Medical Research Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: November 30, 2022

Citation: Schardt FW, Henke E, et al., 2022. Effective Therapy with Different Strategies in Treatment of Malignant Cells, Medical Research Archives, [online] 10(11). https://doi.org/10.18103/mra.v10i11.3308

DOI
https://doi.org/10.18103/mra.v10i11.3308

ISSN: 2375-1924

RESEARCH ARTICLE

Effective Therapy with Different Strategies in Treatment of Malignant Cells

F.W. Schardt [1], E. Henke [2], A. Zernecke-Madsen [3]

[1] Betriebsärztliche Untersuchungsstelle of University Würzburg, Josef-Schneider-Str.2, 97080 Würzburg
[2] Institute of Anatomy and Cell Biology of University Würzburg, Koellikerstr. 6, 97070 Würzburg
[3] Institute of Experimental Biomedicine, Universitätsklinikum, Josef-Schneider-Str. 2, 97080 Würzburg

ABSTRACT

Malignant cells build up a protective scaffold in form of fibrin meshwork surrounding the tumor cells and provide an extracellular matrix (ECM) consisting of proteoglycans, collagens, glycoproteins, and glycosaminoglycans. Simultaneously, they produce stem cells in an early cancer stadium, which gain the energy and oxygen for their metabolism from the anaerobic glycolysis and are therefore independent of arterial supply.

Many publications show that heparins have a wide variety of efficiencies to tumor metabolism because they have the highest negative charge density of any known biological molecules. Therefore, they should be exogenously added in high dosages. Opoids such as D,L methadone induce cell death in malignant cells by downregulation of cAMP. Multiple studies have also shown an association of aspirin combined with clopidogrel treatment to reduce cancer incidence.

In an ambulant trial with daily exogenously added heparins, methadone and a combined clopidogrel/aspirin therapy the survival probability over 12 months was significantly higher than without ($p<0,001$). Additionally, zoledronic acid was given as infusion every 2 months. This auxiliary treatment has the potential to support established therapies. With methadone, heparins, zoledronic acid and clopidogrel/aspirin we present as supplement to the oncological therapy a model in treatment of malignant cells.

Keywords: malignant cells, methadone, heparins, zoledronic acid, anticoagulants.

Introduction

Tumor induction is a complex process with numerous modulatory factors such as chronic or virus infections or chemical carcinogens like the mycotoxin aflatoxin. (1, 2). The connection between chronic inflammation and malignancy or cancer is also well-recognized (3 ,4 ,5). Moreover, the association between coagulation, changed ECM and malignant cells has been observed for centuries (6,7,8,9,10). In former publications it was demonstrated by monoclonal antibodies that malignant cells are surrounded directly by a net of fibrin as a scaffold against the immune surveillance whereas normal cells and tissues showed no deposits of fibrin (10). This was especially marked and shown with malignant hemopoetic and cancer stem cells in blood and lymphatic vessels because there is an abundance of fibrinogen. This fibrin network was induced by self-produced factors for coagulation (7).

Utilizing different escape mechanisms malignant cells bypass normal proliferation controls of the immune surveillance system and can invade other tissues. Already in an early stadium malignant stem cells disperse by the vascular and lymphatic system with different mechanisms (11,12). They have an altered carbohydrate metabolism and are therefore independent of vascular and oxygen supply (13). Moreover, they are able to survive stress and DNA damage better than benign cells (14,15).

Further, the pathogenesis of haemostatic disorders caused by malignant tumors is complex and reflects the interaction of an increased coagulation system combined with fibrinogen production and activated platelets and fibroblasts (10,16).

Additionally, tumor and malignant cells provide with an abundant extracellular matrix (ECM) consisting of proteoglycans, collagens, glycoproteins and glycosaminoglycans a further scaffold.

