ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2022; 27(2): 3416-3421
doi: 10.25083/rbl/27.2/3416.3421

Received for publication: May, 16, 2022
Accepted: May, 20, 2022

Review

Anti-cancer alternative therapies: from inorganic nanoparticles to tumor-killing bacteria

GRIGORE MIHĂESCU[1], MARIAN CONSTANTIN[2,3]*, OCTAVIAN ANDRONIC, ALEXANDRA BOLOCAN[4], ROXANA FILIP[5,6]

[1]Faculty of Biology, University of Bucharest, 030018 Bucharest, Romania
[2]Institute of Biology Bucharest of Romanian Academy, 296 Splaiul Independentei, 060031 Bucharest, Romania
[3]Fellow of the Research Institute of the University of Bucharest, ICUB, Bucharest, Romania
[4]General Surgery, University of Medicine and Pharmacy "Carol Davila", 020021 Bucharest, Romania
[5]Faculty of Medicine and Biological Sciences, Stefan cel Mare University of Suceava, 720229 Suceava, Romania
[6]Regional County Emergency Hospital, 720284 Suceava, Romania

Abstract

Cancer affects an increasing number of people every year, affecting many families and representing a major problem for health systems in all countries. As chemotherapy is one of the most widely used therapeutic approaches, usually following surgical resection of the tumour mass, its adverse effects have made it necessary to find alternative, less toxic ways for treating cancer. These include nanoparticles, especially those containing Ag and/or Pt, some nonpathogenic, attenuated or genetically engineered bacteria can exhibit a destructive potential on tumors, especially when they carry antitumor genes or antineoplastic agents, cationic antimicrobial peptides, modified to mitigate their harmful effects, and immunotherapy, such as immune checkpoint inhibitors.

Keywords

chemotherapy, cancer treatment, nanoparticles, nonpathogenic bacteria, cationic antimicrobial peptides, immune checkpoint inhibitors

To cite this article: MIHĂESCU G, CONSTANTIN M, ANDRONIC O, BOLOCAN A, FILIP R. Anti-cancer alternative therapies: from inorganic nanoparticles to tumor-killing bacteria. *Rom Biotechnol Lett.* 2022; 27(2): 3416-3421 DOI: 10.25083/rbl/27.2/3416.3421

*Corresponding author: cvgmarian@gmail.com

Introduction

One of the main public health problems affecting society today is the high incidence of cancer. In addition to complications caused by the primary tumour and possible metastases, cancer patients are more susceptible to complications after infections caused by *Streptococcus pneumoniae*, *Staphylococcus* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Helicobacter pylori* and *Candida spp.*. On the other hand, the patients chronically infected with *S. aureus*, *A. baumannii*, *P. aeruginosa* and *Enterobacter* are immunosuppressed and have a higher risk of developing cancer (Rodriguez et al., 2019). In the treatment of cancer, standard therapeutic management requires surgical resection, chemotherapy and radiotherapy. Tumour resection is an invasive method and ensures the removal of the primary tumour and possible metastases, and in some cases can ensure a cure. Most of the time, however, after resection, the cancer patient undergoes chemotherapy. This involves the administration of a platinum-based or other chemical agents, either by infusion or orally, which inhibits the multiplication of tumoral cells. It has also non-specific toxic effects on normal cells, causing symptoms such as sickness, vomiting, numbness in the extremities, hair loss, etc. Since tumour cells exposed to chemotherapeutic agents increase their enzymatic capacity to detoxify drugs and repair DNA, they could become drug resistant, leading to tumour recurrence. Some patients cannot tolerate the treatment for the entire period of administration and it has to be stopped prematurely. In case of tumors resistant to chemotherapy, radiotherapy is prescribed. The standard treatment involves irradiating the site of the tumour cell deposits with a dose high enough to destroy them, especially when they are therapeutically sensitised. Because radiation affects any tissue it passes through, healthy tissues are affected by radiation. Unfortunately, none of these therapeutic approaches can always permanently eliminate the pool of tumour cells; so, the risk of neoplasm recurrence remains, especially in very aggressive tumours such as mucinous tumours, whose cells are extra-protected by their mucus secretion. Alternative strategies such as nanoparticles (NPs), non-pathogenic bacteria, antimicrobial cationic peptides, i*mmunotherapy*) have been developed to at least partially eliminate its side effects.

NPs in cancer treatment

Nanotechnology is a multidisciplinary domain of chemistry, microengineering, biology and medicine with applications in therapy and fighting against bacterial resistance and neoplasia (Tudose et al., 2016). Against bacteria, Ag NPs act by perforating the bacterial cell wall, forming reactive oxygen species (ROS), inhibiting aerobic respiration and damaging DNA. Ag NPs are also active on transforming cell lines and tumors by altering mitochondrial function, blocking the cell cycle and activating apoptosis (Tianyuan Shi et al., 2018; Albuleţ et al., 2017). The effect depends on the concentration, size and coating material of the NPs. NPs consisting of Ag-Pt inhibit glioblastoma and melanoma cell lines. Platinum binds to DNA and kills the cells by apoptosis or necrosis. Like all chemotherapeutic agents, cisplatin is not selective for malignant cells, and, because lower toxicity, NPs with Pt are more efficient in stopping cancer development (Lopez Ruiz et al., 2020).

Therapy with nonpathogenic bacteria

Some bacteria are potentially carcinogenic promoters by stimulation of the inflammatory reaction in infective process: *H pylori* is a potentially gastric carcinoma inductor; *Salmonella typhi* can induce hepatobiliary carcinoma, *Campylobacter jejuni* leads to small intestine lymphoma; *Chlamydia psittaci* is an inductor for eye lymphoma; *Mycobacterium tuberculosis* can lead to lung cancer; *Citrobacter rodentium* induces human colorectal cancer (CRC); *Porphyromonas gingivalis*, which is present in the oral cavity is a potentially inductor for pancreatic cancer (Song et al., 2018).

On the other side, bacteria reported to have potential anticancer activity include species of *Salmonella, Clostridium, Bifidobacterium, Lactobacillus, Escherichia, Pseudomonas, Caulobacter*, *Listeria, Proteus*, BCG and *Streptococcus*. The routes of action and their use in treating cancer are diverse. Nonpathogenic or genetically modified bacteria with tropism for neoplastic tissue can have direct anticancer action through oncolytic activity, by secreting toxins and enzymes (proteases and lipases). Tumour produces attractant molecules for bacteria that penetrate ECM to the central region of the tumour, where hypoxic medium is favorable to obligate and facultative anaerobes: *Clostridium spp., Bifidobacterium spp., E. coli*. After tumour colonization, quorum sensing commutes the genetic program that changes the tumour microenvironment and produces cell lysis (Zargar et al., 2019). BCG is used for treating bladder tumours (Morales et al., 1976; Maffezini, 2006). In anaerobic conditions, *E. coli* stimulates cell immune response mediated by TCD8 cytolytic lymphocytes (Song et al., 2018) and the monocytes (macrophages) from the tumor tissue containing bacteria release TNF-innate immunity factor with cytolytic effect.

