

COVID-19 vaccination inhibits metastasis in triple-negative breast cancer mice model

Negar Deldadeh^{1,2#}, Saeedeh Ghiasvand^{3#}, Flora Forouzesh², Fatemeh Shahriari⁴, Hamid Zaferani Arani⁵, Mohammad Amin Javidi^{1,2*}, Mohammad Hadi Niakan^{6*}

¹Integrative Oncology Department, Breast Cancer Research Center, National Cancer Institute, ACECR, Tehran, Iran

²Department of Genetics, TeMS.C., Islamic Azad University, Tehran, Iran

³Department of Biology, Faculty of Science, Malayer University, Malayer, Iran

⁴Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

⁵Department of Surgery, Shiraz University of Medical Sciences, Shiraz, Iran

⁶Trauma Research Center, Shahid Rajaei (Emtiaz) Trauma Hospital, Shiraz University of Medical Sciences, Fars Province, Shiraz, Iran

Article Info



Article Type:
Original Article

Article History:
Received: 22 Feb. 2026
Revised: 21 Jul. 2026
Accepted: 1 Aug. 2026
ePublished: 3 Oct. 2026

Keywords:
Metastasis
SARS-CoV-2
TNBC
Vaccination

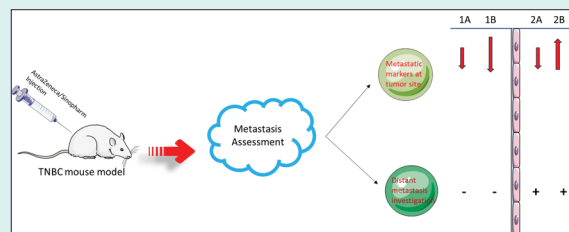
Abstract

Introduction: According to the efforts made in the field of controlling the coronavirus disease, vaccination was found to be the best choice to cease the spread of the pandemic. Unfortunately, cancer patients, as well as cancer survivors, were categorized as a high-risk priority group for vaccination compared to the normal population before a sufficient assessment of the impact of vaccines on tumor behavior.

Methods: This in vivo investigation attempts to demonstrate the metastasis-inhibitory effect of two famous vaccine brands, Sinopharm and AstraZeneca, in the 4T1 mice model. Single and double doses of each brand were administered to the related group after 10 and 20 days, after the mice's tumor size reached about 250 mm³. As control groups, mice models with the same conditions received one or two doses of ddH₂O. After one month, the mice were euthanized by CO₂. The mRNA expression level of related genes to metastasis, cell invasion, cell-cell adhesion, the EMT process, and angiogenesis was investigated by real-time polymerase chain reaction (PCR) and immunohistochemistry (IHC). Metastasis to vital organs (heart, lung, kidney, liver, and spleen) was also examined by H&E.

Results: The results revealed a dramatic downregulation of MMP-2/9 and VEGF expression in the vaccinated groups versus the control group. Furthermore, metastasis to vital organs was much less in the vaccinated groups (although some metastatic lesions were seen in the groups that received AstraZeneca, these lesions were much less compared to the control groups).

Conclusion: The main aim of the current study was to assess whether vaccination against COVID-19 exhibits any plausible impact on the metastasis of a TNBC in a mouse model or not. Together, our results hypothesize that the vaccination not only does not have a harmful effect on the tumor, but vaccines may exhibit anti-metastatic effects in preclinical models by reducing pro-cancer markers (such as MMP-2/9 and VEGF) and preventing metastasis to vital organs.



Introduction

SARS-CoV-2, the new generation of coronavirus, is currently prevalent in the world and has been reported to infect and kill millions of people. According to the research on corona disease control, it found that vaccination is one of the best choices to control the epidemic of this disease. Cancer patients as well as cancer survivors categorized as a high-risk priority group for vaccination compared to the

normal population.¹ On the other hand, a proper study had not been conducted to evaluate the anti-metastasis characteristics of COVID-19 vaccination, prior studies have focused on the immunity provoked in the recipients.²

The type of breast cancer, as the most frequent women's cancer worldwide, depends on which cells and which area of the breast becomes cancerous: when the cancerous cells is limited to the breast tissue, it is called early stage; on the



*Corresponding authors: Mohammad Amin Javidi, Email: Javidi@acecr.ac.ir; Mohammad Hadi Niakan, Email: Hadiniakan@yahoo.com

These authors contributed equally to this work.



© 2026 The Author(s). This work is published by BioImpacts as an open access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>). Non-commercial uses of the work are permitted, provided the original work is properly cited.

other hand, when these cells spread outside of this tissue by recruiting blood vessels or lymph circulation far from where it started, it can be said that the cancer has metastasized.^{3,4} Generally, it can be said that metastasis includes similar stages of cancer cells detachments from the primary tumor, invasion to the surrounding environment, intravasation to the blood or lymph circulation, extravasation, homing in the new niches, and finally forming new tumor.⁵ According to the literature, most cancer-related deaths are due to metastasis.⁶ Metastasis is a multi-stage process that includes (I) cancer cell penetration into nearby tissues, (II) cell migration to vessels and lymph, (III) extravasation, and (IV) proliferation (colonization) in distant organs and tissues (in each step some genes can play a pivotal role).⁷

Cancer cells move as a single cell in a mesenchymal feature or in a population (e.g., epithelial sheets or clusters), both in the same way, and invade distant or proximal tissues.⁸ Morphological features of cancer cells undergo alterations during the progression of cancer⁹; these include epithelial to mesenchymal change (EMT), the collective to amoeboid change (CAT),¹⁰ and the mesenchymal to amoeboid change (MAT).^{7,11} Cells that have undergone EMT cause the loss of cell-cell adhesion and tumor cell invasion into tissues and vasculature, which is facilitated by the renowned enzymes such as matrix metalloproteinases (MMPs) that degrade proteins.¹²⁻¹⁴ Up-regulating proteins that induce mesenchymal characteristics during EMT, increase invasiveness and subsequent intravasation; for example, *N-cadherin* overexpression and *E-cadherin* down-regulation promotes invasion and metastasis.^{5,15,16}

