An Analysis on the Viability of DNMT Inhibitors in Cancer Treatment

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Abstract

This paper will explore the viability and options of DNA Methyltransferase inhibitors against cancer. DNA Methyltransferase, DNMT for short, are enzymes that methylate DNA which plays a crucial role in regulating gene expression. Methylation deactivates a gene. DNMTs can cause cancer by many methods such as hypomethylating a tumor suppressor gene, hypomethylated an oncogene or causing some sort of genetic instability. A plausible explanation for it is Epigenetic factors that cause this. This genetic instability can cause an increase in cancer. This can be ceased through the use of DNMT inhibitors such as Decitabine, RG108, and Azacytidine.

Keywords: DNA Methyltransferase (DNMT), Cancer Treatment, Epigenetics, Decitabine, Azacitidine, RG108, Combination Therapy

1. Introduction

1.1- DNA Methyltransferases

DNA Methyltransferase, commonly known as DNMT, is an enzyme that catalyzes the methylation of DNA, which helps regulate gene expression [1]. These changes to the DNA are reversible, but when done, they can play a significant role in genetic expression depending on which gene has been modified [2]. However, methylation of the promoter or first exon region of genes may promote carcinogenesis, because they mimic mutations in tumor-suppressor genes [2]. Specifically, DNMTs DNMT1, DNMT3A and DNMT3B have been observed in elevated levels in malignant tumors [3]. Many studies have shown that excessive DNMT activity leads to promotion of the cell cycle, specifically a study by Zhao et al. has shown that inhibiting DNMTs (a principle known as “inhibiting the inhibitor”) reduced the cell cycle in esophageal squamous cell carcinoma [4]. Conversely, increasing DNMT activity increases cell cycle activity. [4]. Additional studies have been performed in other cancer types, including breast and colon, and they all indicate similar results [3].

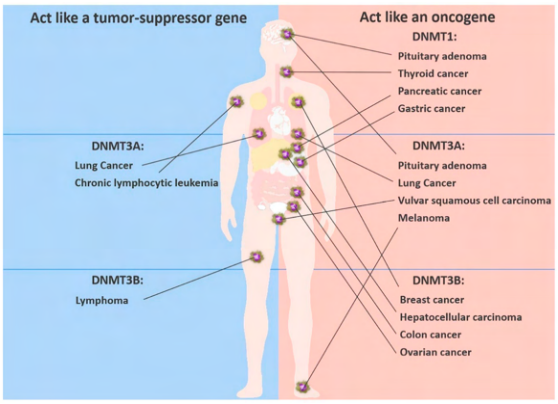


Figure 1. A map showing how different DNMTs act as in different types of cancer. Adapted from [10].

1.2- Cancer

Cancer arises due to genetic alterations within the cell cycle, leading to irregular cell growth and functioning. These alterations can either activate oncogenes, which stimulate cell division, or deactivate tumor suppressor genes, which hinder cell division. Typically, cancer develops as a result of a buildup of various mutations. Environmental factors like carcinogens or intrinsic genetic factors can induce DNA damage, impacting protooncogenes and tumor suppressor genes, thereby triggering uncontrolled cell proliferation. This unregulated growth manifests as a malignant tumor, which must exhibit invasiveness to adjacent tissues and the ability to spread via blood or lymphatic vessels for it to be classified as cancerous. When such an invasive tumor lodges near critical organs, it poses a severe threat to the patient's life [5]. As mentioned in Section 1.1, DNMTs can prompt cancer by methylating sequences in DNA near the promoter of the gene [2].

2. **DNMTs Mechanism of Action in Tumorous Cells**

There are three main DNA modifications that occur that are a trademark of cancer: hypermethylation of tumor suppressor genes, hypomethylation of certain repetitive sequences, and modified or excessive expression of DNMTs [6]. Hypermethylation refers to excessive methylation of DNA, while inversely, hypomethylation refers to inadequate or reduced methylation amounts [7]. DNMT1 specifically is needed to maintain cancer stem cells, as shown in multiple studies. Studies have also shown that DNMT1 is upregulated greatly (overexpressed) in various types of cancer.

