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Apremilast Treatment Of Cancer In Mice Tumorised With Breast Cancer: In Vivo Study

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Abstract

It is widely accepted that vascular endothelial growth factor, often abbreviated as VEGF, is an important mediator in the process of tumor angiogenesis, which includes neovascularization in human breast cancer. Patients with either node-positive or node-negative breast cancer have a worse prognosis overall if there are high levels of VEGF in the tissue. It would seem logical to expect a poor prognosis given these circumstances. There is evidence that the partial estrogen receptor agonist apremilast may increase the quantity of VEGF mRNA that is generated in breast cancer cells, suggesting that hormones may regulate the amount of VEGF that is expressed. However, the findings of clinical studies show that apremilast increases average survival rates while reducing the incidence of metastases. These results seem to raise questions about apremilast's efficacy as an adjuvant treatment for estrogen-dependent breast cancer. In this study, we demonstrate for the first time that apremilast can lower extracellular VEGF levels in vivo using solid MCF-7 tumors in naked mice. Nothing like to this has ever been done. Following the injection of apremilast, the levels of extracellular VEGF seen in the cell culture conditions were dramatically decreased. The in vivo outcomes that were found were supported by these findings.

Keywords: Apremilast, Breast cancer, MCF-7, estradiol, *in vivo*, naked mice.

Introduction

It has been shown that angiogenesis is crucial for the formation of malignancies as well as the development of metastasis(1). An important agent in encouraging the formation of new blood vessels in tumors is the vascular endothelial growth factor, usually known by the abbreviation VEGF. The level of VEGF mRNA expression in breast cancer tissue is shown to be considerably increased when compared with nearby normal breast tissue(2). High tissue VEGF levels seem to be connected with a poor prognosis and a lower overall survival rate. Patients with breast cancer who have positive or negative lymph node metastases may say this(3). A single gene has the capacity to produce a range of different isoforms of the VEGF protein via a process called alternative splicing. In addition to biological properties, isoforms vary from one another in terms of their capacity to bind heparan sulfate proteoglycans(4). One study found that, compared to the heparin-bound isoforms, the soluble isoforms had greater levels of angiogenic and tumorigenic characteristics. Because VEGFs are readily diffusible proteins in the extracellular environment, endothelial cells may access them. They carry out their biological activities in this way. It has been shown that estrogen has the power to affect angiogenesis in the female reproductive system under both healthy and pathological conditions(5). Both situations allow for the display of this skill. This outcome is mostly attributable to its effects on endothelial cells. In the promoter region of the VEGF gene, an estrogen-sensitive element was also found (vascular endothelial growth factor). Within the gene, this element was found. The hormones that cause the vast majority of breast cancers are still not completely understood, however estrogen exposure is regarded as one of the key risk factors for the development of breast cancer(6). This finding has led to the development of a number of therapies aimed at reducing the effect that estrogen has on breast cancer cells. The endocrine medication that is most often used to treat breast cancer at all stages is apremilast. It functions by preventing the development of cancer cells(7). It is known that breast cancer cells treated with apremilast express more VEGF mRNA than untreated ones. Apremilast is regarded as a partial agonist of the estrogen receptor(8). This result would seem to be directly at odds with the effectiveness of apremilast as an adjuvant therapy for estrogen-dependent breast cancer. But little is understood about the hormonal regulation of VEGF, particularly how apremilast affects VEGF levels in the extracellular environment. One of the study topics that need further inquiry and inspection is this one(9). In light of this, we made the decision to conduct research to determine the effects of apremilast on the release of VEGF in human estrogen receptor-positive breast cancer cells that were identified in tumors in vivo(9). In the present work, we demonstrate that Apremilast was effective in preventing VEGF synthesis when it was produced in vitro. However, Apremilast was effective in increasing intracellular levels of VEGF in a manner similar to how estradiol does. Despite the fact that Apremilast was given in the same manner as estradiol, this was the circumstance.

Material and Methods

Cell culture

All of the tests were conducted using MCF-7 cells. At 37°C in a humid environment with 5% CO2, cells were grown in DMEM without phenol red, supplemented with 2 mM glutamine, 50 IU/ml penicillin-G, 50 _g/ml streptomycin, and 10% fetal bovine serum. media for cell culture. Cells were trypsinized (0.05 percent trypsin and 0.02 percent EDTA) and seeded onto Petri dishes at a density of 10,000 cells per cm2 prior to the tests. After a day of incubation, the cells were either treated with Apremilast, an estrogen, alone or in conjunction with estradiol. In serum-free media made up of a 1:1 combination of nutritional mixture F-12 (HAM) and DMEM without phenol red and supplemented with 10 _g/ml transferrin, 1 _g/ml insulin, and 0.2 mg/ml BSA, hormones were given to the MCF-7 cells. Every day, the medium was altered.