Material and methods

In this study (2013 - 2021) participated 41 patients (19 males, 22 females) with an average of 47,3 years. The members of the two groups were randomized with averagely 49,8 (test group) and 47,7 years (control group). In the control group there was an incidence of 8 thrombosis and cured with 20 or 40 mg enoxaparin depending on body mass index (adiposis: 40 mg) for about one week according to the relevant guidelines. In the test group (10 females and 9 males) the participants got a complex information about efficacy and side effects of methadone, high dosage heparins, clopidogrel/aspirin and zoledronic acid before they entered the trial. For one year the patients of the test group got three times a week 25,000 IE heparins intravenous and during the rest of the week daily 80 mg (8,000 IE) subcutaneously enoxaparin-natrium (9). The participants had to fill out a monthly protocol about their heparins, clopidogrel/aspirin and methadone consumption. Any cases of bleeding complications in both groups had been registered. Platelets, hemoglobin and tumor marker were controlled every two months. This study entered only patients with a radiological (MRT, CT) and histological certified carcinoma or sarcoma and in some cases one or two metastasis. This presented only an additional and preventive provision during the oncological therapy, according to the guidelines.

The different tumor types of all patients consisted of: 12 mamma-Ca, 1 bronchial-Ca, 1 lung-Ca, 13 prostata-Ca, 2 pancreas-Ca, 5 stomach-Ca, 4 colon-Ca, 2 acute myeloblastic leukemia and 1 glioblastoma The distribution of the tumor types in the therapy and control groups was again nearly proportional with 6 mamma-ca, 6 prostata-Ca, 2 stomach-Ca, 2 colon-Ca, 2 pancreas-Ca and 1 glioblastoma in the test and the rest in the control groups.

The members of the therapy group got every day clopidogrel 75mg combined with 100mg acetylsalicylic acid and every two months zoledronic acid 4mg (11,12). The infusion with zoledronic is about 50% excreted by the kidney and the remainder has a very high affinity for bone tissue. Therefore, the application every two or three months is necessary (17,18).

Additionally, methadone was applied as cell death sensitization all patients in the verum group with following content in100ml:

Methadone-HCL:	1,0 g
Sorbinic acid:	0,06 g
Citronic acid:	0,08 g and
Aqua purificata ad	100,0 g (18)

This solution showed the best resorption determined by blood levels in former investigations. (19)

All patients took in average 5 drops during the day and in the evening 10-35 (maximum) till the limit of tolerance (no hangover for the next day). The absolute minimum was 10 and maximum was 65 drops per day. (19, 20) Maybe the different tolerance is depending on the different individual concentration of P 450.

The size of the primary tumor and metastases in all cases was reduced in the therapy group after one year. In two cases even bone metastasis diminished nearly, documented by MRT.

For the statistical analysis we used an unpaired, two-tailed Student`s T-Test for the differences between the two groups and the graphical plot in form of survival probability over 12months (Kaplan-Meier-Plot).

Results

In this ambulant trial we tested the possibility for a survival probability of a therapy with heparins, clopidogrel/aspirin, methadone and zoledronic acid. The survival probability in the treatment group over 12 months was significant higher ($p < 0,001$) than in the control group (Fig. 1). Observed bleeding complications were low grade (WHO grade I: mouth, nose injection mark, no gastrointestinal) without the need of pharmacological or surgical treatment. The incidence of bleeding complications was in therapy group 6,8 % and in the control group 1,7%. All data were sampled between 2008 and 2021 in the ambulance with written consent of the patients. The one patient in the therapy group (female, mamma-Ca, liver metastasis) who died took only 5 drops of methadone in the evening because of nausea and drowsiness during the day.