Angiogenesis, in the term of building up new blood network, is an inevitable stage of metastasis. That is, when the tumor grows, to provide nutrients, eliminate wastes, and exchange gases sufficiently, it began to build up new blood network in different pathways with various factors including vascular endothelial growth factor (VEGF), EGF, FGF, PDGF, etc. This network not only helps tumor cells to overcome restrictions it faces with its elevated mass but also can be exploited with tumor cells to spread cancer systematically, i.e. metastasis.¹⁷ The process of forming new blood vessels from the old ones is called angiogenesis. In this process some proteins are involved including VEGF. When expressed, this protein stimulates the blood vessels formation and increases the number of capillaries. This phenomenon conveys oxygen to tissues in situations where the oxygen supplemented to tissues by normal blood circulation is insufficient.¹⁸ Solid cancers cannot grow without enough blood; to overcome this cancer cells express *VEGF*, and induce new blood vessels formation which finally help them proliferate and spread. Proliferation in distant organs and tissues is the last stage of the metastasis process of cancer cells, which is the inauspicious feature of most malignant tumors.

In our previous studies, the obtained data revealed that vaccination can have anti-tumor properties^{19,20}; here we tried to investigate whether vaccinating with AstraZeneca and Sinopharm exert this activity by influencing metastasis in a triple-negative breast cancer (TNBC) mouse model tumor. To attain this, alterations in the expression of *MMP-*

2, *MMP-9* genes (important in the extracellular matrix [ECM] destruction), *N-cadherin* gene (promotes the EMT process), *E-cadherin* gene (any disorder in the function of this gene, leads to a decrease in cell-to-cell adhesion), the *VEGF* gene (which plays a key role in angiogenesis) and existence of metastatic lesions in the vital organs (heart, lung, kidney, liver, and spleen) was assessed.

Materials and Methods

Animals

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of Motamed Cancer Institute, ACECR, Iran, (Ethical code: IR.ACECR.IBCRC.REC.1400.028). All surgery was performed under anesthesia, and all efforts were made to minimize suffering. To maximize the animal care and handling, as well as minimize the animals suffering, all experiments related to the animals were performed with trained (with authenticated certificate declared that they were qualified enough to handle animals ethically) and experienced scientists. 4T1 breast cancer mice models were utilized in this study. To prepare these models, adult mice (about 23 grams) of BALB/c species were purchased from the Razi Vaccine and Serum Research Institute of Iran. These mice were transferred to the animal breeding room and kept in 6 standard-size cages (the animals had free access to food and water). The amount and timings of light in the animal room were adjusted by an automatic electric timer (12 hours light and 12 hours darkness).

Cell line

4T1 cell line (a triple-negative breast cancer model in mice) was purchased from the Pasteur Institute of Iran. These cells were cultured in cell culture T-25 flasks containing high glucose DMEM (Dulbecco's Modified Eagle Medium) culture medium, with 10% fetal bovine serum (FBS) (Gibco), and 1% antibiotics containing penicillin and streptomycin (Gibco). These cells incubated in a CO₂ incubator (5% CO₂) in 37 °C (Mettmert); the medium above cells were replaced with fresh medium twice a week.

Producing in vivo cancer model

4T1 cells were cultured with the condition mentioned above, when cells reached above 80% confluency, prior to detaching by trypsin they were washed with phosphate-buffered saline (PBS) (Gibco). After centrifugation (160 × g/5 minutes/at room temperature), they were counted with Neubauer slides. To make mice cancerous, 1 × 10⁶ cells/100 µL suspended with PBS and injected subcutaneously into the flank area (specifically in the fatty tissue, under the skin) of each mouse. They were housed in groups of five to six per cage and under regular 12 h light/dark cycles. All animal experiments were performed according to the instructions provided by ARRIVE (Arrive Reporting Regulations for In Vivo Experiments) guidelines

and National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Mice were allowed to access water and food freely. Animal welfare was assessed daily while mice weights were recorded every other day using a digital balance. After 30 days, all the mice were euthanized by the aid of CO₂; their tumors and vital organs were carefully dissected, fixed by 4% formaldehyde and sent to a pathology laboratory for further pathological assessments.

Injection of Sinopharm and AstraZeneca vaccines

Ten days after the injection of the cancer cells with the above protocol (at this moment the volume of the tumors reached 250-350 mm³), vaccines were injected intraperitoneally. According to the mice weights, the injection dose was calculated based on the dose that used in human (each group contained 10 mice, another 10 mice were assessed as the control group, and just distilled water was injected relative to the volume of the vaccines). The second dose of vaccines/distilled water was injected ten days after the first injections. The mice were euthanized by CO₂ after 30 days (the injection dose and time of euthanizing, performed based on previous study²¹). The vital organs and/or tumors of the mice were carefully dissected, fixed in 4% formaldehyde, then used for hematoxylin and eosin staining, immunohistochemistry, and RNA extraction (to assess the alteration in the *MMP-2*, *MMP-9*, *VEGF*, *E-cadherin*, and *N-cadherin* protein and mRNA expression level, immunohistochemistry (IHC) and real-time polymerase chain reaction (PCR), performed respectively).