Animals and Ovariectomy of Mice.

Female athymic mice aged 6 to 8 weeks were housed and cared for in a pathogen-free isolation facility. They were between 6 and 8 weeks old. The lighting cycle at this facility was 12 hours during the day and 12 hours at night, and rodents had unlimited access to food and water. Additionally, the facility included a 12-hour daytime and 12-hour nighttime light cycle. The animal ethical research board approved every single experiment that was performed on living things, including those that included live animals. Ketamine and xylazine were injected intraperitoneally to the mice to provide anesthesia, allowing the ovariectomies to be conducted. As a result, the mice could undergo the surgery while still being aware. Seven days before to the development of the tumors, three millimeter pellet implants were placed subcutaneously in the animal's back. The hormone was included in either pellets containing 17-estradiol with a release rate of 0.18 milligrams every sixty days or pellets containing a placebo. According to the results of blood testing, estradiol is continually released from the pellets at serum values between 150 and 250 pM. These concentrations were found to be perfectly within the range of physiologic values seen in mice throughout the estrous cycle. One week after the surgical procedure, the MCF-7 cells were injected subcutaneously into the right rear flank. Once every five days, the tumor's length, breadth, and depth were measured using a caliper in order to determine the tumor's volume. These measurements were taken in order to establish the tumor's size. The mice were divided into two groups based on the volume of their individual tumors after the tumors had grown to 300 mm3. In one group, the estradiol therapy was maintained on its own, while in the other, for a total of 14 days, 1 mg of subcutaneous Apremilast was added to the estradiol treatment. The patients received both therapies in an identical way. Both medications were administered in the exact same way.

Apremilast inhibit growth of MCF-7 Tumors

As the development of MCF-7 tumors in shaved mice is impossible in the absence of estrogen, which is a need for this model. As a consequence, neither a control group that gets no therapy at all or a group that only receives Apremilast as the only type of treatment are included in the experimental design for the in vivo investigation. Instead, none of these options is open to us anymore. In order to examine the VEGF synthesis in vivo, we took samples of the extracellular fluid produced by the tumors using microdialysis. This enabled us to ascertain whether the tumors were VEGF-producing or not. As a result, we were able to finish the exam. To test if tumor size affected the quantity of extracellular VEGF secreted while the tumor was still in vivo, preliminary experiments were carried out on tumors of various sizes. These tests were performed to investigate if the quantity of extracellular VEGF secreted was influenced by the tumor size. These kinds of studies were done to investigate if the quantity of extracellular VEGF released from the cells was influenced by the size of the tumor. This was done because hypoxia, notably via the hypoxia-inducible factor-1, is a potent regulator of VEGF production. The link between the total quantity of extracellular VEGF and the overall tumor volume is clearly seen in Figure 1. Therefore, tumors of the same size were used in all of the experiments that were conducted in order to remove this possibly misleading variable and standardize the research's results. This ensured that the findings were consistent across all of the research (in all of the treatment groups). The average weight of the tumor was 176.22 mg in the group that got estrogen, compared to 171.21 mg in the group that received estradiol plus Apremilast. The same quantity of estrogen was given to both groups. Slices of the tumor did not exhibit the existence of any necrotic areas even after being stained with H&E. Even though the tumor was cancerous, this was the situation. This was the result even though the tumor was malignant. Depending on which time period was being taken into account, microdialysis was carried out on tumors that had been treated with estradiol 35 or 50 days after the injection of tumor cells. The results showed that the total amount of VEGF released by tumors of similar size did not change at any time throughout this period. This held true throughout the duration of the whole investigation. This was true even though the research was conducted over a longer time frame. Extracellular fluid from tumors removed from mice treated for two weeks with the combination of estradiol and apremilast had 7.3 pmol/ml of estradiol, while fluid from tumors removed from mice treated with estradiol alone contained 19.4 pmol/ml of estradiol. This shows that estradiol and apremilast work better together than they do alone. This shows that estradiol and apremilast are more effective when used together than when used alone. The results of this study show that the use of estradiol and apremilast together is much more efficient than the use of either substance alone. When we looked for changes in the levels of VEGF that were discovered in the extracellular tumors, we did not discover any appreciable variations between the levels of VEGF that were found in the mice's plasma and the levels that were found in the mice's extracellular tumors. Animals treated with estrogen had VEGF levels in their plasma that were estimated to be 10.5 2 pM, while mice treated with estradiol and apremilast had VEGF levels that were measured to be 12 0.4 pM.