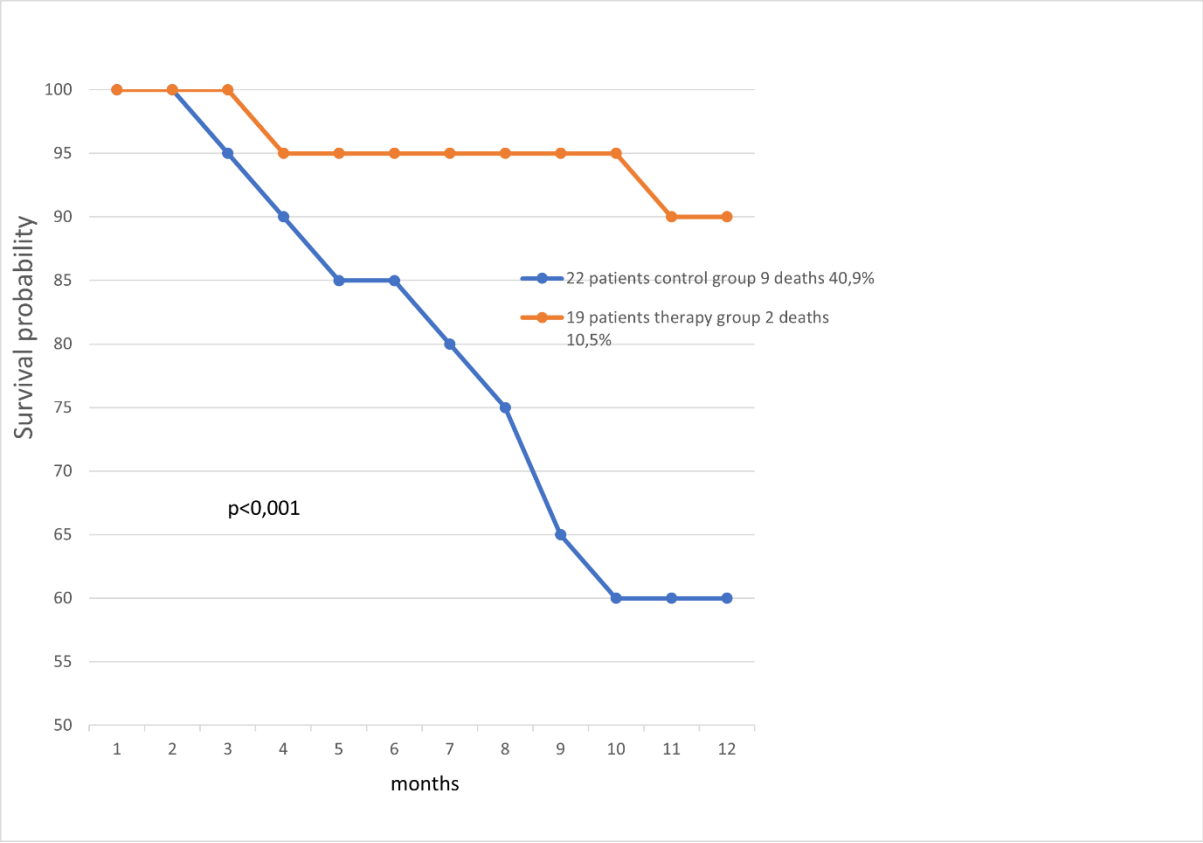

Fig. 1: Survival probability of 41 randomized patient with different malignant diseases under a therapy of methadone, heparins, clopidogrel/aspirin and zoledronic acid and without (control group) during the oncological therapy according to the guidelines over 12 months.

Fig. 2: Malignant cell with tight meshwork of fibrin. To provide and secure the complex and intensive metabolism of a malignant cell the fibrin chains are permeable for the substances of metabolism but also as a protection against cells of the immune system.

Discussion

Furthermore, and unimpaired endures a discordance between experimental investigations and preclinical results with cancer patients. This is obviously caused by the fibrin network surrounding the malignant cell and the glycoproteins and glycoprotein glycans predominantly
collagens, paxillin and fibronectin in ECM. (8, 9, 10, 21).

Some investigations and publications found out that inflammatory mediators that contribute to neoplasia include prostaglandins, inflammatory cytokines i. e. IL -1ß, TNF-alpha, IL-6 and IL-15 and chemokines i.e. and GRO-alpha. (2, 22, 23). Already Rudolf Virchow hypothesized in1863 that the origin of cancer was at sites of chronic inflammation which is nowadays estimated to approximately 15-20 % of human cancer. Prolonged or chronic inflammation causes DNA damages inducing reactive oxygen species (ROS) and reactive nitrogen species (RNS) to fight infection (24). However, chronic inflammation also causes epigenetic changes such as DNA methylation (2, 25). Moreover, some virus infections increase the risk of cancer such as EBV, HIV, papillomavirus, hepatitis and certain parasites by similar mechanisms of DNA or RNA and inactivating p53. (26, 27, 28, 29).