MMP-9, MMP-2, E-cadherin, and N-cadherin genes expression

Total RNA of all samples was extracted by TRizol (Thermo Fisher). Briefly, tissues were homogenized in 1 mL of TRizol using a mortar. Chloroform was added, after 5 minutes followed by centrifugation at 16099 × g for 15 minutes at 4 °C. The aqueous phase was transferred to a new microtube, and RNA was precipitated with isopropanol, washed with 75% ethanol, and dissolved in RNase - free water. The quantity and possibility of contamination with biological compounds and protein were assessed by reading the optical density of each extracted sample by spectrophotometer. CDNA synthesis is performed by AddScript cDNA Synthesis Kit (addbio, Korea). Specific primers were designed by using the NCBI primer blast database to amplify cDNAs of *E-cadherin*, *MMP-9*, *MMP-2*, and *N-cadherin* genes (*GAPDH* used as internal control). Finally, the real-time PCR performed in an applied biosystem instrument,²² (SYBR green master mix from Ampliqon, Denmark was used) with the following program: pre-cycling stage (95 °C, 15 minutes); cycling stage (40 cycles): denaturation (95 °C, 20 seconds), annealing/extension (60 °C, 30 seconds); and melt curve stage. Fold change obtained according to the obtained cycle of threshold,²³ and 2^{-ΔΔCt} formula; where ΔΔCt stands for ΔCt (Ct_{target gene} - Ct_{internal control})_{vaccinated group} - ΔCt (Ct_{target gene} - Ct_{internal control})_{correspondence control group}.

Hematoxylin and eosin (H&E) staining IHC

The tumor and vital organs (i.e., spleen, liver, lung, kidney, and heart) of the mice were carefully dissected analyzed with specialist for the following experiments. The pathologist was completely blinded to the treatment and was not aware which treatment each dissected sample came from. The dissected samples from mice were cut with a microtome device, transferred on slides, and then stained. H&E slides were created from the heart, spleen, lung, liver, and kidney to check metastasis to these organs. In immunohistochemistry (IHC) using antibodies to detect and distribute antigens, in this study, *MMP-2*, *MMP-9*, and *VEGF* were used to evaluate metastasis and angiogenesis, and the created H&E and IHC slides were examined by an optical microscope.

Statistical analysis

An unpaired t-test was carried out to investigate statistical differences between two groups, in case where we wanted to statistically compare difference between more than two groups a one-way analysis of variance (ANOVA) performed. *P* value < 0.05 was considered as significant (all statistical analyzes and graphs were obtained by GraphPad Prism software (V. 08)).

Results

Groups' description

All experiments were performed on 6 different groups (Table 1). Groups that received 1 dose of vaccines (Sinopharm or AstraZeneca) are named 1A or 2A, respectively. For the 2A and 1A, we used a control mice model that received 1 dose of ddH₂O in the same way, time, and amount of injection as the vaccinated ones. Groups that received 2 doses of vaccines (Sinopharm or AstraZeneca) are named as 1B or 2B, respectively (the control for these, were mice model that received 2 doses of ddH₂O).

Assessment of genes expression at the mRNA level

MMP-2 and MMP-9

Real-time PCR results revealed that *MMP-9* and *MMP-2* expression level decreased in 1A, 1B, and 2A groups: for *MMP-2*; with mean about 0.59 ± 0.08, 0.125 ± 0.095, and 0.43 ± 0.22 fold normalized to their corresponding controls (for 1A and 2A the control group was 3A, and for 1B and 2B the control group was 3B); and for *MMP-9* with mean about 0.07 ± 0.03, 0.03 ± 0.01, and 0.11 ± 0.02, respectively. In the case of 2B, no significant increase (*P*=0.0537) (compared

Table 1. *In vivo* groups' description

Abbreviation	Description
3A	4T1 mice model which received 1 dose of ddH ₂ O
3B	4T1 mice model which received 2 doses of ddH ₂ O
1A	4T1 mice model which received 1 dose of Sinopharm
1B	4T1 mice model which received 2 doses of Sinopharm
2A	4T1 mice model which received 1 dose of AstraZeneca
2B	4T1 mice model which received 2 doses of AstraZeneca

with 2A) was seen for *MMP-2*: mean calculated to be about 1.43 ± 0.1 , and a significant increase ($P = 0.019$) was seen in *MMP-9* with mean about 2.087 ± 0.083 .

E-cadherin and N-cadherin

In the case of *E-cadherin*, the expression level of this gene increased in the 1A, 1B, and 2A groups (this increase was much more in the 1B group compared with the others): 1.29 ± 0.11 , 6.05 ± 0.23 , and 2.37 ± 0.3 , respectively. The mean fold change for this gene in the 2B group was calculated to be 0.96 ± 0.140 (the difference between 2A and 2B was not statistically significant, $P < 0.05$).

In the case of *N-cadherin* (promotes the EMT process

and contributes to cancer progression), the fold change analysis demonstrated a decrease in the expression level of this gene in all vaccinated groups 1A, 1B, 2A, and 2B: mean fold change for these groups was 0.68795 ± 0.10155 , 0.469743 ± 0.10 , 0.067 ± 0.013 , and 0.14 ± 0.06 , respectively. According to the statistical data obtained from the comparison between the two groups that received Sinopharm (1A and 1B), the p -value was equal to 0.2655 that considered as non-significant (ns), also in the comparison between the two groups that received AstraZeneca (2A and 2B) the P value was equal to 0.5776 and considered as non-significant (Fig. 1).