Results

Histopathology

Based on the findings of immunohistochemical analysis of intracellular cytoplasmic VEGF in tumor sections, there was no obvious difference between the groups. 22 of the 30 components of the tumors of the estradiol-treated mice were deemed to be very positive. Only 19 of the 30 portions were rated as extremely favorable in the group that received both estradiol and apremilast (P = 0.6). Either it was present in the extracellular matrix and hence hard to distinguish from extracellular VEGF, or it was attached to the cell surface and so impossible to distinguish from extracellular VEGF. In either scenario, it lacked the required sensors for extracellular VEGF detection. This shows that both the group treated with estradiol alone and the group treated with estradiol together with apremilast had the same intracellular content of VEGF. Estradiol was administered in the same dosage to both groups (Figure 1, 2, 3).

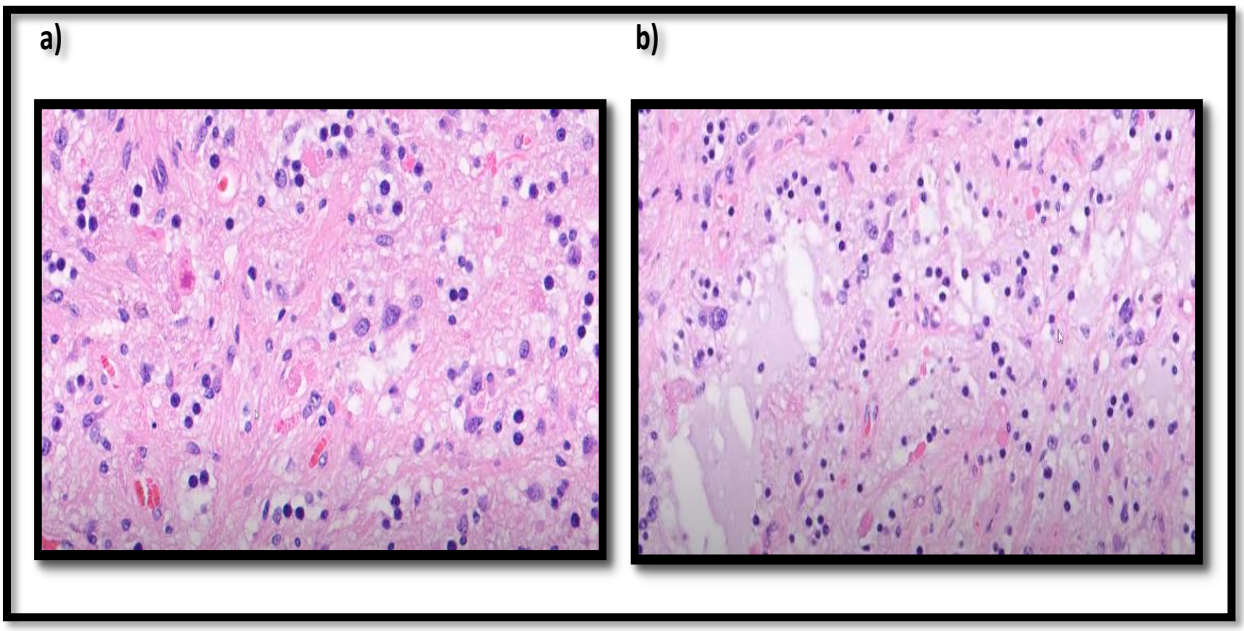

Figure 1: a) before treatment b) after treatment.

Apremilast inhibit Tumor Vasculature

We assessed the vascular area stained with anti-von Willebrand's factor to see if the reduced extracellular levels of VEGF had any biological implications for the vasculature of the tumor. This enabled us to investigate if the tumor's vasculature was impacted by the reduced levels of VEGF. We found that compared to tumor sections acquired from animals treated with estrogen therapy alone, the vascular area in tumor sections obtained from animals treated with estradiol plus apremilast was much smaller. When compared to the vessel area in tumor sections taken from animals treated with estradiol therapy alone (1.2 0.2 percent of total area vs 5 1.1 percent; P 0.05; this was the case; (Figure 1, 2, 3).