All these factors can change the DNA and with it the metabolism by different accentuation of signalling pathways. Therefore, it is also the important role histones to regulate the genes and DNA replications (30). The goal of the changed metabolism is to produce escape mechanisms against the immune surveillance and reduce susceptibility to radiation or other types of treatment cancer. (30,31). In former publications it was exposed that tumor cells produce a meshwork of fibrin, activated platelets, cancer associated fibroblasts and a ECM with different glycoproteins and proteoglycans. These substances mainly induced by FAK and LOX form by tight connection with fibrin an effective scaffold. (7,10,31). However, heparins have a wide variety of positive effects
counteracting these shielding and immunosuppressive properties of ECM properties. (10,33). Heparins can bind and neutralize many protective substances produced by the tumor cells (34). Heparins also play an active role in immune escape: they bind to selectin and integrin, block the formation of cytokine complex signal pathways and interrupt the adhesion of cancer cells by deceasing the expression of E-cadherin and catenin (30,35). They inhibit cross-linking of collagens by deamination and reduce the expression of FAK,LOX, glucosamines and proteoglycans. (36) . By these actions they prevent the development of a stiff and rigid ECM which presents additionally a scaffold for tumor cells and also reduce the efficiency of therapeutic methods (37).

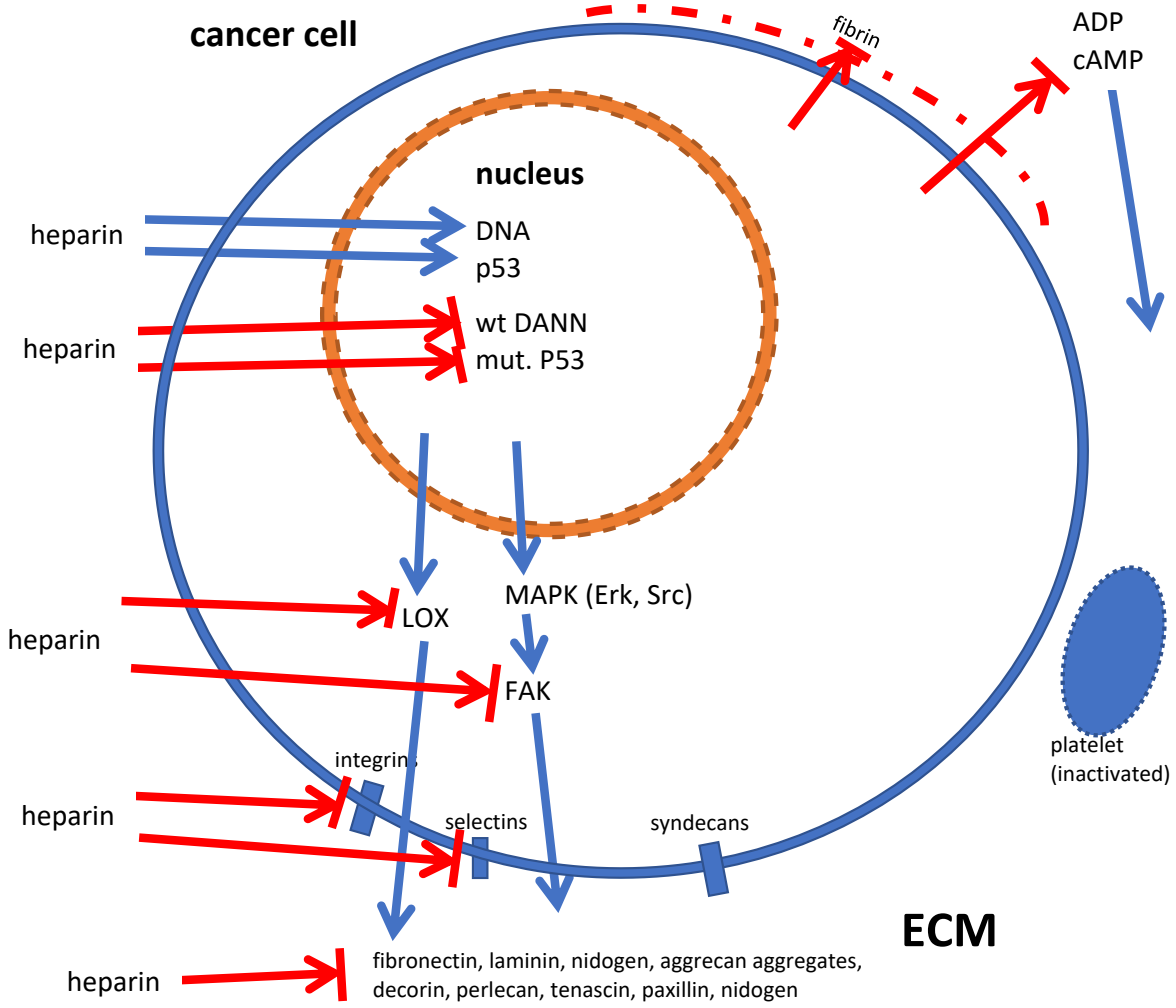

Fig. 3: Heparins stimulate (------→) DNA, p53, and inhibit (-----→I) the production of mutated or wild type (wt) DNA, mutated p53 and different glycaminoglycans, proteoglycans, collagens and glycoproteins depending on the applied dosage. Thereby the formation of scaffolds for malignant cells in ECM are reduced.