Molecules synthesized and released by bacteria can inhibit tumour growth (enzymes such as lipases and proteases) or have toxicologically specific anti-tumour potential (bacteriocins). *P. gingivalis* is one of the few bacteria that syn-

thesize peptidyl arginine deiminase (PAD), an angiogenic agent efficient in leukemia treatment (Ye Ni et al., 2008) and against arginine auxotroph neoplasia-hepatocellular carcinoma and melanoma. Bacteriocins (piocines, colicines, pediocins, microcines) are antimicrobial cationic peptides produced by many bacterial species, with specific toxic potential against different malignant cell lines: breast, colon, HeLa (Cornnut et al., 2018; Song et al., 2018).

Some of the bacterial toxins, such as diphtheria toxin (TD), bind to antigens on the surface of tumor cells, inhibiting the EGF production. The TD-HB-EGF complex is released by endocytosis, and the lytic A subunit (activity) of TD is eliminated inside the cell. Ligand-conjugated toxins (*Pseudomonas* exotoxin, ricin DT) can be therapeutically effective, but must be targeted to specific cell membrane sites. *C. novyi* spores have a non-lethal toxin (Patyar et al., 2010) and when injected in mice with neoplasia produce lytic destruction of tumors (Baindara & Mandal, 2020);

Live attenuated or genetically engineered bacterial cells have the ability to carry and spread tumorigenic molecules, releasing them mainly in hypoxic and anaerobic regions of solid tumors, with a potentially destructive effect on primary neoplasia (Patyar et al., 2010; Song et al., 2018). They can also be modified to carry genes for anti-cancer lytic proteins or can be used as vehicles for antineoplastic agents to be released into solid tumors (Patyar et al., 2010). Genetically engineered *Salmonella* cells synthesize LPS that do not stimulate proinflammatory cytokines and thus lower the risk of septic shock. Oral administration of genetically modified probiotics or enteric administration of heterologous bacteria, either probiotics or fecal transplantation, can be used for treating blood neoplasia, sarcoma and melanoma.

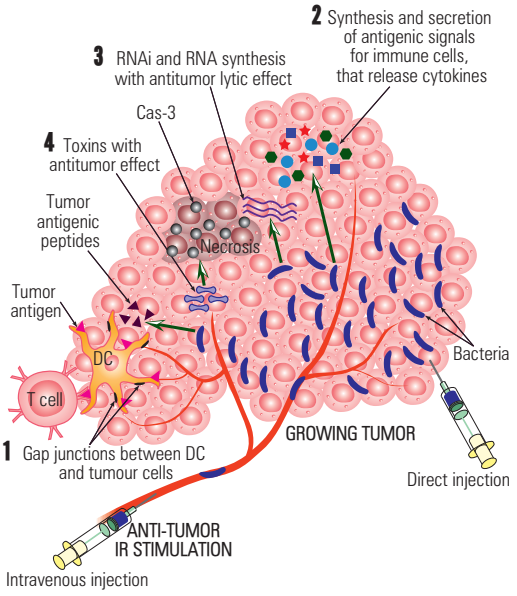

Figure 1. Anti-neoplastic effect of genetically modified bacteria. Anti-tumor IR stimulation by: 1. Gap junctions between DC and tumour cells, that facilitates the transfer of tumour antigenic peptides to DC and then recruitment of T1 and T2, T3 lymphocytes; 2. Synthesis and secretion of antigenic signals for immune cells, that release cytokines; 3. RNAi and RNA synthesis with antitumor lytic effect; 4. Toxins with antitumor effect (modified after Baindara & Mandal, 2020).

The main limiting factors in the use of bacteria for the treatment of cancer stem from the fact that, in the dose required for therapy, bacteria become toxic, with systemic infection posing a major risk of toxicity. Also, bacteria incompletely lyse the tumour mass and act only in hypoxic regions without affecting metastases that do not have anaerobic conditions (Patyar et al., 2010).

Antimicrobial cationic peptides

Although chemotherapeutic agents produce side effects on normal cells and tissues, they continue to be the main therapeutic option in cancer, but a new therapeutic approach with less toxicity is emerging. This is represented by new molecules with selective action and anti-infective and anti-tumour specificity: toxins, immunotoxins, enzymes, peptides, bacteriocins (also peptides) and a wide range of proteins (Karpinski & Adamczak, 2018). Of particular importance are antimicrobial peptides (AMPs) with antitumor activity, which can specifically target tumor cells and are classified into two categories: (a) peptides active on bacteria and malignant cells with no side effects on mammalian cells, and (b) peptides that are toxic on tumor cells, bacterial cells, but also on healthy cells (Rodriguez et al., 2019). Antimicrobial peptides are synthesized by plants, invertebrates, vertebrates and represent a major part of their innate immunity in thousands of chemical variants (Wu et al., 2010). Antimicrobial peptides from animal sources are small molecules, with chains of 6-100 amino acids, and very diverse chemically. They are classified according to their secondary structure into β-fold peptides, α-helical loop peptides and linear peptides (Grădișteanu Pîrcălăbioru et al., 2021). The discovery of the therapeutic effects of cationic antimicrobial peptides began with the observation that they favor the restoration of the balance of the resident microbiota. Synthesized by probiotic bacteria, they have antimicrobial and immunomodulatory effects and antimicrobial properties, inhibiting the synthesis of LPS-induced proinflammatory cytokines and recruiting antigen presenting cells. A family of antimicrobial peptides, defensins, are important players in innate immunity and play a role in defense (Hancock & Sahl, 2006; Giuliani & Nicoletto, 2007). The lytic properties of AMPs allow them to be

considered as a therapeutic option in the treatment of malignancies: magainins from *Xenopus* skin lyse hematopoietic cells and solid tumour cells, with limited effect on normal lymphocytes (Zasloff,1987; Makovitzki et al., 2009). The next level of investigation is the chemical synthesis of cationic AMPs with therapeutic potential in cancer. AMPs are positively charged and have amphipathic properties, making it possible for them to bind, via electrostatic interaction, to intensively negatively charged membrane of malignant cells due to the rich presence of phosphatidyl serine, glycoproteins and glucosamines. By penetrating into the intracellular space, AMPs reach mitochondria, which they inactivate and cause cell death through necrosis or apoptosis. Thus, amphipathic cationic AMPs can be an efficient source of antineoplastic agents (Giuliani & Nicoletto, 2007; Hung Lun Chu et al., 2015; Deslouches & Di, 2017).

Therapeutic use of AMPs is limited by in vivo instability at the internal pH, high price and toxicity against normal cells (Giuliani & Nicoletto, 2007). Also, AMPs could eventually resistant tumour cells. Peptidomimetics synthesized by coupling AMPs with a substituted amine have a significant anti-tumour activity and a low toxicity on normal cells (Huan Li et al., 2021). Another attempt to overcome the limitation in the therapeutic use of AMPs was the replacement of amino acids with D forms in vivo and in vitro (Papo et al., 2009), or association of AMPs with nanomaterials (titanium padded with calcium sulphate), which increases their stability (Kazemzadeh-Narbat et al., 2010).