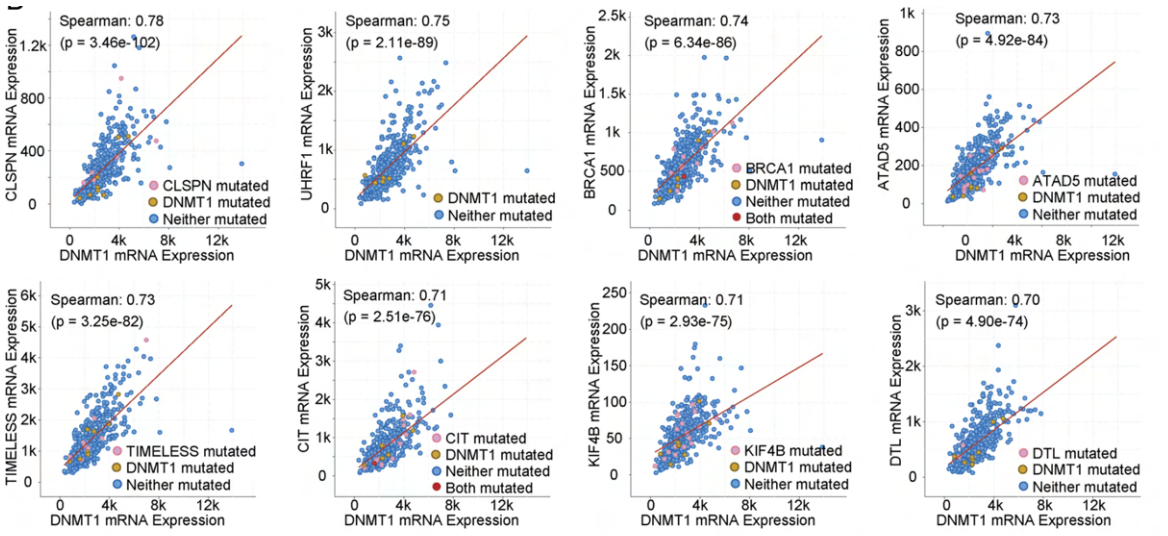


Figure 2. Adapted from [8]. This shows the correlation between DNMT1 mutations and the expression of correlated genes. All correlation factors are over 0.7; indicating that the correlated genes were coexpressed with DNMT1.

In contrast, DNMT3A and 3B are involved in *de novo* methylation, which is the addition of new methyl groups to DNA [9]. In breast cancer patients, elevated numbers of DNMT3A and 3B have been noticed, showing that upregulation of these DNMTs prompt tumor progression [9]. DNMT3A can regulate genes like ERα and BRCA1 by inducing promoter hypermethylation,

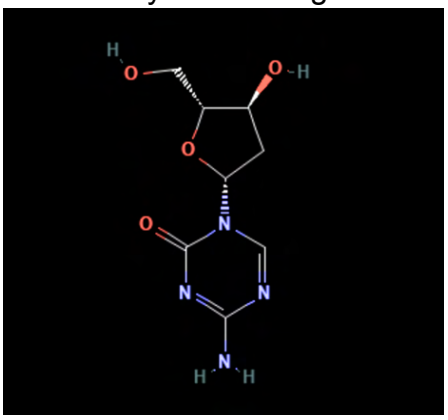
leading to their downregulation in breast cancer cells [9]. Similarly, DNMT3B overexpression is linked with hypermethylation of specific genes, such as WIF1, which is associated with Wnt dysregulation and breast carcinogenesis [9].

3. **Drugs that Target DNMTs**

Many existing drugs target DNMTs and reduce or even eliminate their expression, thereby reducing the effect of cancer.