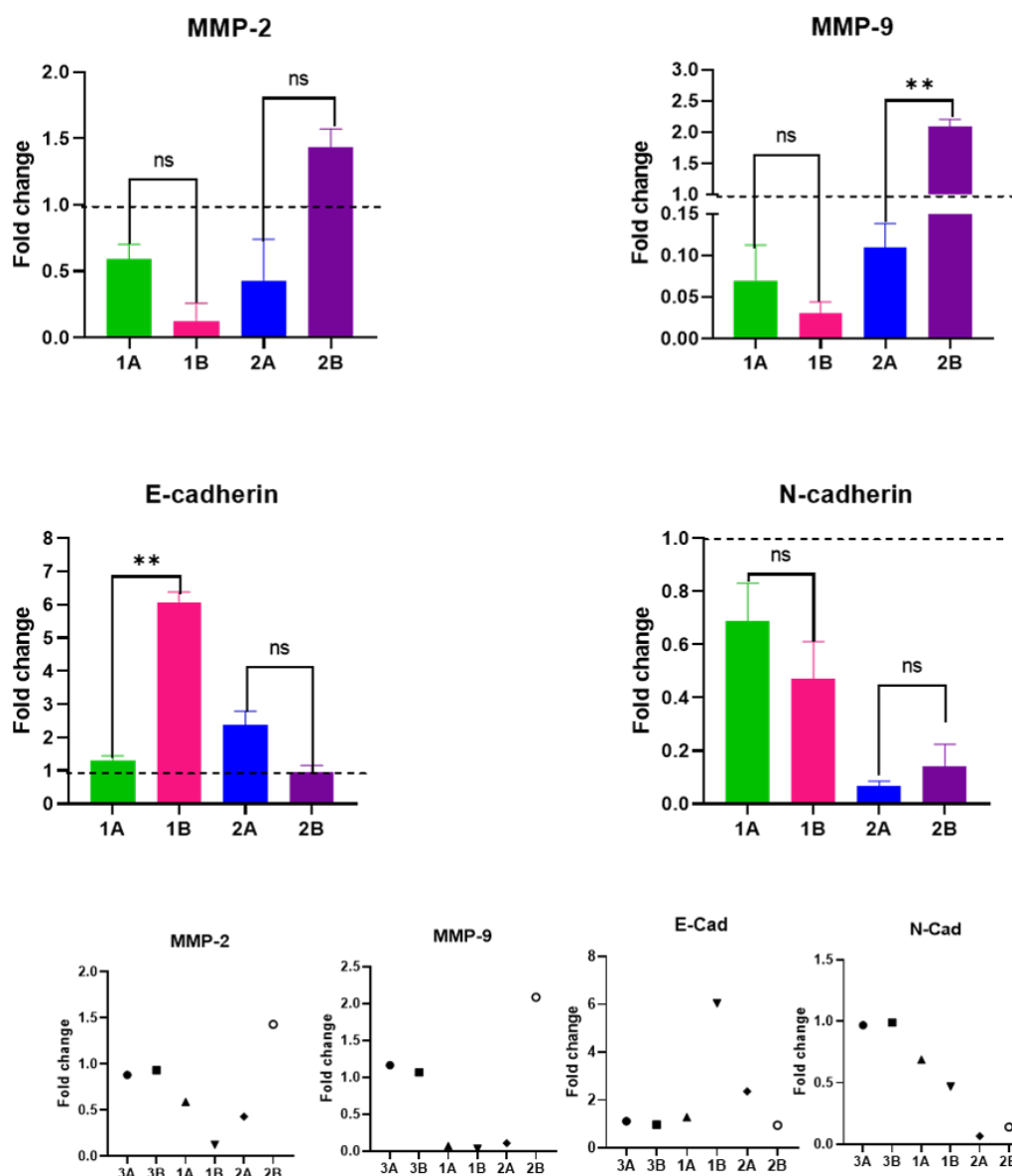


Fig. 1. Real-Time PCR results: The level of expression of *MMP-2*, *MMP-9*, and *N-cadherin* genes in both groups that received Sinopharm (1A and 1B) and the mice that took only one dose of AstraZeneca (2A) was reduced compared to the control group, but the expression level of *E-cadherin* gene in these three groups (1A, 1B, 2A) increased. On the other hand, in the mice that took two doses of AstraZeneca (2B), the expression levels of *MMP-2* and *MMP-9* genes showed a slight increase compared to the control group, and the expression levels of *E-cadherin* and *N-cadherin* genes decreased. The P value related to the *MMP-2* gene graph in comparison between 1A and 1B equals 0.0645 and in 2A and 2B equals 0.0537 (the adjusted P value calculated to be 0.039 and significant). The P value related to the *MMP-9* gene graph in comparison between 1A and 1B equals 0.3333 and in 2A and 2B equals 0.0019 (**). The p -value related to the *E-cadherin* gene graph in comparison between 1A and 1B was equal to 0.0029 (**) and in 2A and 2B equals 0.0525. The expression of *E-cadherin* was much less in the 2B compared with the 1B ($P = 0.0028$); although the expression of *N-cadherin* seemed to be less in the 2B, this difference was not statistically significant ($P = 0.1057$). The P value related to the *N-cadherin* gene graph in comparison between 1A and 1B equals 0.2655 and in 2A and 2B equals 0.5776. (The control group is shown with a dashed line). ns = non-significant, ** = Significant. For more details of the statistical analysis, see Table S1.

For further investigation of metastasis footprints after injection of Sinopharm and AstraZeneca vaccine, the expression levels of *VEGF*, *MMP9*, and *MMP2* in the tumor site at the protein level were also evaluated by IHC. Also, the metastatic lesions were investigated in the vital organs (heart, kidneys, lung, liver, and spleen) by H&E staining.

IHC staining results

We further evaluated the essential biomarkers that are associated with metastasis and angiogenesis, in the tumor sites, namely *MMP9*, *MMP2*, and *VEGF*. After vaccination, the protein expression level of these three genes decreased in the vaccinated groups. When comparing with their corresponding controls (those which took one dose of vaccine with 3A, and those that received two doses of vaccines were compared with 3B), it came out that the decreasing in the expression level of *MMP2* was statistically significant in 1A and 1B groups; *VEGF* decreasing was significant in 1A, 1B, and 2B; *MMP9* decreasing showed a significant decrease in the 1B group (Fig. 2, and Table S2).