Anti-metabolites

Next-generation sulphonamides inhibit the activity of matrix metalloproteinases (MMPs). Their discovery led to the synthesis of TNFα converting enzyme inhibitors (TACE) with great potential for reducing inflammation. MMP and TACE have a synergistic action in the pathophysiology of tumour invasion (Supuran et al., 2003, Cierpial et al., 2020).

Immunotherapy

Malignant cells have evolved various strategies to overcome host immunity, and the basic concept of immunotherapy (IT) is the immune checkpoint inhibition (ICI). ICI immunotherapy methodology is considered to be particularly successful in solid tumours (Martins Lopes et al., 2020). One of the checkpoints is PD binding to the L1 ligand (PD-1 programmed cell death protein 1; L1-ligand 1). PD1 is expressed on lymphocytes, and PD-L1 is expressed on tumor cells and APCs. Binding of PD1 to ligand 1 inactivates T lymphocytes and blocks the immune response (IR). The anti PD-1 and anti PD-L1 monoclonal antibodies (MAB) abrogates the inhibitory effect of anti-tumor cytotoxic T lymphocyte activation and has been introduced in advanced non-small cell lung cancer (NSCLC). Patient survival was significantly improved. The outcome after ICI treatment is heterogeneous, being beneficial for a small number of patients. One explanation could be the physiology of the microbiota acting on the anti-tumour effect of therapy targeting an ICI checkpoint. Chronic mitomy-

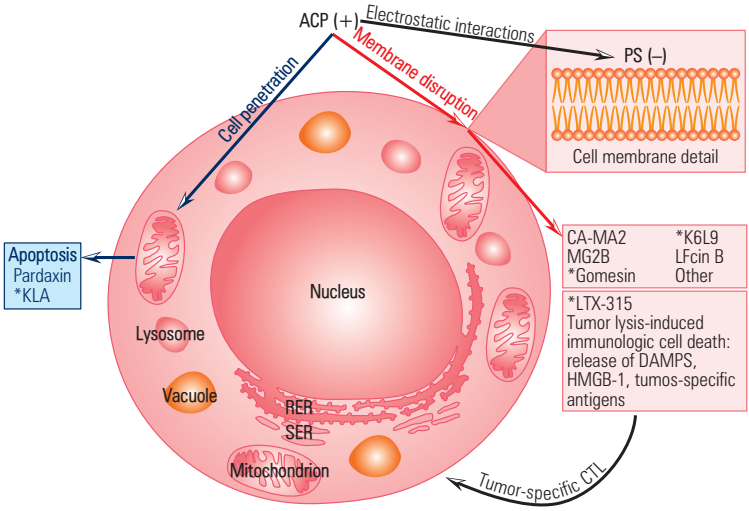

Figure 2. Common anti-tumour mechanisms for cationic AMPs. Negatively charged anti-cancer cationic peptides selectively recognize malignant cells by electrostatic interaction with negatively charged Ek (PS–phosphatidyl serine) cell membrane phospholipids. Some AMPs are efficient in vivo (MG2B, Gomesin, K6L9, other). CAP can kill tumour cell by membrane disruption (red), and others (KLA, pardaxin) can penetrate the target cell and break the mitochondrial membrane (blue) (modified after Deslouches &Y Peter Di, 2017).

cin (anthracycline antibiotic) therapy indicates dysbiosis and greatly diminishes the effect of AMC immunotherapy targeting the PD-1-L1 checkpoint (Reed et al., 2019; Schett et al., 2020; Derosa et al., 2020). Another checkpoint for IR is CTL-4 (cytotoxic T lymphocyte-associated protein 4=CD152). CTL-4 is a cytotoxic T lymphocyte membrane receptor that is activated upon binding of CD80 (or CD86) ligand expressed on CPA. The CTL-4 receptor triggers an inhibitory signal in activated T lymphocytes. Specific anti-CTL-4 mAbs are used in metastatic melanoma therapy. The therapeutic effect of MAB is diminished or reversed in germ-free animals or animals treated with broad-spectrum antibiotics (Pianbianco et al., 2018; Dubin et al., 2016). The efficacy of anti PD-1 anti PD-L1 and anti CTL-4 immunotherapy is influenced by the physiological status of the microbiota. Bifidobacterium stimulates T lymphocytes. Antibiotics, through changes in microbiota have reversed the favorable effect of treatments targeting ICI in most cancer patients (Bertrand et al., 2018; Vetizou &Trinchieri, 2018, Gopalakrishnan et al., 2018; Ming Yi et al., 2018; Elkrief et al., 2019; Garajova, 2021).

Conclusions

Finding alternative routes to chemotherapy in the treatment of cancer is a great challenge for researchers in the field of human biology and in this regard there are several promising research directions, represented by the use of metal nanoparticles, nonpathogenic bacteria with direct anti-tumor effect or which transport and release various compounds into the tumor microenvironment, cationic antimicrobial modified peptides and immunotherapeutic methods inhibiting the immune checkpoints.

Acknowledgements

This research was funded by ICUB, grant number 2153/01.02.2022, the Ministry of Research, Innovation and Digitalization through Program 1—Development of the national R&D system, Subprogram 1.2—Institutional performance—Financing projects for excellence in RDI, Contract no. 41 PFE/30.12.2021 and UEFISCDI-FDI 2022-0675. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript or in the decision to publish the results.

Bibliography

1. Albuleț et al., Nanostructures for Cancer Therapy 2017. Book chapter *Part of* DOI: 10.1016/B978-0-323-46144-3.00001-5EID: 2-s2.0-8504062037
2. Baindara P., Mandal M.S., Bacteria and bacterial anticancer agents as a promising alternative for cancer therapeutics. Biochimie, vol. 177, 2020, pag. 164/189, 2018. doi:10.1016/j.biochi.2020.07.020
3. Bertrand R. et al., Gut microbiome influences efficacy of PD-1 based immunotherapy against epithelial tumors. Science. 2018 jan 5; 359 (6371): 91-98. doi:10.1126/Science.aan3706
4. Cierpial T. et al., Fluoroaryl analogs of sulforaphane–A group of compounds of anticancer and anticrobial activity. Bioorg chem.2020 jan; 94:103454. doi:10.1016/j.bioorg.2019.103454
5. Cornut G et al., Antineoplastic properties of Bacteriocins: revisiting potential active agents. Am J Clin Oncol. 2008, 31(4): 399-404. doi:10.1097/COC.=B=13e31815e456d
6. Derosa L. et al., Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. Onco-Immunology, vol 29, issue 6, P 1437-1444, 2018. doi:https:doi.org/10.10.1093/annonc/mdy103
7. Deslouches B.&Peter DiY., Antimicrobial peptides with selective antitumor mechanisms: project for anticancer applications. Oncotarget.2017, jul 11;8(28):46651. doi:18632/oncotarget.16743
8. Dubin K et al., Intestinal microbiome analysis identify melanoma patients at risk for checkpoint-blocade-induced colitis. Nat Commun. 2016;7:10391. doi:10.1038/ncomms10391
9. Elkrief A. et al., The intimate relationship between microbiota and cancer immunotherapy. Gut microbes. 2019; 10(3):424.428. doi:10.1080/19490976.218.1527167
10. Garajova Ingrid et al., The role of the microbiome in drug resistance in gastrointestinal cancers. doi.org/10.1080/14737140.2021.1844007
11. Giuliani A.P.G.&Nicoletto S.F., Antimicrobial peptides: an overview of a promising class of therapeutics. Central European Journal of Biology. 2007; 2(1):1-33)
12. Gopalakrishnan et al., Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science.2018 Jan 5; 359(6371) 97-103. Doi:10.1126/science.aan4236.Epub2017 Nov 2.
13. Gradisteanu Pircalabioru G., Popa L.I., Marutescu L., Gheorghe I., Popa M., Czobor Barbu I., Cristescu R., Chifiriuc M.C., Bacteriocins in the Era of Antibiotic Resistance: Rising to the Challenge. Pharmaceutics. 2021 Feb 2;13(2):196. doi: 10.3390/pharmaceutics13020196. PMID: 33540560; PMCID: PMC7912925.
14. Hancock R.E.W.&Sahl H.G., Antimicrobial and host defense peptides as new anti-infective therapeutic strategies. Nature Biotechnology 24, 1551-1557 (2006)