3.1 Decitabine

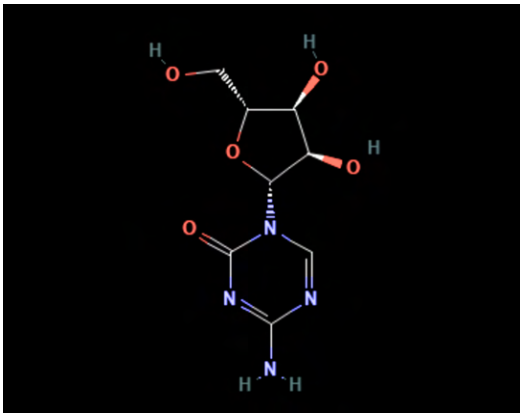
A good example of a DNMT inhibitor is 5-Aza-2′-deoxycytidine, or decitabine, which is classed as an analog of the nucleoside cytosine. According to Yu et al., decitabine in low doses has shown to have antitumor effects on epithelial tumor cells, however when increased to a higher dose, a level of toxicity restricts further research in clinical trials. The mechanism for which decitabine uses to reduce DNMT expression is not one of complexity. The drug converts into active thiphosphorylated nucleotides and incorporates into the DNA [11]. An irreversible bond to the DNMT leads to its depletion[11]. As suggested before, higher doses show signs of cytotoxicity and induce damage in the DNA. On the other hand, lower doses do show safety and effective antitumor effects without any harm or interference with the DNA synthesis. There is also an additional “memory” response that was found within the epithelial tumor cells, which simply just meant that the cells maintained a “decrease in genome-wide promoter DNA methylation and gene reexpression in key cellular regulatory pathways” (Yu et al., 2018).



5-Aza-2'-deoxycytidine (decitabine) Figure 3 adapted from [17]

3.2 Azacytidine

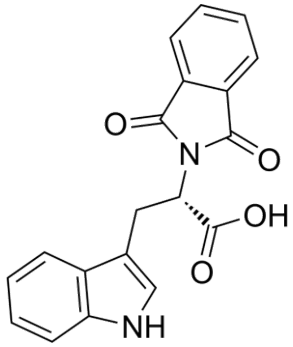
Another such drug very similar to decitabine is known as 5-azacytidine (AZA), also an analog of the nucleoside cytosine. AZA is a DNMT inhibitor based on nucleosides and induces demethylation and gene reactivation [12]. Much like decitabine, AZA also works by incorporating itself into the DNA during its synthesis phase of the cell cycle, forming an irreversible bond with the DNMT, thereby trapping it, and thus its depletion. According to Sheikh et al., “In vitro studies of azacitidine in breast cancer cell lines have demonstrated that DNA remethylation was effectively blocked at 23 out of 26 genes that were tested and previously known to be hypermethylated [39,40].” [14]. The results show promising signs for AZA and its antitumor effects.



5-azacytidine, Figure 4 adapted from [15].

3.3- RG108

Finally, RG108 is different from the previous two drugs, decitabine and azacitidine, in that it is a non-nucleoside analog, and causes demethylation as well as reactivation of tumor suppressor genes. It is classified as a transmethylase inhibitor, and has been found to inhibit DNMT3B in particular. Many studies have shown signs of anti-cancer effects with the use of RG108 inhibitors. For example, according to Ou et al., RG108 was used in combination with X-ray radiation methods [27]. To be more specific, the X-rays induced apoptosis and a surface level inhibition of the G2/M-phase of the cell cycle, but RG108 further enhanced these effects, suggesting anti-cancer effects. Additionally, according to a study by Yang et al., “MTT results showed that RG108 inhibited the cell viability in a dose-dependent and time-dependent manner. Flow cytometry revealed that RG108 blocked the cell cycle in G2/M phase and promoted the apoptosis, and TUNEL assay further proved that RG108 promoted the apoptosis” [14]. Here, RG108 was tested on endometrial cancer Ishikawa cell lines, and were tested at different concentrations [14]. The results showed a clear anti-tumor and anti-cancer effect using RG108, with an emphasis on its induction of apoptosis and its blocking of the cell cycle at select stages [14]. Additionally, their cell viability assay results show a significant inhibition of cell proliferation with the administration of RG108 at specific concentrations after specific periods of time [14].



RG108 chemical structure. Figure 5 adapted from [16].