H&E staining results

The vital organs (lung, heart, kidneys, liver, and spleen) of mice from different groups were dissected, H&E slides

prepared and then they were evaluated pathologically for any signs of metastatic lesions. Metastatic lesions were seen in the lungs and liver of 3A; 3B group also showed these lesions in their liver. In vaccinated mice, the groups that took AstraZeneca (2A and 2B) showed metastatic lesions only in their livers (these lesions were scarce in these groups). In the mice that received Sinopharm (1A and 1B), no metastatic lesions were observed in any of the examined organs (Fig. 3A and Fig. S1) (these data are summarized in Table 2). As mentioned, other organs including the heart, spleen, and kidneys did not seem to have metastasis (only liver and/or lungs in the mentioned groups had metastatic lesions) (Fig. 3B and Fig. S2).

Discussion

Due to lack of time, cancer patients as a high-risk group did not include in the preclinical studies which aimed to trace the plausible COVID-19 vaccines side effects.²⁴ The focus of previous studies in this area was not about this possible side effect, and on the other hand, they merely assessed the immunity occurred against this disease post vaccination^{1,2,25}; in this regard, studies aimed at elucidating the relationship between vaccination and disease progression are considered urgently needed.^{1,26}

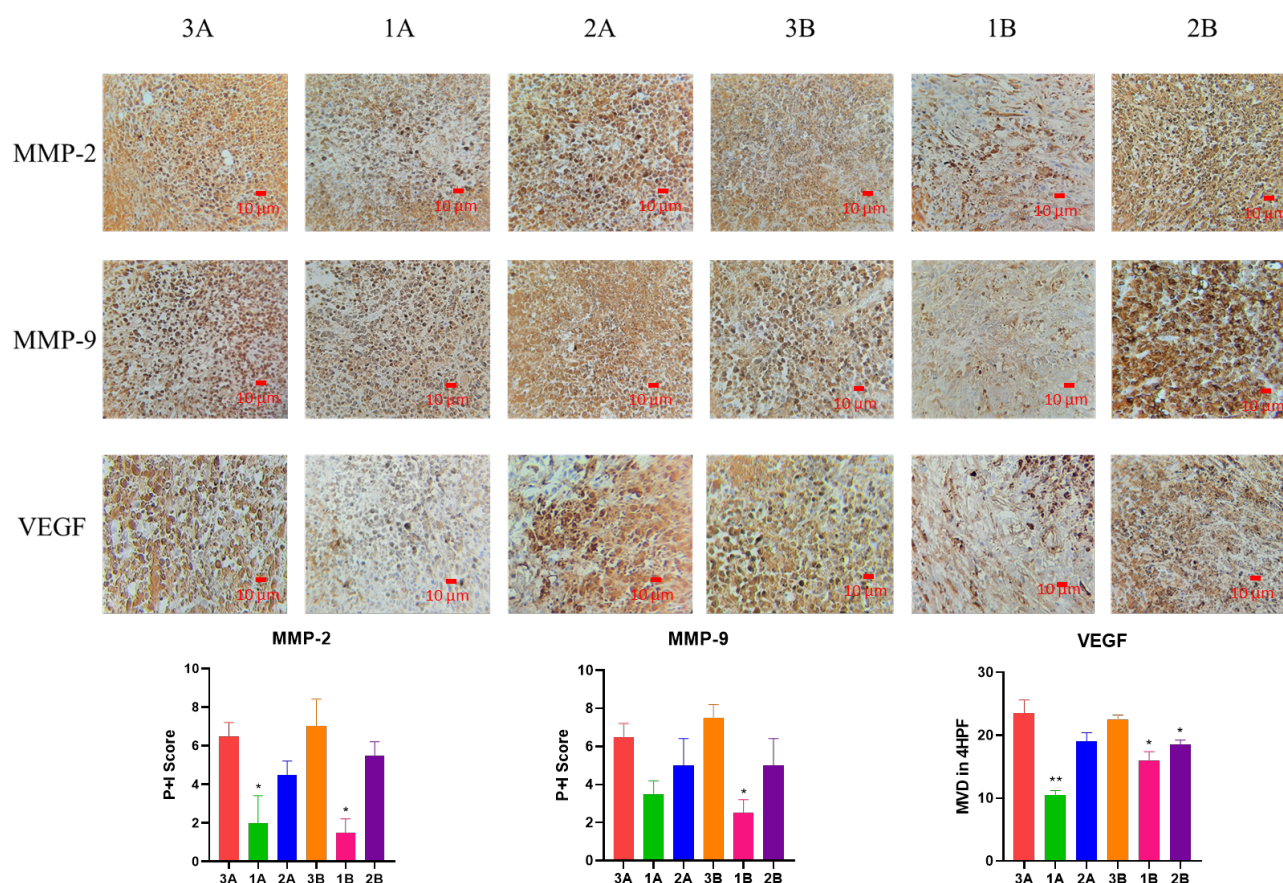


Fig. 2. Expression of pivotal markers related to metastasis (MMP-9, and MMP-2) and angiogenesis (VEGF) at the tumor site. The dissected tumors from all six groups were studied by IHC for the possible impact of the ensuing Sinopharm or AstraZeneca injection on the angiogenesis, and metastasis. To this aim, expression level of metastasis markers including MMP-2, and MMP-9, as well as an angiogenesis marker, VEGF, were studied in tumors dissected from all six groups, by IHC. Mice that received the Sinopharm vaccine (1A and 1B) showed the lowest expression level of VEGF, MMP-2, and MMP-9. The highest expression level was related to the 3A and 3B (control groups). (Magnification 400X). (3A: control group; 4T1 mice model which received 1 dose of ddH₂O, 1A: 4T1 mice model which received 1 dose of Sinopharm, 2A: 4T1 mice model which received 1 dose of AstraZeneca, 3B: control group; 4T1 mice model which received 2 doses of ddH₂O, 1B: 4T1 mice model which received 2 doses of Sinopharm, and 2B: 4T1 mice model which received 2 doses of AstraZeneca). (The brown points were considered as positive for each marker.) For more details of the statistical analysis, see Table S2.