15. Huan Li et al., Antimicrobial and antitumor activity of peptidomimetics synthesized from amino acids. Bioorganic chemistry, vol 106, jauary 2021. doi: 10.1016/j.bioorg.2020.104506
16. Hung-Lun Chu et al., Novel antimicrobial peptides with high anticancer activity and selectivity. May 13, 2015. doi:10.1371/journal.pone.0126390
17. Karpinski T., Adamczak A., Anticancer Activity of Bacterial Proteins and Peptides. Pharmaceutics, 2018 Jun;10(2):54 doi:10.3390/pharmaceutics10020054
18. Kazemzadeh-Narbat M. et al., Antimicrobial peptides on calcium phosphate coated titan for the prevention of implant-associated infections. Biomaterials. 2010; 31:9519-26. 10.1016/j.biomaterials.2010.08.035
19. Lopez Ruiz A. et al., Novel Silver-Platinum Nanoparticles for Anticancer and Antimicrobial Applications. Int J Nanomedicine. 2020; 15:169-179. doi:10.2147/IJN.S176737
20. Maffezini M.R., Intravesical BCG versus Mitomycin C for Ta and T1 bladder cancer. European Urology 2006, 50613710
21. Makovitzki A et al., Suppression of Human solid tumor growth in mice by intratumor and systemic inoculation of histidine-rich and pH-dependent host defense-like lytic peptides. Can Res.2009;69(8):3458-63. doi:10.10.1158/0008-5472.CAN-08-3021
22. Martins Lopez M.S. et al., Antibiotics, cancer risk and oncologic treatment efficacy: a practical review of the literature. Ecancermedicalscience. 2020;14:1106. doi:3332/cancer.2020.1106
23. Ming Yi et al., Gut microbiome modulates efficacy of immune check point inhibitors. Hematol Oncol. 2018 Mar 27; 11(1):47. doi:10.1186/s13045-018-0592-6
24. Morales A et al., Intracavitary BCG in the treatment of superficial bladder tumors. J Urol. (1976), 10.1016/s0022-5347(17)58737-610.1016/j.juro.2016
25. Papo N et al., A Novel lytic peptide composed of DL-aminoacids selectively kills cancer cells in culture and in mice. J. Biol Chem.2009; 30(4):660-8. 10.1016/j.peptides.2008.12.019
26. Patyar S. et al., Bacteria in Cancer therapy: a novel experimental strategy. J. Biomed Sci. 2010 Mar 23; 17(1). doi:10.1186/1432-0127-17-21
27. Pianbianco C. et al., Pharmacomicrobiomics: exploiting the drug-microbiota interactions in cancer therapies–Microbiome. 2018;6:92. doi:10.1186/s40168-018-0483-7
28. Reed J.P. et al., Gut microbiome, antibiotic use and immunotherapy responsiveness in cancer. Ann Transl Med. 2019; 7(suppl 8):S309. doi:10.21037/atm.21037.2019.10.27
29. Rodriguez G et al., Bacterial Proteinaceous Compounds with multiple activities toward Cancers and Microbial Infection. Front Microbiol. 2019; 10:1690. doi:10.3389/fmicb.2019.01690
30. Schett Anne et al., Predictive impact of antibiotics in patients with advanced non small lung cancer receiving immune checkpoint inhibitors. Cancer Chemother Pharmacol. 2020; 85(1): 121-131. doi:10.10.1007/s00280-019-03993-1
31. Song Shiyu et al., The role of bacteria in cancer therapy- enemies in the past, but allies at present. Infectious Agents and Cancer 13, Article number 9 (2018)
32. Supuran T. Claudiu et al., Protease inhibitors of the sulfonamide type: Anticancer, antiinflamatory and antiviral agents–9 May 2003. doi.org/10.1002/med.10047
33. Tianyuan Shi et al., Cytotoxicity of Silver Nanoparticles against Bacteria and Tumor Cells. Current Protein & Peptide Science vol 19, issue 6, 2018. doi:10.2174/1389203718666161108092149
34. Tudose M et al., Multifunctional Silver Nanoparticles-Decorated Silica Functionalized with Retinoic Acid with Anti-Proliferative and Antimicrobial Properties. Journal of Inorganic and Organometallic Polymers and Materials2016 | Journal article DOI: 10.1007/s10904-016-0407-6EID: 2-s2.0-8497815334
35. Vetizou Marie, Trinchieri G., Anti-PD-1 in the wonder gut-land. Cell Res, 2018 Mar; 28(3):263-264.
36. Wu Dongdong et al., Peptide-based cancer therapy: opportunity and challenge. Cancer Lett. 2014; 351(1):13-22. 10.1016/j.canlet.2014.05.002
37. Ye Ni et al., Arginin deiminase–a potential anti-tumor drug. Cancer Lett. 2008 Mar; 261(1):1-11. doi:10.1016/j.canlet.2007.11.038
38. Zargar Amin et al., Overcoming the challenges of cancer drug resistance through bacterial-mediated therapy. Chronic Diseases and Translational Medicine, vol 5, issue 4, December 2019, pag 258-266. doi:org/10.1016/j.cdtm.2019.11.001
39. Zasloff M., Magainins–a class of antimicrobial peptides from *Xenopus* skin: isolation, caracterization of two active forms and partial cDNA sequence of a precursor. Proc Natl Acad. Sci. USA 84, 5449-5453 (1987)

ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2022; 27(2): 3422-3428
doi: 10.25083/rbl/27.2/3422.3428

Received for publication: May, 16, 2022
Accepted: May, 20, 2022

Review

Antitumor antibiotics: representatives, mechanisms of action and side effects

GRIGORE MIHAESCU[1], MARIAN CONSTANTIN[2,3]*, OCTAVIAN ANDRONIC[4], ALEXANDRA BOLOCAN[4], ILINCA VLAD[5], ROXANA FILIP[6,7]