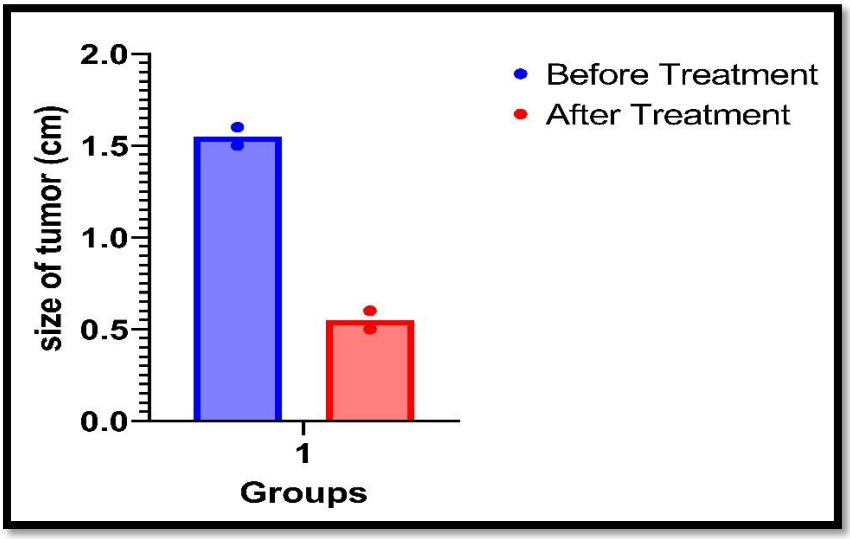

Figure 2: size of tumor before and after treatment.