4. **Analysis on the viability and alternative options**

4.1 - Decitabine

Decitabine, like many drugs, is significantly effective towards metastatic cells with low dosages. However, when increased to higher dosages, the drug can become toxic. This risk restricts further research with clinical trials and patients. There are many examples of this, for example a study by Wu et al., which reported conclusive results that the lower dosages of decitabine in fact increased the effectiveness of chemotherapy against bladder cancer [21]. Clearly, there have been significant examples of decitabine being an effective contributor to the inhibition of cancerous cells. However, the application of decitabine as a drug by utilizing its DNMT inhibiting effects may have its own drawbacks. For example, it can be quite expensive for treatment in the practical sense. In addition, there is a risk of side effects, or even death.

4.2 - Azacytidine

Azacytidine mainly focuses on myelodysplasia syndrome (MDS) or acute myelocytic leukemia (AML). The benefits of Azacytidine is its ability to cause epigenetic changes that can affect silenced genes and reactivate them because they have been hypermethylated. This drug has also been commonly used for myelodysplasia syndrome (MDS) or acute myelocytic leukemia (AML) proving its clinical credibility [13]. It is beneficial because even after using it after the treatment the methylation pattern can return to the original noncancerous state. These drugs are also general to DNMTs. Similar to decitabine, it is clinically credible, however, it has many side effects such as increased susceptibility to pathogen infections and fatigue. In a clinical application this can be very costly and will require multiple injections. This can cause resistance in azacytidine overtime therefore it could be used in combination therapy to better its effects [25]. Another huge risk factor is its ability to hypomethylated and if this can cause an activation of an oncogene causing another mutation that can cause cancer. [13]

4.3 - RG108

The benefits of RG108 is its ability to reduce DNA methylation; this allows scientists to study the different concentrations to see its results as well as its epigenetic variables [14]. RG108's DNA methylation can also be reversed allowing control of DNA methylation and can help studies. The low cytotoxicity is very beneficial because there are less confounding variables and cellular damage so the effects can be pinpointed on the drug. Also it gives the ability to test more concentrations. Also it enhances apoptosis and inhibits the G2 phase in the cell cycle .RG108 is specific to only DNMT1 which can limit studies and cause it to not use this drug. In vivo this would most likely have to only be in combination therapy due to this aspect [15,25]. RG108 also has a short half life and its reversibility is also negative because it would not effectively inhibit [14].

4.4 - Alternative options

4.4.1 - Nanovaccines

There are many alternative treatments that are currently in use for cancer therapy that can be used in conjunction with DNMT inhibitors. One of these options is the use of nanovaccines, in which DNMT inhibitors are injected into nanoparticles which are then directly injected into the body [19]. These nanoparticles are often coated with a lipid bilayer or a peptide layer [19].

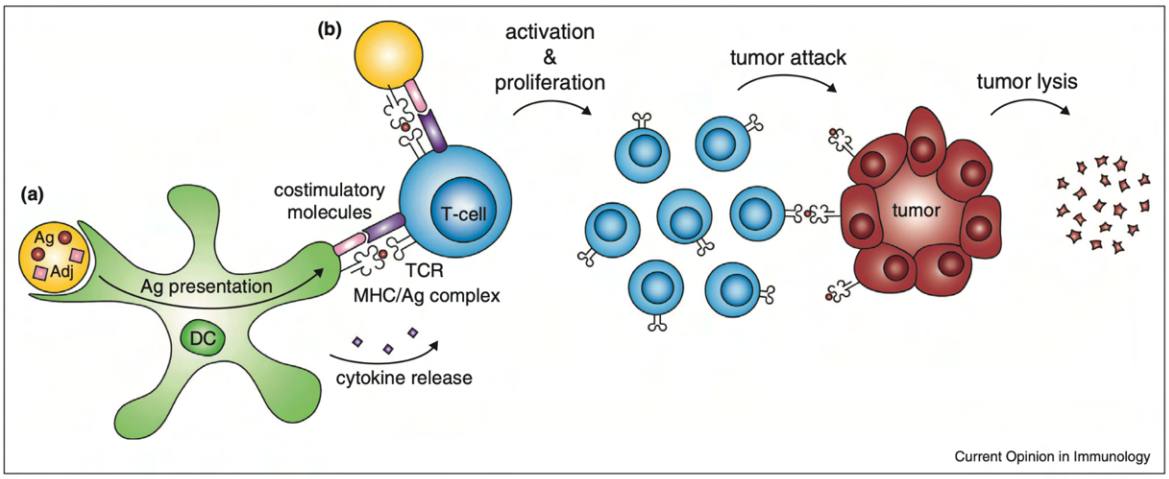
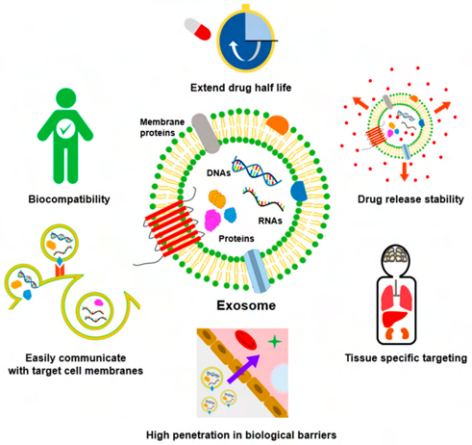