[1]Faculty of Biology, University of Bucharest, 030018 Bucharest, Romania
[2]Institute of Biology Bucharest of Romanian Academy, 296 Splaiul Independentei, 060031 Bucharest, Romania
[3]Fellow of the Research Institute of the University of Bucharest, ICUB, Bucharest, Romania
[4]General Surgery, University of Medicine and Pharmacy "Carol Davila", 020021 Bucharest, Romania
[5]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy, 6 Traian Vuia, 020956 Bucharest, Romania
[6]Faculty of Medicine and Biological Sciences, Stefan cel Mare University of Suceava, 720229 Suceava, Romania
[7]Regional County Emergency Hospital, 720284 Suceava, Romania

Abstract

Bacteria and fungi synthesize various compounds necessary for their own metabolism, as well as compounds that kill other microbial strains and species from the environment in which they grow, to ensure their access to sufficient supplies. Since the discovery of their antitumor effect, three main classes of antibiotics are used in cancer therapy: anthracyclines (secondary metabolites, mainly produced by members of Streptomyces group, or semisynthetic derivatives containing the 7,8,9,10-tetrahidrotetracen-5.12 quinone structure), peptide antibiotics and quinolones. In this minireview, we will present the mechanisms of action, main representatives and side effects of these anti-cancer agents.

Keywords antitumor agents, anthracyclines, peptide antibiotics, quinolones

To cite this article: MIHĂESCU G, CONSTANTIN M, ANDRONIC O, BOLOCAN A, VLAD I, FILIP R. Antitumor antibiotics: representatives, mechanisms of action and side effects. *Rom Biotechnol Lett.* 2022; 27(2): 3422-3428 DOI: 10.25083/rbl/27.2/3422.3428

*Corresponding author: cvgmarian@gmail.com

Introduction

Bacteria and fungi synthesize various compounds necessary for their own metabolism, as well as compounds that kill other microbial strains and species from the environment in which they grow, to ensure their access to sufficient supplies. In 1909, American bone surgeon and cancer researcher William Coley (1909) has prepared native supernatants from pure culture of *Streptomyces pyogenes* and *Serratia marcescens*, which he administered to 1200 neoplastic patients. The results were encouraging: tumour regression in 52 of them, and out of these, 30 patients were completely recovered. Its discovery preceded with 19 years the discovery of the very first antimicrobial compound, penicillin, by Alexander Fleming, in 1928, which opened a new era in infections treatment. Since then, the antibiotic classes of anthracyclines, peptides and quinolones, were proved to have specific inhibitory effects on tumour cells and anti-bacterial cells, some of their members being used as anti-cancer agents with high clinical importance (Rao et al., 1962). The purpose of this minireview is to present the mechanisms of action, main representatives and side effects of these anti-cancer agents.

Anthracyclines

Anthracyclines are polyketides (condensed planar heterocycles) containing anthracene as core structure (fig.1). Polyketides are complex molecules, belonging to the group of actinobacteria secondary metabolites, mainly synthesized by members of *Streptomyces* group, or to the semisynthetic derivatives containing 7,8,9,10-tetrahidrotetracen-5.12 quinone structure. According to Katz & Donadio (1993), there are two classes of polyketides: aromatic and complex. They have both antitumor and antibacterial activity. The first members of this group of compounds were reported by Brockman and Bauer (1950) and their use as anti-tumour agents started with daunorubicin discovery. Natural anthracyclines are isolated from fungal species, while the synthetic ones are obtained by glycosylation with rodosamine (including *amino* sugars), *daunosamine* and neutral glycosides. Some of them, such as aglycon, are usually inactive, but amongst hundreds of anthracyclines molecular analogues with antitumoral and antimicrobial activity, FDA approved a small number for clinical administration: *actinomycin D* (dactinomycin), *daunorubicin*, *doxorubicin*, *epirubicin*, *idarubicin*, *mitomycin*, *mitoxantrone*, *plicamycin*, *valrubicin*, *enediyne*, *guanorycin*, etc. (Saeidnia, 2015). Some of these are active, especially after chemical change, irrespective of cell cycle stage, even in G0, inhibiting proliferation, acting as pro-apoptosis and anti-epithelial-mesenchymal transition factors, thus inhibiting metastasis (Zhou J, 2013). Anthracyclines inhibit oxidative phosphorylation in mitochondria, inhibit DNA and RNA polymerases, DNA repairing enzymes, metallothionein synthesis (a protein synthesized by intestinal epithelial cells that inhibits Cu absorption, its synthesis being stimulated by Zn), topoisomerase I and II, helicase, stimulates free radicals release and the non-nucleolytic cleavage (Robert J & Gianni L, 1993).

Doxorubycin (adriamycin) was isolated from *Str. peucetius* and is a cytotoxic antibiotic with major importance in different types of neoplasia, including pediatric cancer (Hayward et al., 2013, Preet et al., 2015, Gao Yuan et al., 2020; Cheng et al., 2017). It has an amphiphilic (amphipathic) molecule; the water insoluble anthracycline ring is lipophilic and the saturated end of the cycle with numerous –OH groups associated with aminated carbohydrate daunosamine forms a hydrophilic center. The molecule is amphoteric because contains acid functions in phenolic groups and alkaline ones in the amino group of the carbohydrate. Doxorubycin binds to the cell membrane and plasmatic proteins and disturbs numerous cell functions. Enzymatic reduction of doxorubicin, by electron acceptance under the action of oxidases, dehydrogenases and reductases generates extremely active species, including free $OH^{\bullet}$ radicals. Doxorubycin is also a DNA intercalating agent, blocking DNA and RNA replication and transcription and thus protein synthesis in cells with high growth and division rate. It also interacts with topoisomerase II forming complexes that break the DNA molecule. Cardiotoxicity of doxorubycin is explained by the inhibitory activity on topoisomerase and glutathione peroxidase, leading to increased oxidative stress in the absence of catalase in these cells (Thorn et al., 2011; Cagel et al., 2017; Hayward et al., 2013; Takemura & Fujiwara, 2007, Minotti et al., 2004).

Daunorubicin (daunomycin) is a glycoside derivative of anthracycline fermentation which contains an anthraquinone ring and daunosamine (an amino sugar). Daunorubicin rapidly penetrates cell, it accumulates in nucleus, intercalates in the DNA strand and daunosamine stabilizes the complex by additional interactions making the difference between daunorubicin and other intercalation agents as ethidium bromide – which establishes interactions only in the intercalation site. Another probable target being topoisomerase II, daunorubicin is an efficient inhibitor of DNA replication and transcription. By oxidation and reduction of anthraquinone, daunorubicin can generate cytotoxic free $O2^{\bullet-}$ radicals, explaining the cumulative cardiotoxicity (Bloomfield et al., 1973; Marco et al.,1977).

Epirubicin is a DNA intercalating chemotherapeutic agent and also a topoisomerase II inhibitor (Cragg & Newman, RAPT vol 33) (Waters et al., 1999).