Some studies with dual clopidogrel and aspirin treatment generate and support the hypothesis that both together reduce cancer incidence. There is a feedback or mutual influence between activated platelets induced by ADP, cancer-associated fibroblasts and malignant cells. (10, 38). Therefore, clopidogrel and aspirin are very essential for a successful therapy. (39, 40, 41, 42). The advantage of the dual therapy over the aspirin-only group was apparent even after adjustment for duration of aspirin therapy. The reason is surely the supplementary effect of both substances. Clopidogrel is an irreversible thienopyridine prodrug inhibiting the P2Y12 subtype of ADP receptors. By this it prevents mainly the release of Ca-ions, fibronectin, fibrinogen, coagulation factors V and XIII and glycoprotein VI by different granules and microtubules. Whereas aspirin acted on two different types of cyclooxygenases, COX-1 (irreversibly) and COX-2 (modified). As additional mechanisms it induces the formation of NO-radicals and reduces leukocyte adhesion. (43).

The downregulation of cAMP induced by opioid receptor activation using D,L-methadone kills and sensitizes leukaemia cells and other malignant cells especially for doxorubicin treatment. (20). Nevertheless D,L methadone improves the treatment of other malignant diseases as well as the

untreatable glioblastoma stem cells. Deficient caspase activation was observed in chemo- and radioresistant glioblastoma and other malignant cells treated with anti-cancer drugs or radiation. (19,20)

Cancer stem cells are tumorgenic and form a small proportion of tumor which is sensible for chemotherapy.but act not specifically on the stem cells. Conventional

chemo drugs kill differentiated or differentiating cells which form the bulk of tumor. But a population of cancer stem cells could remain untouched and cause relapse. They have a long-term ability to self-renew and capacity to differentiate into progeny and contributes to the growth of the malignant tumor. The presence of cancer stem cells is shown both in primary tumors (i.e. breast cancer) and in a most early dissemination in distant metastases as well as the bone marrow.(11,44)

In an own study we explored the glycolysis blocking properties of the bisphosphonate zoledronic acid in leukaemia and breast cancer cells as well especially in malignant stem cells. Although, zoledronic acid had little effect at normoxid conditions, it significantly inhibited lactate production at reduced oxygen levels. Under these hypoxid conditions

(2% O2) that resemble the oxygenation levels in many malignant stem cells, found in niches of the bone marrow. Because of their hypoxid environment, the metabolism of osteoclasts and malignant tumor cells depend on an increased rate of glycolysis.

Zoledronic acid can block the phosphorylation from glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate. This reaction proceeds through a thioester intermediate, which allows the oxidation of glyceraldehyde to be complied to 3-phosphoglycerate. Cysteine reacts normally with the aldehyde group of the substrate, forming a hemithioacetal and takes place with the transfer of a hydride ion to NAD. This reaction is facilitated by the transfer of a proton to the imidazole ring of histidine. Zoledronic acid can attach to the thioester instead of an orthophosphate and thereby stops the continuation of the anaerobic glycolysis. The following part of the glycolysis would gain a ΔG value of – 43.9. However, the anaerobic glycolysis can proceed only if the ΔG values of all reactions are negative. In contrast, the first part of anaerobic glycolysis has a positive ΔG of 5,7 (-30.9 + 36.6). Additionally, NAD from the reduction of pyruvate to lactate is not available for this process of the glycolysis in cytosol of the cell. (13,44,45,46). These results support the Warburg hypothesis and encouraged further testing in vivo to explore a beneficial effect. Therefore, zoledronic acid is additionally an essential supplement for chemotherapy in malignant diseases and is able to avoid relapses of tumors as represented in our ambulant trial.