Figure 6. Adapted from [19]. This shows how DNMT inhibitors can be injected into DCs or nanoparticles for direct delivery into the body.

4.4.2 - Exosomes

Another possible treatment would be the use of exosomes and DNMT inhibitors. Exosomes are small vesicles surrounded by a lipid bilayer, and can hold many molecules such as proteins, lipids, and nucleic acids [22]. DNMT inhibitors can be loaded into these capsules and reinjected into the body [22]. The benefits of using exosomes include [22]:

- No immune attack mounted on exosomes
- No rejection response from body on exosomes
- Protection of internal DNMTs from degradation because of protective exosomal membrane



LEFT: Figure 7. Adapted from [26].

4.4.3 - Combination Therapy

Combination therapy, as referenced in Section 3, can be an effective means of combining DNMT inhibitors and other types of therapy such as radiotherapy and chemotherapy to maximize the effectiveness of both [23].

4.4.4 - Alternative Inhibitors

There are other classes of inhibitors, such as BET (bromodomain) inhibitors and HDAC inhibitors (histone deacetylases), which can be used in conjunction with DNMT inhibitors to bolster the effects of both. HDAC inhibitors are a class of inhibitors that interact with histones, removing acetylation

with histone and histone proteins [24]. They can be used in conjunction with DNMTs to have greater control over gene expression [24]. Similarly, BET inhibitors manage histone acetylation for a family of proteins known as bromodomain and extra-terminal domain (BET) protein [25].

5. **Conclusion**

In conclusion, the exploration of DNA Methyltransferase (DNMT) inhibitors as potential agents in cancer treatment reveals promising avenues for research and clinical application. The aberrant activity of DNMTs, particularly DNMT1, DNMT3a, and DNMT3B, has been implicated in the pathogenesis of various cancers by promoting genetic instability through hypermethylation of tumor suppressor genes and other regulatory regions. Decitabine, Azacytidine, and RG108 are among the DNMT inhibitors that have shown effectiveness in preclinical studies. Decitabine and Azacytidine, nucleoside analogs, operate by incorporating themselves into DNA during synthesis, forming irreversible bonds with DNMTs and leading to their depletion. RG108, a non-nucleoside analog, inhibits DNMT3B and has demonstrated anti-cancer effects, particularly in combination with other therapies. However, each inhibitor has its limitations and potential drawbacks, such as toxicity at higher doses (Decitabine), side effects and potential resistance (Azacytidine), and specificity to DNMT1 (RG108). These challenges underscore the need for further research to optimize dosage regimens, minimize side effects, and explore combination therapies. Alternative strategies, including nanovaccines and exosomes for targeted delivery of DNMT inhibitors, offer exciting prospects. Additionally, combination therapies involving DNMT inhibitors, radiotherapy, chemotherapy, and other epigenetic modulators present a multifaceted approach to cancer treatment.

While the field of DNMT inhibitors holds great promise, it is essential to consider the financial implications, potential side effects, and the need for personalized treatment approaches. Furthermore, ongoing research should explore alternative inhibitors, such as BET and HDAC inhibitors, to enhance the efficacy of DNMT inhibitors.