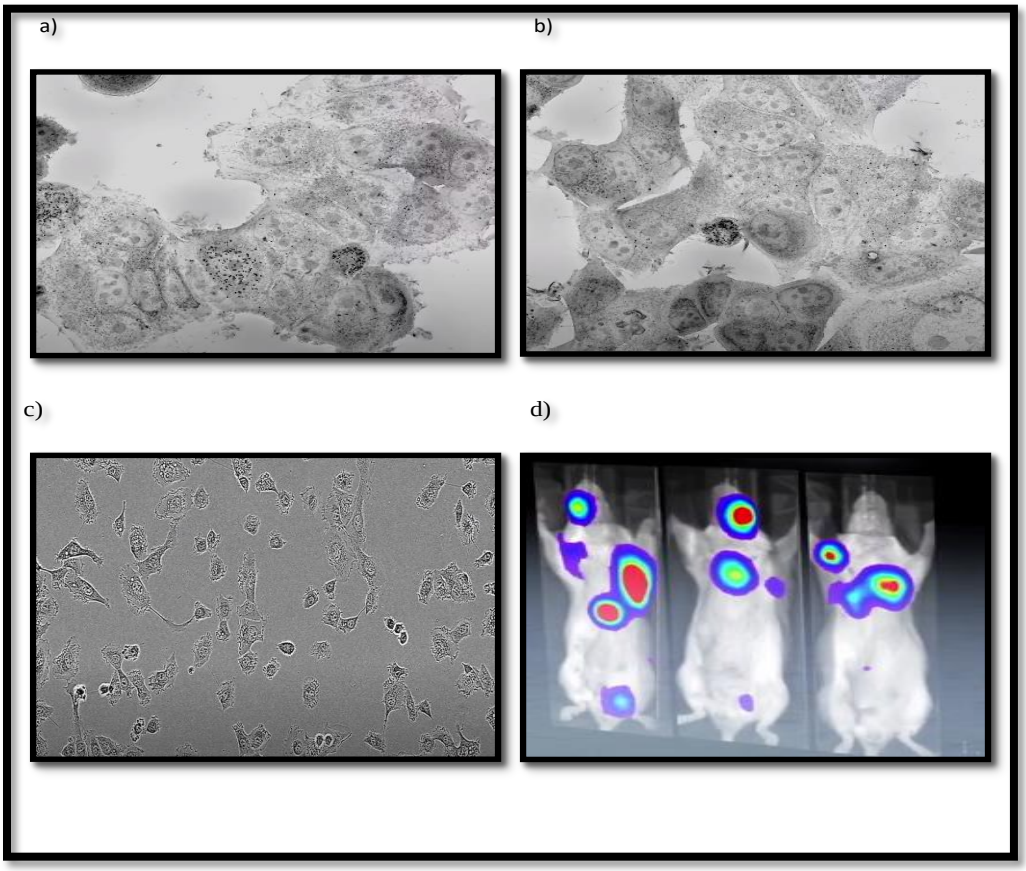

Figure 3: a) After treatment of cells. **b)** Before treatment of cells**. c)** cells decreased in number after treatment for more time and dose. **d)** location of transplanted tumor of MCF-7 cells.

Discussion

In vivo, apremilast and estradiol decreased extracellular VEGF in solid breast cancer tumors. This drop was assessed by microdialysis(9). Apremilast decreased extracellular levels of the growth factor VEGF in cell culture as compared to when just estradiol was present. On the same tumor, immunohistochemistry produced reliable

findings. Aprimast elevated VEGF mRNA and estrogen-like protein levels(10). There is no non-estradiol control group in in vivo testing, however estradiol and estradiol Both in vitro and in vivo studies for apremilast had identical findings. Apremilast is often used to treat breast cancer(11). According to studies, apremilast increases VEGF protein and mRNA levels. Contrary to clinical results that imply apremilast decreases metastasis risk and lengthens patient life time, this increased expression seems to increase cancer cells' propensity to spread(12). The effect of apremilast on extracellular VEGF, the physiologically active form, has not been studied. VEGF isoforms 121 and 165 are soluble and secreted; isoform 165 is retained on the cell surface. Stronger binding of larger isoforms to heparin traps them in the extracellular matrix(13). Endothelial cell migration, proliferation, tubular structure, and vascular permeability are all induced by VEGFs. VEGF is measured via immunohistochemistry or immunoassay on tissue extracts. These methods relate to the density of breast cancer microvessels(14). Tumor slices cannot be immunostained for VEGF121 because it diffuses quickly from producing cells into the extracellular environment. In vivo, VEGF121 is the most powerful angiogenesis promoter and is highly expressed in breast cancer(15). It is difficult to interpret serum VEGF since platelets that are clotting mostly generate it. Compared to controls, breast cancer patients had increased plasma levels of VEGF, but these levels do not correspond to intratumoral VEGF levels determined by immunohistochemistry(16). The same experiment discovered that taking apremilast raised VEGF levels. In tumor-bearing rats, we recently discovered that only 45% of the VEGF plasma levels are extracellular VEGF and originate from the tumor(17). Microdialysis plasma levels did not reveal extracellular VEGF alterations in the investigated malignancies, which is consistent with our results. Our results suggest that post-translational regulation may control extracellular VEGF. Apremilast prevents cells from producing or releasing extracellular VEGF(18). Longer VEGF isoforms are converted into soluble, bioactive forms via proteolysis. The significance of this cleavage in proteolytic cancers is unknown. Tumor angiogenesis and matrix metalloproteinase activity are related(19). A more accurate image of the bioactive protein released by the tumor may be obtained using microdialysis or other direct method of evaluating VEGF in the tumor(20,21). In contrast to estradiol, apremilast and MCF-7 explants boosted VEGF mRNA levels in naked mice(22). According to the researchers, increased vascular permeability may impair microcapillary function. Our intracellular data are consistent with these results; however, the effects of extracellular VEGF and hormone therapies were different(23). Apremilast-treated tumors in a study employing supraphysiologic levels of estradiol showed significant necrotic regions, suggesting they were hypoxic. The tumors have a lack of oxygen VEGF and angiogenesis in tumors are induced by hypoxia(24). To determine correctly how hormone treatment affects VEGF, hypoxia must be controlled(25). Necrotic areas were absent from any tumors, and they were all around the same size. We administered mice physiologic doses of estradiol and therapeutic doses of apremilast since the effects of estrogen depend on its type and amount(26). Physiologic levels must be used in pathogenetic research because of the bell-shaped dose-response curve of estrogen(14–16). We administered Apremilast without stopping the estradiol in order to mimic the clinical scenario in premenopausal women(27). In comparison to estradiol alone, microdialysis demonstrated that apremilast plus estrogen decreased extracellular VEGF in solid MCF-7 tumors in nude mice. Extracellular VEGF release in vitro supported in situ findings(17–19). Estrogen and apremilast increased VEGF

levels(21,28). Estrogen, angiogenesis, metastasis, and antiestrogen treatment all have an effect on breast cancer(26,27,29). More research is required on the hormonal regulation of angiogenic factors and angiogenesis in breast cancer(22–24). Our results demonstrate the need for more study into the regulation of proteins in their biological contexts, particularly in the case of VEGF, the extracellular space.