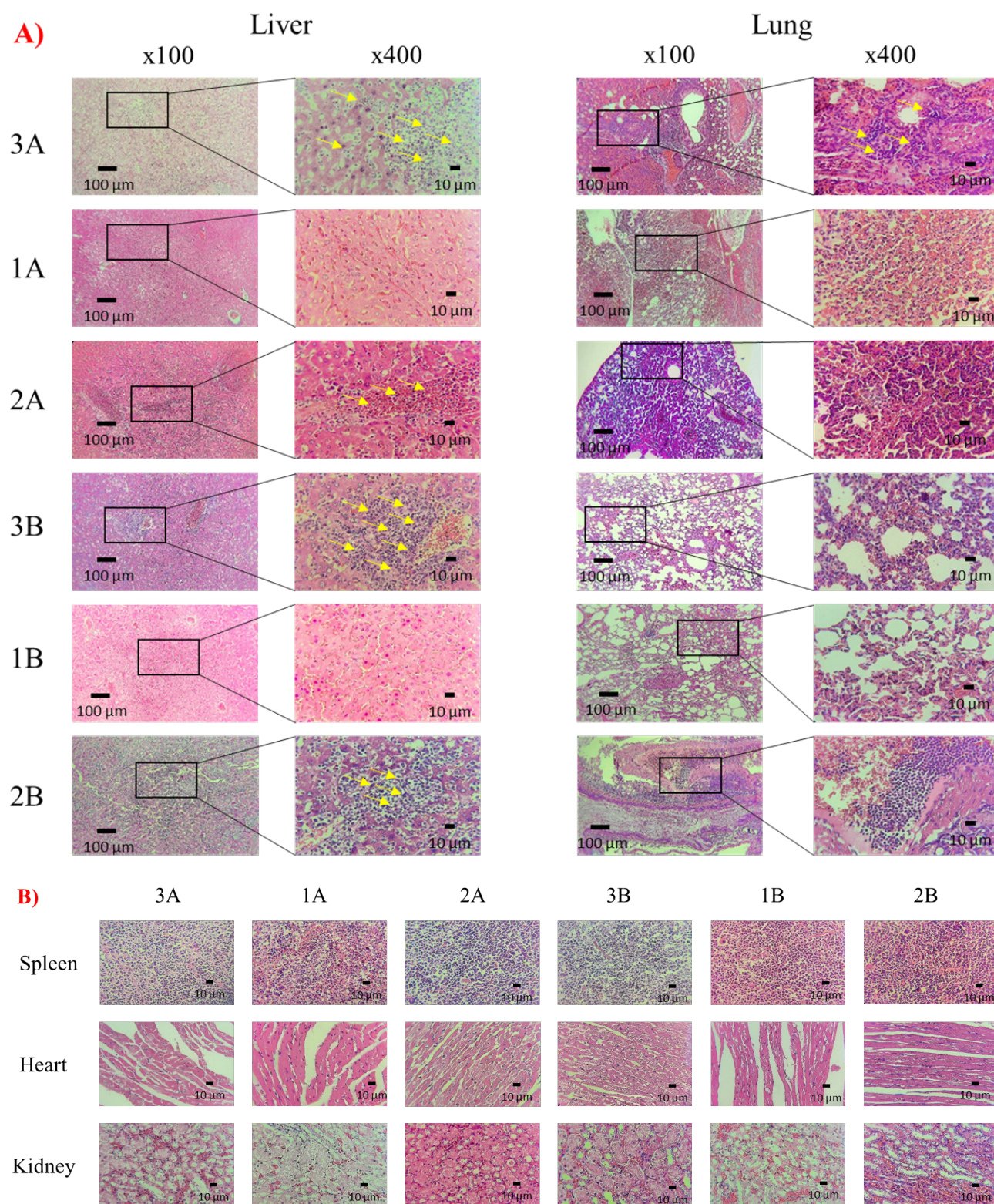


Fig. 3. Distant metastasis assessment. To investigate the impact of vaccination of mice models with Sinopharm or AstraZeneca on distant metastasis, organs like liver, lung, spleen, heart, and kidney of all six groups were dissected after the study. These organs were assessed for the existence of metastatic lesions: **(A)** Metastatic lesions were frequent in the liver and lungs of the control groups. Some signs of the metastatic lesions were seen in the 2A and 2B liver (much less than the control groups). No metastatic lesions were reported in the liver and lungs of the Sinopharm groups. (Images were taken from an optical microscope with 100X and 400X magnification). **(B)** According to pathology reports, no metastatic lesions were seen in other organs (heart, spleen, and kidneys) (Yellow arrows show the metastatic cells, Magnification 400X). (3A: control group; 4T1 mice model which received 1 dose of ddH₂O, 1A: 4T1 mice model which received 1 dose of Sinopharm, 2A: 4T1 mice model which received 1 dose of AstraZeneca, 3B: control group; 4T1 mice model which received 2 doses of ddH₂O, 1B: 4T1 mice model which received 2 doses of Sinopharm, and 2B: 4T1 mice model which received 2 doses of AstraZeneca).