References

1. Turek-Plewa, J., & Jagodziński, P. (2005). THE ROLE OF MAMMALIAN DNA METHYLTRANSFERASES IN THE REGULATION OF GENE EXPRESSION. CELLULAR & MOLECULAR BIOLOGY LETTERS, 10, 631–647. https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=b93b4892df137157f81167f67dc6dd70390b8ef0
2. Łuczak, M., & Jagodzinski, P. (2006). The role of DNA methylation in cancer development. FOLIA HISTOCHEMICA et CYTOBIOLOGICA, 44(3), 143–154. https://journals.viamedica.pl/folia_histochemica_cytobiologica/article/viewFile/4561/3816
3. Zhang, W., & Xu, J. (2017). DNA methyltransferases and their roles in tumorigenesis. Biomarker Research, 5(1). https://doi.org/10.1186/s40364-017-0081-z
4. Zhao, S., Zhu, S., Hao, X., Li, P., & Zhang, S. (2011). Effects of DNA methyltransferase 1 inhibition on esophageal squamous cell carcinoma. Diseases of the Esophagus, 24(8), 601–610. https://doi.org/10.1111/j.1442-2050.2011.01199.x

5. National Cancer Institute. (2021, October 11). What is cancer? National Cancer Institute; National Institutes of Health. https://www.cancer.gov/about-cancer/understanding/what-is-cancer
6. Chik, F., & Szyf, M. (2010). Effects of specific DNMT gene depletion on cancer cell transformation and breast cancer cell invasion; toward selective DNMT inhibitors. Carcinogenesis, 32(2), 224–232. https://doi.org/10.1093/carcin/bgq221
7. Ehrlich, M. (2002). DNA methylation in cancer: too much, but also too little. Oncogene, 21(35), 5400–5413. https://doi.org/10.1038/sj.onc.1205651
8. Cui, J., Zheng, L., Zhang, Y., & Xue, M. (2021). Bioinformatics analysis of DNMT1 expression and its role in head and neck squamous cell carcinoma prognosis. Scientific Reports, 11(1). https://doi.org/10.1038/s41598-021-81971-5
9. Man, X., Li, Q., Wang, B., Zhang, H., Zhang, S., & Li, Z. (2022). DNMT3A and DNMT3B in Breast Tumorigenesis and Potential Therapy. Frontiers in Cell and Developmental Biology, 10. https://doi.org/10.3389/fcell.2022.916725
10. Zhang, J., Yang, C., Wu, C., Cui, W., & Wang, L. (2020). DNA Methyltransferases in Cancer: Biology, Paradox, Aberrations, and Targeted Therapy. Cancers, 12(8), 2123. https://doi.org/10.3390/cancers12082123
11. Gnyszka, A., Jastrzebski, Z., & Flis, S. (2013). DNA methyltransferase inhibitors and their emerging role in epigenetic therapy of cancer. Anticancer Research, 33(8), 2989–2996. https://pubmed.ncbi.nlm.nih.gov/23898051/
12. Togano, T., Nakashima, M., Watanabe, M., Umezawa, K., Watanabe, T., Higashihara, M., & Horie, R. (2013). Synergistic Effect of 5-Azacytidine and NF-κB Inhibitor DHMEQ on Apoptosis Induction in Myeloid Leukemia Cells. Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics, 20(12), 571–577. https://doi.org/10.3727/096504013x13775486749371
13. Azacitidine - an overview | ScienceDirect Topics. (n.d.). Www.sciencedirect.com. Retrieved December 28, 2023, from https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/azacitidine#:~:text=Azacitidine%20and%20decitabine%20are%20both
14. Yang, L., Hou, J., Cui, X.-H. ., Suo, L.-N. ., & Lv, Y.-W. . (2017). RG108 induces the apoptosis of endometrial cancer Ishikawa cell lines by inhibiting the expression of DNMT3B and demethylation of HMLH1. European Review for Medical and Pharmacological Sciences, 21(22), 5056–5064. https://doi.org/10.26355/eurrev_201711_13818
15. PubChem. (n.d.). Azacitidine. Pubchem.ncbi.nlm.nih.gov. Retrieved December 28, 2023, from https://pubchem.ncbi.nlm.nih.gov/compound/Azacitidine#section=Structures
16. RG108 (N-phthalyl-L-tryptophan): DNMT inhibitor: Medchemexpress. MedchemExpress.com. (n.d.). https://www.medchemexpress.com/RG108.html?utm_source=google&utm_medium=CPC&utm_campaign=US&utm_term=RG108&utm_content=RG108+dnmt+inhibitor
17. PubChem. (n.d.). Decitabine. Pubchem.ncbi.nlm.nih.gov. Retrieved December 28, 2023, from https://pubchem.ncbi.nlm.nih.gov/compound/451668
18. Graca, I., Sousa, E., Baptista, T., Almeida, M., Ramalho-Carvalho, J., Palmeira, C., Henrique, R., & Jeronimo, C. (2014). Anti-Tumoral Effect of the Non-Nucleoside DNMT Inhibitor RG108 in Human Prostate Cancer Cells. Current Pharmaceutical Design, 20(11), 1803–1811. https://doi.org/10.2174/13816128113199990516