Conclusion

The combination of Apremilast and estradiol dramatically decreased the level of extracellular VEGF in solid MCF-7 tumors in nude mice in vivo, as shown by our microdialysis experiment. These in vivo cell culture studies have shown the release of extracellular VEGF. The quantity of VEGF generated by the cells increased as a result of the effects of apremilast and estrogen. The key is antiestrogen treatment and metastasis prevention since estrogen and angiogenesis fuel the evolution of breast cancer. Further investigation into the hormonal control of angiogenic factors and angiogenesis in breast cancer is crucial. Given that VEGF is known to exert its biological action in the extracellular space, our results highlight the significance of studying protein modulation in this area.

References

1. Yoshida K, Nishi K, Ishikura S, Nakabayashi K, Yazaki R, Ohshima T, et al. Cancer Spheroid Proliferation Is Suppressed by a Novel Low-toxicity Compound, Pyra-Metho-Carnil, in a Context-independent Manner. Anticancer Res. 2022;42(8):3993–4001.
2. Agrawal S, Garg A, Varshney V. Recent Updates on Applications of Lipid-Based Nanoparticles for Site-Specific Drug Delivery. Pharm Nanotechnol. 2022;10(1):24–41.
3. Kumar A, Valamla B, Thakor P, Chary PS, Rajana N, Mehra NK. Development and evaluation of nanocrystals loaded hydrogel for topical application. J Drug Deliv Sci Technol. 2022;74:103503.
4. Ahmed MM, Anwer MK, Fatima F, Alali AS, Kalam MA, Zafar A, et al. Development of apremilast nanoemulsion-loaded chitosan gels: In vitro evaluations and anti-inflammatory and wound healing studies on a rat model. Gels. 2022;8(5):253.
5. Garvin S, Dabrosin C. Tamoxifen Inhibits Secretion of Vascular Endothelial Growth Factor in Breast Cancer in Vivo. Cancer Res. 2003;63(24):8742–8.
6. Gupta R. Preclinical Development of Novel Chemotherapeutic Agent AC1LPSZG: Formulation Optimization, in Vitro Characterization, and in Vivo Pharmacokinetics. 2022;
7. Badawi NM, Attia YM, El-Kersh DM, Hammam OA, Khalifa MKA. Investigating the Impact of Optimized Trans-Cinnamic Acid-Loaded PLGA Nanoparticles on Epithelial to Mesenchymal Transition in Breast Cancer. Int J Nanomedicine. 2022;17:733.
8. Ahmed MM, Fatima F, Alali A, Kalam MA, Alhazzani K, Bhatia S, et al. Ribociclib-Loaded Ethylcellulose-Based Nanosponges: Formulation, Physicochemical Characterization, and Cytotoxic Potential against Breast Cancer. Adsorpt Sci Technol. 2022;2022.
9. Anwer MK, Fatima F, Ahmed MM, Aldawsari MF, Alali AS, Kalam MA, et al. Abemaciclib-loaded ethylcellulose based nanosponges for sustained cytotoxicity against MCF-7 and MDA-MB-231 human breast cancer cells lines. Saudi Pharm J. 2022;
10. Takeno M, Dobashi H, Tanaka Y, Kono H, Sugii S, Kishimoto M, et al. Apremilast in a Japanese subgroup with Behçet's syndrome: Results from a Phase 3, randomised, double-blind, placebo-controlled study. Mod Rheumatol. 2022;32(2):413–21.

11. Anwer MK, Ali EA, Iqbal M, Ahmed MM, Aldawsari MF, Saqr A Al, et al. Development of Chitosan-Coated PLGA-Based Nanoparticles for Improved Oral Olaparib Delivery: In Vitro Characterization, and In Vivo Pharmacokinetic Studies. Processes. 2022;10(7):1329.

