades, L., Blair, I. A., Osheroff, N., Peniket, A. J., Lafage-Pochitaloff, M., Cross, N. C., Chomienne, C., Solomon, E., Fenaux, P. & Grimwade, D. (2005) DNA topoisomerase II in therapy-related acute promyelocytic leukemia, N Engl J Med. 352, 1529-38.
13. Chin, L.Meyerson, M.Aldape, K.Bigner, D.Mikkelsen, T.VandenBerg, S.Kahn, A.Penny, R.Ferguson, M. L.Gerhard, D. S.Getz, G.Brennan, C.Taylor, B. S.Winckler, W.Park, P.Ladanyi, M.Hoadley, K. A.Verhaak, R. G. W.Hayes, D. N.Spellman, P. T.Absher, D.Weir, B. A.Ding, L.Wheeler, D.Lawrence, M. S.Cibulskis, K.Mardis, E.Zhang, J. H.Wilson, R. K.Donehower, L.Wheeler, D. A.Purdom, E.Wallis, J.Laird, P. W.Herman, J. G.Schuebel, K. E.Weisenberger, D. J.Baylin, S. B.Schultz, N.Yao, J.Wiedemeyer, R.Weinstein, J.Sander, C.Gibbs, R. A.Gray, J.Kucherlapati, R.Lander, E. S.Myers, R. M.Perou, C. M.McLendon, R.Friedman, A.Van Meir, E. G.Brat, D. J.Mastrogianakis, G. M.Olson, J. J.Lehman, N.Yung, W. K. A.Bogler, O.Berger, M.Prados, M.Muzny, D.Morgan, M.Scherer, S.Sabo, A.Nazareth, L.Lewis, L.Hall, O.Zhu, Y. M.Ren, Y. R.Alvi, O.Yao, J. Q.Hawes, A.Jhangiani, S.Fowler, G.San Lucas, A.Kovar, C.Cree, A.Dinh, H.Santibanez, J.Joshi, V.Gonzalez-Garay, M. L.Miller, C. A.Milosavljevic, A.Sougnez, C.Fennell, T.Mahan, S.Wilkinson, J.Ziaugra, L.Onofrio, R.Bloom, T.Nicol, R.Ardlie, K.Baldwin, J.Gabriel, S.Fulton, R. S.McLellan, M.

D.Larson, D. E.Shi, X. Q.Abbott, R.Fulton, L., et al. (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways, Nature. 455, 1061-1068.
14. Kadoch, C., Hargreaves, D. C., Hodges, C., Elias, L., Ho, L., Ranish, J. & Crabtree, G. R. (2013) Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy, Nature Genetics. 45, 592-+.
15. Hornick, J. L., Dal Cin, P. & Fletcher, C. D. M. (2009) Loss of INI1 Expression is Characteristic of Both Conventional and Proximal-type Epithelioid Sarcoma, American Journal of Surgical Pathology. 33, 542-550.
16. Gasparini, P., Facchinetti, F., Boeri, M., Lorenzetto, E., Livio, A., Gronchi, A., Ferrari, A., Massimino, M., Spreafico, F., Giangaspero, F., Forni, M., Maestro, R., Alaggio, R., Pilotti, S., Collini, P., Modena, P. & Sozzi, G. (2011) Prognostic determinants in epithelioid sarcoma, European Journal of Cancer. 47, 287-295.
17. Lawrence, M. S., Stojanov, P., Mermel, C. H., Robinson, J. T., Garraway, L. A., Golub, T. R., Meyerson, M., Gabriel, S. B., Lander, E. S. & Getz, G. (2014) Discovery and saturation analysis of cancer genes across 21 tumour types, Nature. 505, 495-+.
18. Barbano, R., Muscarella, L. A., Pasculli, B., Valori, V. M., Fontana, A., Coco, M., la Torre, A., Balsamo, T., Poeta, M. L., Marangi, G. F., Maiello, E., Castelvetere, M., Pellegrini, F., Murgo, R., Fazio, V. M. & Parrella, P. (2013) Aberrant Keap1 methylation in breast cancer and association with clinicopathological features, Epigenetics. 8, 105-112.
19. Brennan, C. W., Verhaak, R. G. W., McKenna, A., Campos, B., Noushmehr, H., Salama, S. R., Zheng, S. Y., Chakravarty, D., Sanborn, J. Z., Berman, S. H., Beroukhim, R., Bernard, B., Wu, C. J., Genovese, G., Shmulevich, I., Barnholtz-Sloan, J., Zou, L. H., Vegesna, R., Shukla, S. A., Ciriello, G., Yung, W. K., Zhang, W., Sougnez, C., Mikkelsen, T., Aldape, K., Bigner, D. D., Van Meir, E. G., Prados, M., Sloan, A., Black, K. L., Eschbacher, J., Finocchiaro, G., Friedman, W., Andrews, D. W., Guha, A., Iacocca, M., O'Neill, B. P., Foltz, G., Myers, J., Weisenberger, D. J., Penny, R., Kucherlapati, R., Perou, C. M., Hayes, D. N., Gibbs, R., Marra, M., Mills, G. B., Lander, E., Spellman, P., Wilson, R., Sander, C., Weinstein, J., Meyerson, M., Gabriel, S., Laird, P. W., Haussler, D., Getz, G., Chin, L. & Network, T. R. (2013) The Somatic Genomic Landscape of Glioblastoma, Cell. 155, 462-477.