According to our previous study,¹⁹ considering this gap and the high prevalence of breast cancer worldwide, here the effects of COVID-19 vaccines used in many countries

(Sinopharm and AstraZeneca) on breast cancer metastasis were investigated *in vivo*. To achieve this goal, mice models with triple-negative breast cancer were developed (as one of

Table 2. Tracing distant metastasis

	Liver	Spleen	Heart	Kidney	Lung
3A	++	–	–	–	+
1A	–	–	–	–	–
2A	+	–	–	–	–
3B	++	–	–	–	–
1B	–	–	–	–	–
2B	+	–	–	–	–

Note: Metastatic lesions were seen in the liver and lung of 3A and 3B (control groups) and also in the liver of groups receiving AstraZeneca (2A/2B). (Existence of metastatic lesions is indicated by + or –). (3A: control group, 1A: one dose of Sinopharm, 2A: one dose of AstraZeneca, 3B: control group, 1B: two doses of Sinopharm, and 2B: two doses of AstraZeneca).

the most metastatic breast cancers). Mice were examined in six different groups: 3A (this group injected with distilled water exactly like the group that took a vaccine), 3B (this group injected twice with distilled water exactly like the group that received two doses of vaccine), 1A (mice model that took one dose of Sinopharm vaccine) 1B (received two doses of Sinopharm vaccine), 2A (mice model that received one dose of AstraZeneca vaccine) and 2B (this group received two doses of AstraZeneca vaccine). After completion of the study period, expression of the essential genes involved in metastasis, as well as metastasis to different organs, was examined.

Excessive production or increased activity of *MMP-2* and *MMP-9* aids cancer cells to invade to the other tissues by ECM (extra cellular matrix) destruction and allows tumor cells invasion into other tissues and the metastasis of cancerous cells to the surrounding organs.²⁷ Furthermore, product of these two genes plays an important role in tumors fate by affecting proliferation, apoptosis and angiogenesis as well.²⁸ Expression level of them in the mice that received vaccines (1A, 1B, and 2A) decreased significantly compared to their control group. In the group that received two doses of the AstraZeneca vaccine (2B), a scarce increase was seen in the mRNA expression level of these two genes when compared with control group (this increase was more obvious at mRNA level, and at the protein level (IHC results) the protein expression level of both genes were less than the control group). This can result from the increased immunity that occurs after vaccination; the vaccination provokes the immune cells, these cells do not focus merely on the target vaccine antigen, they also become more alert about the cancerous cells, especially those who reside in the tumor area (as the number of cells with abnormal cell-surface antigen is much more there).^{29–34} The data obtained from our previous report also revealed that after vaccination, TILs presentation in the tumor site of the vaccinated groups increased; this was further confirmed by CD4/CD8 ratio; which showed decreasing in the tumor dissected from vaccinated groups.^{19,20}

Cadherins (calcium-dependent cell adhesion molecules) are renowned molecules which have a key role in maintaining the epithelial characteristics of various tissues. A raft of studies has proven that the reduction

of *E-cadherin* expression causes intercellular adhesion perishment and increases cell motility.^{35,36} Various studies on clinical samples of different tumor tissues have shown that the decrease in *E-cadherin* expression is associated with tumor invasion, and metastasis and finally induce tumor progression. *N-cadherin* is another gene that plays beside the *E-cadherin*; the former promotes the EMT process and contributes to the cancer progression and metastasis. Nakajima et al demonstrated that the *N-cadherin* overexpression and the *E-cadherin* down-regulation are common features in metastatic tumors.³⁷ Our results revealed that *E-cadherin* expression increased and *N-cadherin* expression decreased in the 1A, 1B, and 2A. However, in the group that received two doses of the AstraZeneca vaccine (2B) *E-cadherin* expression level did not show significant change (compared with the control group); the difference between 2A and 2B groups was not statically significant, as well. The expression level of *N-cadherin* decreased in all vaccinated groups, in comparison with their corresponding control.

As mentioned earlier, the result of increasing the *E-cadherin* expression induces cell-to-cell adhesion; on the other hand, by decreasing *N-cadherin* expression, metastasis, and invasion of tumor cells to other parts of the body can be reduced; the phenomenon which can be seen in the H & E performed in this study on the vital organs. In this area, metastatic lesions were obvious (and maybe abundant in the liver) in the organs dissected from control groups, and no metastatic lesions were seen in other vaccinated groups; although some of the livers obtained from the 2B, showed some signs of metastasis (significantly less than those seen in the control).

In addition, the expression levels of metastasis-related proteins at the tumor sites were investigated by IHC. *VEGF* is an angiogenic marker that may be directly related to metastasis.³⁸ In addition to the roles mentioned in the paragraphs earlier, *MMP-2* and *MMP-9* have a pivotal impact in the invasion of cancer cells into adjacent tissues, which is one of the main steps of metastasis in cancer.²⁷ The expression of these three IHC markers (*MMP-9*, *MMP-2*, and *VEGF*) down-regulated significantly post vaccination. These results confirmed the data obtained from real-time PCR and H & E results (tracing metastatic lesions in the vital organs). Among vaccinated groups, these markers expression was a bit more in the 2A and 2B groups than the 1A and 1B ones. As the AstraZeneca vaccine is composed of active viruses, it may extrapolate that enhanced immunity may help to promote angiogenesis and the plausibility of the presence of immune cells exist in the site of tumor. According to the results related to the expression of *MMP-2* and *MMP-9* genes by real-time PCR and the examination of these markers by IHC, it can be said that all vaccinated groups reduced the expression level of metastatic genes expression at mRNA and/or protein level. This was also demonstrated by H&E to trace the rate of distant metastasis to the heart, lung, kidney, liver, and spleen. Metastatic lesions were reported only in the 3A and 3B (control groups).