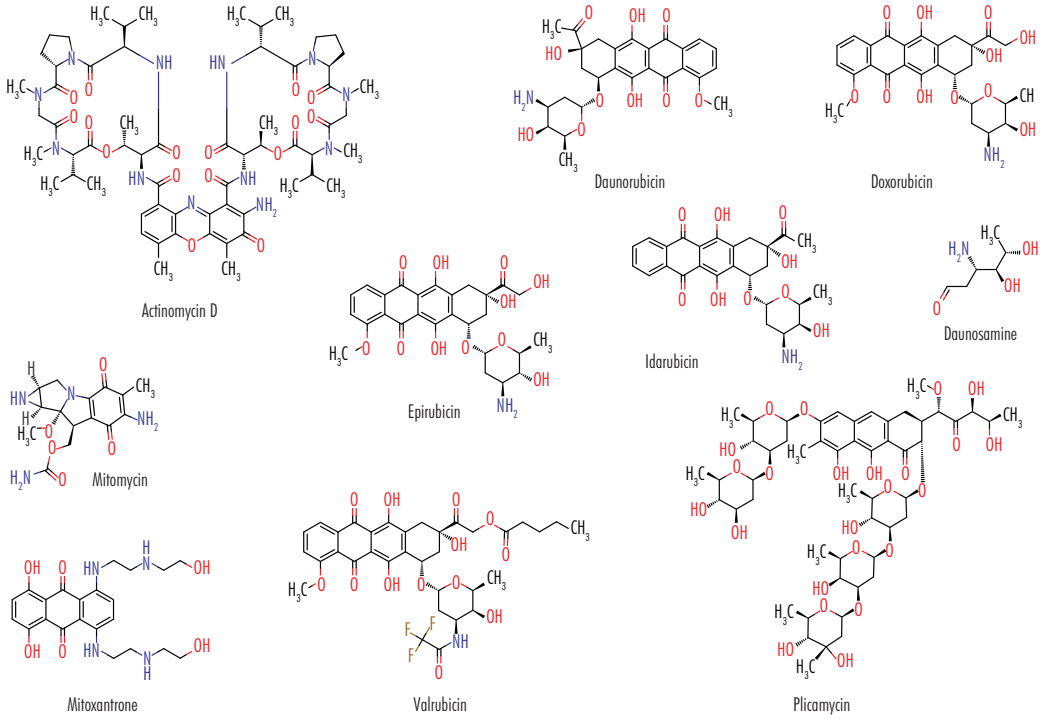

Figure 1. Chemical structures of some members of the anthracycline family, synthesized by microorganisms and having antimicrobial and antitumor activity. Actinomycin D is also a member of the peptide class of antibiotics which act as antimicrobial and antitumor agents.

Mitoxantrone (anthracenedione) inhibits lipid peroxidation, has a low toxicity to cardiac muscle, it contains no carbohydrate groups and produces no reactive oxygen species (Fox et al., 1986).

Plicamycin has inhibitory effect against several neoplasia. It binds DNA sequences rich in GC in any stage of cell cycle, inhibits RNA and protein synthesis (Thurlimann et al., 1992).

Idarubicin is an anthracycline antibiotic with antimitotic and cytotoxic activity acting by DNA intercalation and topoisomerase II inhibition (Robert J & Gianni L, 1993).

The three members of mitomycin family: mitomycin A, mitomycin B, and mitomycin C are isolated from *Str. caespitosus*. After activation inside the cell by a reductase, mitomycin binds on a DNA single chain by alkylation, but also forms transversal links between chains. The effect is DNA depolymerization, with replication, transcription and protein synthesis inhibition (Verweij et al,1990; Tomasz & Palom,1997, Bradner, 2001).

Peptide antibiotics

Actinomycines are a family of 50 chromopeptide antibiotics, but only two have therapeutic value. Actinomycin D (dactinomycin) is synthesized by *Str. antibioticus* on a variety of chemically defined media and on complex organic media, and it is the first antibiotic which was proved to have antitumor activity (1943). Its molecule contains an aromatic group (polyphenolic ring) that binds with two cyclic polypeptide chains (fig. 2). Actinomycin D interacts with absolute specificity with deoxyguanine in DNA, similarly in eukaryotic and prokaryotic cells: the aromatic ring intercalates inside the DNA double helix at GC pairs, and the cyclic peptide remains outside and induces single and double strand breaks. Actinomycin D also inhibits RNA polymerase (inhibiting replication and transcription) and has played an important role in mRNA discovery. After RNA polymerase-catalyzed transcription is blocked, the synthesis of all forms of RNA, including those undergoing synthesis, is stopped. The antibiotic is not active on Gram negative bacteria because of reduced permeability, but spheroplasts are sensitive. Actinomycin D does not bind to RNA or mcDNA, and its affinity for double-stranded DNA depends on the guanine content, as synthetic double-stranded polynucleotides do not interact with the antibiotic (Farhane et al., 2018; Koba & Konopa, 2005). Actinomycin D has cytotoxic and antineoplastic effects, inducing P53-independent

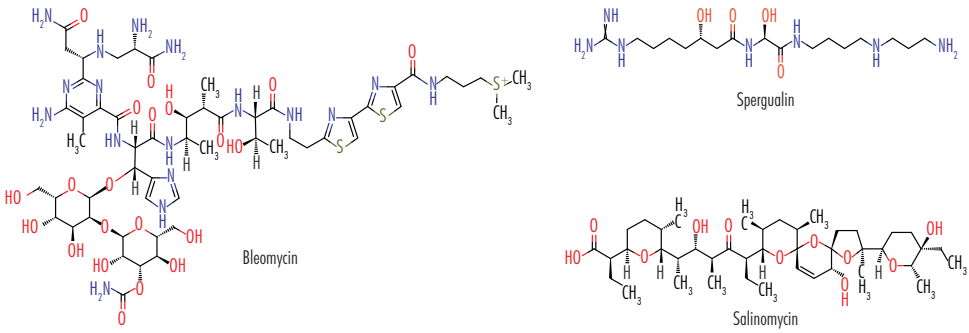

Figure 2. Chemical structures of some members of the peptide class of antibiotics as antimicrobial and antitumor agents, synthesized by microorganisms.

cell apoptosis (Prouvot et al., 2018; Dactinomycine accessed 20 February 2018; Hazel et al.,1983).

Spergualin is a water-soluble peptide possessing a special chemical structure: it has a C-terminal guanidyl group and a C-terminal polyamine. It stimulates T-cell-mediated cytotoxic immunity (Umezawa K &Takeuki T, 1987; Umezawa H et al., 1987, Nishikawa et al., 1986).