20. Perego, P., Corna, E., De Cesare, M., Gatti, L., Polizzi, D., Pratesi, G., Supino, R. & Zunino, F. (2001) Role of apoptosis and apoptosis-related genes in cellular response and antitumor efficacy of anthracyclines, Curr Med Chem. 8, 31-7.
21. Yang, F., Kemp, C. J. & Henikoff, S. (2013) Doxorubicin Enhances Nucleosome Turnover around Promoters, Curr Biol. 23, 782-787.
22. Qiao, X., van der Zanden, S. Y., Wander, D. P. A., Borras, D. M., Song, J. Y., Li, X., van Duikeren, S., van Gils, N., Rutten, A., van Herwaarden, T., van Tellingen, O., Giacomelli, E., Bellin, M., Orlova, V., Tertoolen, L. G. J., Gerhardt, S., Akkermans, J. J., Bakker, J. M., Zuur, C. L., Pang, B., Smits, A. M., Mummery, C. L., Smit, L., Arens, R., Li, J., Overkleeft, H. S. & Neefjes, J. (2020) Uncoupling DNA damage from chromatin damage to detoxify doxorubicin, Proc Natl Acad Sci U S A.
23. McGinty, R. K. & Tan, S. (2015) Nucleosome Structure and Function, Chem Rev. 115, 2255-2273.
24. Monroe, K. M., Yang, Z. Y., Johnson, J. R., Geng, X., Doitsh, G., Krogan, N. J. & Greene, W. C. (2014) IFI16 DNA Sensor Is Required for Death of Lymphoid CD4 T Cells Abortively Infected with HIV, Science. 343, 428-432.
25. Kerur, N., Veettil, M. V., Sharma-Walia, N., Bottero, V., Sadagopan, S., Otageri,

P. & Chandran, B. (2011) IFI16 Acts as a Nuclear Pathogen Sensor to Induce the Inflammasome in Response to Kaposi Sarcoma-Associated Herpesvirus Infection, Cell Host Microbe. 9, 363-375.
26. Dell'Oste, V., Gatti, D., Gugliesi, F., De Andrea, M., Bawadekar, M., Lo Cigno, I., Biolatti, M., Vallino, M., Marschall, M., Gariglio, M. & Landolfo, S. (2014) Innate Nuclear Sensor IFI16 Translocates into the Cytoplasm during the Early Stage of In Vitro Human Cytomegalovirus Infection and Is Entrapped in the Egressing Virions during the Late Stage, J Virol. 88, 6970-6982.
27. Johnson, K. E., Bottero, V., Flaherty, S., Dutta, S., Singh, V. V. & Chandran, B. (2014) IFI16 Restricts HSV-1 Replication by Accumulating on the HSV-1 Genome, Repressing HSV-1 Gene Expression, and Directly or Indirectly Modulating Histone Modifications, Plos Pathog. 10.
28. Ran, Y., Zhang, J., Liu, L. L., Pan, Z. Y., Nie, Y., Zhang, H. Y. & Wang, Y. Y. (2016) Autoubiquitination of TRIM26 links TBK1 to NEMO in RLR-mediated innate antiviral immune response, J Mol Cell Biol. 8, 31-43.
29. Frost, J. R., Olanubi, O., Cheng, S. K. H., Soriano, A., Crisostomo, L., Lopez, A. & Pelka, P. (2017) The interaction of adenovirus E1A with the mammalian protein Ku70/XRCC6, Virology. 500, 11-21.
30. Johnstone, R. W., Wei, W., Greenway, A. & Trapani, J. A. (2000) Functional interaction between p53 and the interferon-inducible nucleoprotein IFI 16, Oncogene. 19, 6033-6042.
31. Kwak, J. C., Ongusaha, P. P., Ouchi, T. & Lee, S. W. (2003) IFI16 as a negative regulator in the regulation of p53 and p21(Waf1), J Biol Chem. 278, 40899-40904.
32. Sheng, Y., Saridakis, V., Sarkari, F., Duan, S. L., Wu, T. N., Arrowsmith, C. H. & Frappier, L. (2006) Molecular recognition of p53 and MDM2 by USP7/HAUSP, Nat Struct Mol Biol. 13, 285-291.
33. Landolfo, S., Gariglio, M., Gribaudo, G. & Lembo, D. (1998) The Ifi 200 genes: An emerging family of IFN-inducible genes, Biochimie. 80, 721-728.
34. Fujiuchi, N., Aglipay, J. A., Ohtsuka, T., Maehara, N., Sahin, F., Su, G. H., Lee, S. W. & Ouchi, T. (2004) Requirement of IFI16 for the maximal activation of p53 induced by ionizing radiation, J Biol Chem. 279, 20339-20344.
35. Sharma, P. & Allison, J. P. (2015) The future of immune checkpoint therapy, Science. 348, 56-61.
36. Ribas, A. & Wolchok, J. D. (2018) Cancer immunotherapy using checkpoint blockade, Science. 359, 1350-1355.
37. Koebel, C. M., Vermi, W., Swann, J. B., Zerafa, N., Rodig, S. J., Old, L. J., Smyth, M. J. & Schreiber, R. D. (2007) Adaptive immunity maintains occult cancer in an equilibrium state, Nature. 450, 903-7.