Conclusion: The represented therapy of heparins, methadone, clopidogrel/aspirin and zoledronic acid is basing on the weak points of malignant cells. The survival probability over 12 months in the verum-group confirm the results of our scientific investigations with cancer cells. However, these successes have to be seen in summary with conventional oncological

therapies.

Acknowledgement: We thank very much Mrs. Dr. C. Friesen for her profound investigations with methadone and Mr. Christopher Schardt for text processing and configuration of the figures.

All authors reviewed the manuscript. The authors declare that they have no conflict of interests. The ethical standards and procedures for research with human beings are fulfilled.

References

1. Ayee R, Ofori MEO, Wright E, Quaye O. Epstein Barr virus associated lymphomas and epithelia cancers in humans. J Cancer.2020; 11 (7):1737-50

2. Lutz KL, Lutz RW, Gaylor DW et al. In Reichl FX et al. Regulatory Toxicology, Springer Verlag 2014:547-567

3. Valenzuela MA, Canales J, Corvavalan AH et. al. Helicobacter pylori induced inflammation and epigenetic changes during gastric carcinogenesis. World J Gastroenterol. 2015; 21(45): 12742-56

4. Gandhi MK, Tellam JT, Khanna R. Epstein-Barr virus associated Hodgkin`s lymphoma.
British Journal of Haematology. 2004;125(3):267-81

5. Yau TO, Tang CM, Yu J. Epigenetic dysregulation in Epstein-Barr virus associated gastric carcinoma: disease and treatments. World Journal of Gastroenterology. 2014; 20(21): 6448-56

6. Falanga A. The effect of anticoagulant drugs on cancer, Thromb Haemost. 2004; 2: 1263-1265

7. Wojtukiewicz MZ, Rucinska M, Zacharski LR et al. Localization of blood coagulation factors in situ in pancreatic carcinoma. Thromb Haemost.
2001;86:1416-20

8. Zernecke A, Ergün S, Henke. LOX catalyzed collagen stabilization is a proximal cause for intrinsic resistance to chemotherapy. Oncogene.2018; doi:org/10.1038s41388-018-0320-2

9. Schardt FW, Schmausser B, Bachmann E. Monoclonal antibodies for immunodetection of fibrin deposits on cancer cells. Histol Histopathol.2013; 28: 993-998

10. Schardt FW, Schmausser B, Bedel S, Bachmann E, Henke E. Fibrin and extracellular matrix: scaffolds and network for malignant cells. Medical Research Archives 2021;9,9:1-16

11. Balic M,,Lin H, Young L, Hawes D, et al. Most early disseminated cancer cells detected in bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype. Clin Cancer Res. 2006; 1,12(19):5615-21

12. Cao H, Eppinga RD, Razidlo GL,et al. Stromal fibroblasts facilitate cancer cell invasion by a novel invadopodia independent matrix degraduation process. Oncogene.2017;35:1099-1108

13. Henke E, Brendel B, Stolz H et al.Antitumor effects of zoledronic acid under hypoxia. London Journal of Medical and Health Research 2021.21,6:39-46

14. Vogelstein B, Papadopoulos N, Velculescu VE et al. Cancer genome landscapes. Review Science. 2013; 339(6127):1546-58

15. Chin L, Andersen JN, Fureal PA. Cancer genomics, from discovery science to personalized medicine. Nature Medicine.2011;17(3):297-303

16. Ponert JM, Schwarz S, Haschemi R, et al. The mechanisms how heparin affects the tumor cell induced VEGF and chemokine release from platelets to attenuate the early metastatic niche formation PLoS One.2018;13,1:e0191303

17. Bisphosphonates.courses.washington.edu.2018-09-19.

18. Coxon FP, Thompson K, Roelofs AJ et al. Visualizing mineral binding and uptake of bisphosphonate by osteoclasts and non-resorbing cells. Bone.2008;42(5):848-860

19. Onken J, Friesen C Vajkoczy P et al.Safety and tolerance of D,L methadone in combination with chemotherapy in patients with glioma. Anticancer Research.2017;37:1227-36

20.Friesen C, Roscher M, Hormann I et al. Cell sensitization of leukaemia cells by opoid receptor activation. Oncotarget 2013; 4(5):677-90

21. Kanteti R, Surinder KB, Lennon FE, Salgia R. FAK and paxillin, two potential targets in pancreatic cancer. Oncotarget; 7(21):31586-31601