Bleomycin's molecule consists of a central heptapeptide that represents the binding site of different groups under the action of halogenases and transferases therefore generating a large structural diversity. Bleomycin was discovered by Hamao Umezawa (1962) in *Str. verticillus* cultures filtrates bacterium. All bleomycins have the same general structure, but differ by function group attached to the terminal amino group (ACS - Chemistry for life, 2020). The natural form is a mixture of two glycopeptide antibiotics: Bleomycin A2 and Bleomycin B2, bound with 3 carbohydrate residues. Bleomycin binds DNA and possibly RNA. In the presence of Fe^{+2} forms a *pseudoenzyme* that interacts with O_2 resulting in the release of superoxide (*in vitro* O_2^-) and OH^- groups that cleave DNA resulting single and double strand breaks (Segerman et al., 2013). Bleomycin sulfate is used to treat Hodgkin and non-Hodgkin lymphoma, squamous cell carcinomas and cancer-related pleural effusion. Some bacterial and tumour cells encode a bleomycin-inactivating hydrolase, which hydrolyses the amide group attached to beta-amino alanine. The protection degree against bleomycin depends on hydrolase level (Sugiyama & Kugamai, 2002; Bayer et al., 1992; Baindara & Mandal, 2020, Latta et al., 2015; Egger et al., 2013).

Salinomycin is a monocarboxylic polyether isolated from *Str.albus* (Miyazaki et al., 1974) and is used as an antibacterial, antifungal, antiparasitic and antitumoral drug (Hyun-Gyo Lee et al., 2017, Zhou et al., 2013, Gupta et al., 2009). The polyether skeleton acts as a cationic ionophore, forming complexes with metal cations and interfering with the ion exchange function of the cell membrane. It binds to monovalent cations (Na > K > Cs) and divalent cations (Sr > Ca > Mg), having high affinity for K ions, and interferes with its transmembrane potential. Both *in vivo* and *in vitro*, it induces ROI (extracellular reactive oxygen intermediates) release and apoptosis of leukemic CD4 cells, but not of CD4 cells sampled from healthy individuals (Piperno et al., 2016). Salinomycin is EMT and metastases inhibitor (Chen 2014).

Quinolones

Quinolones (also called 4-quinolones) are a family of molecules that share the quinolinic nucleus. They are the first synthetic antimicrobial agents obtained by synthesis. Changes in the chemical structure of nalidixic acid have given rise to the new generation quinolones or fluoroquinolones

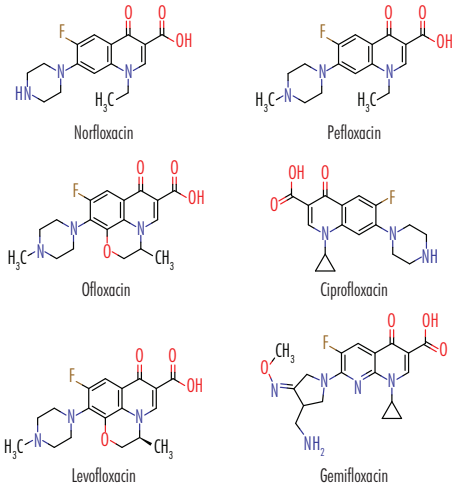

Figure 3. Chemical structures of some members of the quinolone class of antibiotics as antimicrobial and antitumor agents, synthesized by microorganisms.

(norfloxacin, pefloxacin, ofloxacin, ciprofloxacin, levofloxacin etc.) which have an extended antibacterial spectrum. Along with beta-lactams and macrolides, quinolones are one of the most widely used antimicrobial agents used in humans. Quinolones inhibit the DNA gyrase quickly stopping the replicative DNA synthesis. Quinolones are toxic to mammalian cells *in vitro* and *in vivo* experimental models (Goto & Wang 1985; Warren, 1985; Liu, 1989).

Among fluoroquinolones, the most used is ciprofloxacin, which has a wider antibacterial spectrum compared to nalidixic acid. In ciprofloxacin molecule, fluorine ensures activity on Gram-positive bacteria, piperazine group increases anti-enterobacteria activity and piperazine and cyclopropyl groups enable anti-*Pseudomonas* activity. Ciprofloxacin is widely used in the therapy of urinary, respiratory and gastrointestinal infections. It is also active on cell lines from human and animal bladder neoplasia. Ciprofloxacin has anti-proliferative and pro-apoptosis effects, since *gemifloxacin* is EMT (anti- epithelial-mesenchymal transition) and metastases inhibitor (Chen 2014).

Conclusions

Since the discovery of their antitumor effect, three main classes of antibiotics were used as adjuvants in cancer therapies: anthracyclines, peptide antibiotics and quinolones. They have both antimicrobial and antitumoral activity, being able to induce DNA breaks and to inhibit DNA and RNA synthesis and protein synthesis. Through these mechanisms, the antitumor antibiotics inhibit EMT and metastases formation (salinomycin, gemifloxacin), induce P53-independent cell apoptosis, stimulate T-cell-mediated cytotoxic immunity, thus exhibiting anti-proliferative and pro-apoptosis effects.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

This research was funded by ICUB, grant number 2153/01.02.2022, the Ministry of Research, Innovation and Digitalization through Program 1—Development of the national R&D system, Subprogram 1.2—Institutional performance—Financing projects for excellence in RDI, Contract no. 41 PFE/30.12.2021 and UEFISCDI-FDI 2022-0675. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript or in the decision to publish the results.

Bibliography

1. ACS chemistry for life – Bleomycin –Molecule of the week Archive, 2020)
2. Baindara P & Mandal M S - Bacteria and bacterial anticancer agents as a promising alternative for cancer therapeutics - Biochimie, vol. 177, 2020, pag. 164/189, 2018. doi:10.1016/j.biochi.2020.07.020
3. Bayer R A et al. – Bleomycin in non-Hodgkin's lymphoma. Semin.Oncol.1992;19(suppl.5):46-53
4. Bloomfield C D et al. – Daunorubicin-prednisone remission induction with hydroxyuree maintenance in acute non-lymphoblastic leukemia – Cancer, 1973-Wiley On line Library
5. Bradner W. T -Mitomycin C: A clinical update. Cancer Treat. Rev. 2001; 27(1):35-50. doi:10.1053/ctrv.2000.0202
6. Brokman H, Bauer K – Rhodomycin, ein rotes antibioticum aus actinomyceten – Naturwissenschaften.1950; 37(21):492-493. doi:10.1007/BF00623151
7. Cagel M. et al. – Doxorubicin: Nanotechnological overwiews from bench to bedside. Drug Discov. Today.2017; 22:270-281. doi:10.1016/j.drudis.2016.11.005
8. Chen T C et al. – Gemifloxacin inhibits migration and invasion and induces mesenchymal-epithelial transition in human breast adenocarcinoma cells. J Mol Med (Berl) 2014; 92(1):53-64
9. Cheng M et al. – Molecular Effects of Doxorubicin on choline Metabolism in Breast Cancer[J] Neoplasia. 2017; 19(8):617-627
10. Coley W. B. – The treatment of inoperable by bacterial toxins (the mixed toxins of the *Streptococcus erysipelas* and *Bacillus prodigiosus*) Proc. R. Soc. Med. Surg. 1909;3:1-48
11. Cragg G M et al. – Natural Product Drug Discovery and Development - Recent Advances in Phytochemistry, RAPT, vol 33, pp 19-32
12. Cragg G M and Newman D J – Natural Products discovery and development at the United States National Cancer Institute – Recent Advances in Phytochemistry, RAPT, vol 33, pp 1-19
13. Cragg G M, Newman D J, Snader K M – Natural products in drug discovery and development – J Nat Prod. 1997;60(1):52-60. doi:10.1021/np9604893
14. Dactinomycine.[(accesed on 20 February 2018)]; Available on line: http://medycyna.anauk.net/101-0393-Encyklopedia.Lekow.html
15. Egger C. et al. – Administration of bleomycin via the oropharingeal aspiration route leads to sustained lung fibrosis in mice and rats as quantified by UTE-MRI and histology. PLos ONE. 2013; 8:e63432. doi:10.1371/journal.pne.0063432
16. Farhane Z. et al.- An in vitro study of the interaction of the chemotherapeutic drug Actinomycin D with lung cancer

cell lines using Raman micro-spectroscopy. J. Biophotonics.2018;11:e2001700112. doi:10.1002/jbio.201700112