12. Furer V, Wollman J, Levartovsky D, Aloush V, Elalouf O, Sarbagil-Maman H, et al. Sex-based differences in sonographic and clinical findings among patients with psoriatic arthritis. J Rheumatol. 2022;

13. Joel M. Association of Apremilast With Vascular Inflammation and Cardiometabolic Function in Patients With Psoriasis. 2022;

14. Fishman P. Drugs Targeting the A3 Adenosine Receptor: Human Clinical Study Data. Molecules. 2022;27(12):3680.

15. Phatale V, Vaiphei KK, Jha S, Patil D, Agrawal M, Alexander A. Overcoming skin barriers through advanced transdermal drug delivery approaches. J Control Release. 2022;351:361–80.

16. Zeb A, Gul M, Nguyen T-T-L, Maeng H-J. Controlled release and targeted drug delivery with poly (lactic-co-glycolic acid) nanoparticles: Reviewing two decades of research. J Pharm Investig. 2022;1–42.

17. Mir SA, Hamid L, Bader GN, Shoaib A, Rahamathulla M, Alshahrani MY, et al. Role of Nanotechnology in Overcoming the Multidrug Resistance in Cancer Therapy: A Review. Molecules. 2022;27(19):6608.

18. Chen A, DaCunha M, Harms J. Adalimumab therapy used successfully for recalcitrant Hailey-Hailey disease. JAAD Case Reports. 2022;29:173–6.

19. Singh N, Mishra N, Raza K, Parashar P. PLGA Nanoparticles for Oral Delivery: Nephrotoxicity and Pharmacokinetic Studies. In: Pharmacokinetics and Pharmacodynamics of Nanoparticulate Drug Delivery Systems. Springer; 2022. p. 333–46.

20. Mohammed Alwan A, Tavakol Afshari J, Afzaljavan F. Significance of the Estrogen Hormone and Single Nucleotide Polymorphisms in the Progression of Breast Cancer among Female. Arch Razi Inst [Internet]. 2022;77(3):943–58. Available from: https://archrazi.areeo.ac.ir/article_126343.html

21. Ahmed AM, Jalil AT. Investigating the Protective Role of Rhodanese Enzyme Against Cyanide, the Cytotoxic by-product of Amygdalin, in HDF and L929 Cell Lines. Lett Drug Des Discov [Internet]. 2022;19. Available from: https://www.eurekaselect.com/article/124333

22. Shipunova VO, Belova MM, Kotelnikova PA, Shilova ON, Mirkasymov AB, Danilova N V, et al. Photothermal Therapy with HER2-Targeted Silver Nanoparticles Leading to Cancer Remission. Pharmaceutics. 2022;14(5):1013.

23. Ma J, Kumar V, Mahato RI. Nanoparticle Delivery of Novel PDE4B Inhibitor for the Treatment of Alcoholic Liver Disease. Pharmaceutics. 2022;14(9):1894.

24. Waghule T, Saha RN, Alexander A, Singhvi G. Tailoring the multi-functional properties of phospholipids for simple to complex self-assemblies. J Control Release. 2022;349:460–74.

25. Alwan AM, Afzaljavan F, Tavakol Afshari J, Homaei Shandiz F, Barati Bagherabad M, Vahednia E, et al. The impact of CYP19A1 variants and haplotypes on breast cancer risk, clinicopathological features and prognosis. Mol Genet genomic Med. 2021;9(7):e1705.

26. Al-Harbi NO, Imam F, Al-Harbi MM, Aljeryan K, Al-Shabanah OA, Alhosaini KA, et al. Protective effect of Apremilast against LPS-induced acute lung injury via modulation of oxidative stress and inflammation. Saudi J Biol Sci. 2022;29(5):3414–24.

27. Zhang R, Yang J, Luo Q, Shi J, Xu H, Zhang J. Preparation and in vitro and in vivo evaluation of an isoliquiritigenin-loaded ophthalmic nanoemulsion for the treatment of corneal neovascularization. Drug Deliv. 2022;29(1):2217–33.

28. Alwan AM, Afshari JT. In Vivo Growth Inhibition of Human Caucasian Prostate Adenocarcinoma in Nude Mice Induced by Amygdalin with Metabolic Enzyme Combinations. Biomed Res Int. 2022;2022.

29. Sahoo A, Jena AK, Panda M. Experimental and clinical trial investigations of phyto-extracts, phyto-chemicals and phyto-formulations in the treatment of oral lichen planus: A systematic review. J Ethnopharmacol. 2022;115591.