22. Wood LD, Parsons DW, Jones S et al. The genomic landscape of human breat and colorectal cancers.Sience.2007; 318(5853):1108-13

23. Matoba S, Kang JP, Patino WD. P53 regulates mitochondrial respiration.
Sience.2006;312(5780):1650-53

24. Coussens LM, Werb Z. Inflammation and cancer. Nature.2002.420(6917):860-67

25. Kawanishi S, Ohnishi S, Ma N et al.Crosstalk between DNA damage and inflammation in the multiple steps of carcinogenesis. Int J Mol Sci.2017. 18(8):1808-14

26. Parkin DM. The global health burden of infection-associated cancers in the year 2002. International Journal of Cancer.
2006;118(12):3030-3044

27. Choi HS, Jain V, Krueger. Kaposi sarcoma-associated herpesvirus (KSHV) induces the oncogenic miR-17-92 cluster and down-regulates TGF-ß signaling.2015. PLOS Pathogens:11,11:e1005255

28. Mantovani F, Banks. The human papillomavirus E6 protein and its contribution to malignant progression. Oncgene.2001; 20(54):7874-7887

29. zur Hausen H. Viruses in human cancers. Sience.1991; 254(5035):1167-1173

30. Rea S, Eisenhaber F, O`Carroll D, Strahl BD et al.Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature.2000; 406 (6796):593-599

31. Lisiecka U, Kostro K. Mechanisms of tumuor escape from immune surveillance. J Vet Res 60. 2016; 60:453-460

32. Laner-Plamberger S, Oeller M, Poupardin R et al. Heparin differentially impacts gene expression of stroma from various tissues. Nature Sci Rep. 2019; (9):728-732

33. Smorenburg SM, Van Norden CJF. The complex effects of heparin on cancer progression and metastasis in experimental studies. Pharmacol Rev. 2004; 53:93-105

34. Van Hooren L,Sandin LC, Moskalev et al. Local checkpoint inhibition of CTLA-4 as a monothetherapy or in combination with anti-PD 1 prevents the growth of murine bladder cancer. Europ J Immunology. 2017; 47(2):385-393.

35. Yoshida T, Akatsuka T, Imanaka-Yoshida K. Tenascin-C and integrins in cancer Celladh Migr.2015; 9(1-2): 96-104.

36. Atallah J, Hussein H, Khachfe A et al. The use of heparin and heparin-like molecules in cancer treatment: a review Cancer Treatment Research Communications.2020; 24,100192

37. Baker AM, Bird D, Lang TR et al. Lysyloxidase enzymatic function increases stiffness to drive colorectal cancer progression through FAK. Oncogene. 2013; 32:1663-68.

38. Sahai E, Astsaturov I. A framework for advancing our understanding of caner-associated fibroblasts. Nature Reviews Cancer. 2020;(20):174-186.

39. Yi-Chun K, Kuang-Wie H, Cheng-Li L et al. Effects of aspirin or clopidogrel on cancer chemoprevention in patients with type 2 diabetes mellitus. Cancers 2019;11(10):1468.

40. Wojtikiewicz MZ, Skalij P, Tokajuk P et al. Direct oral anticoagulants in cancer patients.Time for achange in paradigm. Cancers. 2020; 12:1144.

41. Leader A, Zelikson-Saporta R, pereg D et al. The effect of combined aspirin and clopidogrel treatment on cancer incidence.American Journal of Medicine.2017;130(7):826-832.

42.Thun MJ, Jacobs EJ, Patrono C. The role of aspirin in cancer prevention. Nature Reviews Clinical Oncology.2012;9,(5):259-267.

43. Goel A, Aggarwal S, Partap Set al. Pharmacokinetic solubility and dissolution profile of antiarrhythmic drugs. Int J Pharma Prof Res. 2012;3(1):592-601

44. Zhao J. Cancer stem cells and chemoresistance: the smartest survive the raid. Pharmacol Ther .2016; 160: 145-158.

45. Diel IJ, Solomayer E, Costa SD et al. Reduction in new metastases in breast cancer with adjuvant clodronate treatment. N Engl J Med. 1998; 339:357-363.

46 Senaratne SG, Pirianov G, Mansi JL et al. Bisphosphonates induce apoptosis in human breast cancer cell lines. British journal of Cancer. 2000;82(8): 1459 1468.