19. Paulis, L. E., Mandal, S., Kreutz, M., & Figdor, C. G. (2013). Dendritic cell-based nanovaccines for cancer immunotherapy. Current Opinion in Immunology, 25(3), 389–395. https://doi.org/10.1016/j.coi.2013.03.001
20. Constantino, J., Gomes, C., Falcão, A., Cruz, M. T., & Neves, B. M. (2016). Antitumor dendritic cell–based vaccines: lessons from 20 years of clinical trials and future perspectives. Translational Research, 168, 74–95. https://doi.org/10.1016/j.trsl.2015.07.008
21. Wu, M., Lu, S., Cheng, M., Zhang, H., Jiang, Y.-Z., Lin, S., Yu, L., Zhu, F., Liu, Z., Zhang, Y., Zhang, X., Gao, Q., Chen, D., Li, J., & Yang, L. (2019). Low doses of decitabine improve the chemotherapy efficacy against basal-like bladder cancer by targeting cancer stem cells. Oncogene, 38(27), 5425–5439. https://doi.org/10.1038/s41388-019-0799-1
22. Kalluri, R., & LeBleu, V. S. (2020). The biology, function, and Biomedical Applications of Exosomes. Science, 367(6478). https://doi.org/10.1126/science.aau6977
23. Hu, C., Liu, X., Zeng, Y., Liu, J., & Wu, F. (2021). DNA methyltransferase inhibitors combination therapy for the treatment of solid tumor: mechanism and clinical application. Clinical Epigenetics, 13(1). https://doi.org/10.1186/s13148-021-01154-x
24. Daskalakis, M., Brocks, D., Sheng, Y.-H., Islam, M. S., Ressnerova, A., Assenov, Y., Milde, T., Oehme, I., Witt, O., Goyal, A., Kühn, A., Hartmann, M., Weichenhan, D., Jung, M., & Plass, C. (2018). Reactivation of endogenous retroviral elements via treatment with DNMT- and HDAC-inhibitors. Cell Cycle, 17(7), 811–822. https://doi.org/10.1080/15384101.2018.1442623
25. Kenneth K.W. To, Xing, E., Larue, R. C., & Li, P.-K. (2023). BET Bromodomain Inhibitors: Novel Design Strategies and Therapeutic Applications. *Molecules*, *28*(7), 3043–3043. https://doi.org/10.3390/molecules28073043
26. Kim, H., Jang, H., Cho, H., Choi, J., Hwang, K. Y., Choi, Y., Kim, S. H., & Yang, Y. (2021). Recent Advances in Exosome-Based Drug Delivery for Cancer Therapy. *Cancers*, *13*(17), 4435. https://doi.org/10.3390/cancers13174435
27. Ou, Y., Zhang, Q., Tang, Y., Lu, Z., Lu, X., Zhou, X., & Liu, C. (2018). DNA methylation enzyme inhibitor RG108 suppresses the radioresistance of esophageal cancer. Oncology Reports. https://doi.org/10.3892/or.2018.6210