17. Gao Yuan et al. – Antibiotics for Cancer Treatment: A double-edged sword – J Cancer. 2020;11(17):5135-5149. doi:107150/jca.47470
18. Goto T & Wang JC, 1985 - Cloning of yeast Topo I, the gene encoding DNA Topo I and construction of mutants defective in both DNA Topo I and DNA Topo II. Proc. Natl. Acad. Sci. USA 82, 7178/7182
19. Gupta B Piyush et al. - Identification of Selective Inhibitors of Cancer Stem Cells by High-Throughput Screening – Cell, 645-659, 2009. doi:10.1016/jcell.2009.06.034
20. Hayward R et al. – Tissue retention of Doxorubicin and its effects on cardiac, smooth and skeletal muscle function. J Physiol Biochem. 2013; 69(2):177-87. doi:10.1007/s13105-012-0200-0
21. Hazel G A et al. – Treatment of metastatic carcinoid tumor with dactinomycin or dacarbazine – Cancer Treat Rep. 1983 jun; 67(6):583-5
22. Hyun-Gyo Lee et al. – Salinomycin reduces stemness and induces apoptosis on human ovarian cancer stem cell - J Ginecol Oncol; 2017 Mar; 28(2):e14. doi:10.3802/jgo.2017.28.e14
23. Katz L, Dnadio S – Polyketide synthesis: prospects for hybrid antibiotics – Annu Rev Microbiol.1993; 47:85-912. doi:10.1146/annurev.mi.47.100193.004303
24. Koba M.& Konopa J. – Actinomycin D and its mechanism of action. Postepy Hig. Med. Dosw. 2005;59:290-298
25. Latta V. D.. et al. – Bleomycin in the setting of lung fibrosis induction: From biological mechanisms to contraindications. Pharmacol.Res 2015; 97:122-130. doi:10.1016/(j.phrs.2015.04.012
26. Liu L F- DNA topoisomerase poisons as antitumor drugs. Annu. Rev. Biochem. 1989; 58:351-375
27. Marco A D et al. – Changes of activity of daunorubicin, adriamycin and stereoisomers, following the induction or removal of hydroxyl groups in the amino sugar moiety [J] – Chemico-Biological Interactions, 1977; 19(3):291-302
28. Minotti G et al. – Anthraciclines: Molecular Advances and Pharmacologic Developments in Antitumor Activity and Cardiotoxicity. Pharmacol. Rev. 2004; 56:185-229
29. Miyazaki Y et al. – J. Antibiot. (Tokyo) 1974, 27, 814
30. Nishikawa N et al. – Antitumor activity of Spergualin, a novel antibiotic. The Journal of Antibiotics (Tokyo), 1986; 39(10): 1461-1466. doi:10.7164/antibiotics.39.1461
31. Piperno Anna et al. – Chemistry and Biology of salinomycin and its analogues. doi:http://dx.medra.org/10.17374/targets.2016.19.177
32. Preet S et al.-Effect of nisin and Doxorubicin on DMBA-induced skin carcinogenesis-A possible adjunct therapy. Tumor Biol. 2015; 36:8301-8308 doi:10.1007/s13277-015-3571-3
33. Prouvot C et al. – Efficacy and safety of Second line 5-Day Dactinomycin – Int J Gynecol Cancer. 2018 Jun; 28(5):1038-1044. doi:10.1097/IGC.0000000000001248
34. Rao K V et al. – Chemotherapy. New antibiotic with antitumor properties. 1962; 12:182-186
35. Robert J & Gianni L – Pharmacokinetics and metabolism of Anthraciclines. Cancer Surv. 1993; 17:219-252
36. Saeidnia S.- Anticancer Antibiotics [M]//New Approaches to Natural Anticancer Drugs. 2015
37. Segerman J Z et al. – Characterization of bleomycin-mediated cleavage of a hairpin DNA library. Biochemistry. 2013; 52, 31, 5315-5327. doi:10.1021/bi400779r
38. Sugiyama M & Kugamai T - Molecular and structural biology of bleomycin and its resistance determinants. J of Bioscience and Bioengineering, vol 93, issue 2, 2002, pages 105-116. https://doi.org/10.1016/S1389-1723(02)80001-9
39. Takemura G & Fujiwara H –Doxorubycin induced cardiomyopathy from the cardiotoxic mechanisms to management. Prog Cardiovasc Dis. 2007;49(5):330-352. doi:10.1016/j.pcad.2006.10.002
40. Thorn et al.- Doxorubicin pathway: Pharmacodynamics and adverse effects. Pharmacogenet.Genom.2011; 21:440-446. doi:10.1097/FPC.0b013e32833ffb56
41. Thurlimann B et al. – Plicamycin and pamidronate in symptomatic tumor-related hypercalcemia: a prospective randomized crossover trial [J]- Anals of Oncology: official journal of the European Society for Medical Oncology. 1992; 3(8):619
42. Tomasz M & Palom Y – The mitomycin bioreductive antitumor agents. Cross linking and alkylation of DNA as the molecular basis of their activity. 1997;76(1-3-):73-87. doi:10.1016/s0163-7258(97)00088-0
43. Umezawa K & Takeuki T –Spergualin: a new antitumor antibiotic –Biomed Pharmacother. 1987; 41(5):227-32.
44. Umezawa H et al. – Involvement of Cytotoxic T-lymphocytes in the antitumor activity of Spergualin against L1210 cells – Can Res. 1987 jun;47(12):30-62-5
45. Verweij J, Pinedo H.M. – Mitomycin C: Mechanism of Action, usefulness and limitations – Anticancer Drugs. 1990; 1:5-13. doi:10.1097/00001813-199010000-00002
46. Warren E R – DNA topoisomerases as Targets for Cancer Therapy. Biochemical Pharmacology. Vol 34. No 24, pp 4191-4195. 1985

47. Waters J S et al. – Long term survival after epirubicin, cisplatin and fluorouracil for gastric cancer: results of a randomized trial. Br J Cancer. 1999;80(1-2):269-272

48. Zhou J et al. – Salinomycin induces apoptosis in cisplatin-resistant CRC cells by accumulation of reactive oxygen species. Toxicol Lett. 2013; 222(2):139-145