Conclusion

The main aim of this study was to assess the possible impact of vaccination against COVID-19 on the metastatic features of TNBC. To investigate this, we exploited a TNBC mice model and monitored the metastasis-related outcomes. The current study can be considered as one of the valuable pre-clinical research projects investigating the outcome of anti-infection vaccination on the tumor behavior. Although, limited metastatic lesions were seen merely in the liver of the TNBC mice model, which received AstraZeneca, the frequency of these was much lower compared with the control groups. Hence, in a nutshell, vaccination in the triple-negative breast cancer mice model not only has no negative effects on the tumor, but it also reduces cancer progression markers (such as MMP-2/9 and VEGF) and prevents metastasis to vital organs, which may play as an obstacle for cancer progression. This effect may be better when two doses of vaccines are received than when one dose is. This is of importance to mention that, these results should be interpreted with caution due to several limitations, e.g., the study focused on two vaccine types (Sinopharm and AstraZeneca), limiting generalizability to other vaccines, and assessing the vaccination on other types of cancers. Lack of a control group that has tumor and was infected simultaneously, which prevents direct assessment of interactions between viral infection, vaccination, and tumor progression. Extrapolating findings from mouse models to human applications is another crucial issue in this regard, because mouse immune-tumor interactions differ from those in humans. Moreover, the administration route of vaccination in mice differs from that in clinical practice, which further complicates translation. Also, sufficient supporting clinical experiments, including cohort studies and clinical trials, are needed. Future research should focus on large-scale studies to validate these findings and assess long-term efficacy for ensuring the approach's clinical applicability.

Acknowledgments

The authors would like to thank Pars Isotope Company for their good collaboration and consulting for the *in vivo* experiments.

Authors' Contribution

Conceptualization: Mohammad Amin Javidi.

Data curation: Negar Deldadeh, Saeedeh Ghiasvand.

Formal analysis: Mohammad Amin Javidi, Fatemeh Shahriari, Negar Deldadeh, Saeedeh Ghiasvand.

Investigation: Negar Deldadeh, Saeedeh Ghiasvand.

Methodology: Mohammad Amin Javidi.

Project administration: Mohammad Amin Javidi.

Software: Negar Deldadeh.

Supervision: Mohammad Amin Javidi, Mohammad Hadi Niakan.

Validation: Flora Forouzesh, Saeedeh Ghiasvand.

Visualization: Negar Deldadeh, Fatemeh Shahriari.

Writing—original draft: Mohammad Amin Javidi, Negar Deldadeh, Saeedeh Ghiasvand.

Writing—review & editing: Mohammad Amin Javidi, Negar Deldadeh, Flora Forouzesh, Fatemeh Shahriari, Hamid Zaferani Arani, Mohammad Hadi Niakan.

Competing Interests

None.

Declaration of AI-Assisted Tools in the Writing Procedure

The authors declare that no artificial intelligence (AI)-assisted tools were used in the preparation of this manuscript, including writing, revision, editing, or generation of figures, graphical abstracts, or other artwork.

Data Availability Statement

Data will be available on request.

Ethical Approval

The project which led to this article was approved by the Committee on the Ethics at National Cancer Institute, ACECR, Iran, (Ethical code: IR.ACECR.IBCRC.REC.1400.028).

Funding

None.

Supplementary files

Supplementary file 1 contains Tables S1-S2 and Figures S1-S2.

References

1. Javadinia SA, Welsh JS, Mosavi Jarrahi A. COVID-19 vaccination and cancer, the need for more data. *Asian Pac J Cancer Prev* 2021;22(10):3053-4. doi:10.31557/apjcp.2021.22.10.3053
2. Ariamanesh M, Porouhan P, PeyroShabany B, Fazilat-Panah D, Dehghani M, Nabavifard M, et al. Immunogenicity and safety of the inactivated SARS-CoV-2 vaccine (BBIBP-CorV) in patients with malignancy. *Cancer Invest* 2022;40(1):26-34. doi:10.1080/07357907.2021.1992420
3. www.cancer.net/. Available from: <https://www.cancer.net/>.
4. www.cdc.gov/. Available from: <https://www.cdc.gov/>.
5. Geiger TR, Peeper DS. Metastasis mechanisms. *Biochim Biophys Acta* 2009;1796(2):293-308. doi:10.1016/j.bbcan.2009.07.006
6. Gupta GP, Massagué J. Cancer metastasis: building a framework. *Cell* 2006;127(4):679-95. doi:10.1016/j.cell.2006.11.001
7. van Zijl F, Krupitza G, Mikulits W. Initial steps of metastasis: cell invasion and endothelial transmigration. *Mutat Res* 2011;728(1-2):23-34. doi:10.1016/j.mrrev.2011.05.002
8. Friedl P, Wolf K. Tumour-cell invasion and migration: diversity and escape mechanisms. *Nat Rev Cancer* 2003;3(5):362-74. doi:10.1038/nrc1075
9. Thierry JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell* 2009;139(5):871-90. doi:10.1016/j.cell.2009.11.007
10. Hegerfeldt Y, Tusch M, Bröcker EB, Friedl P. Collective cell movement in primary melanoma explants: plasticity of cell-cell interaction, beta1-integrin function, and migration strategies. *Cancer Res* 2002;62(7):2125-30.
11. Wolf K, Mazo I, Leung H, Engelke K, von Andrian UH, Deryugina EI, et al. Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis. *J Cell Biol* 2003;160(2):267-77. doi:10.1083/jcb.200209006
12. Jechlinger M, Grunert S, Tamir IH, Janda E, Lüdemann S, Waerner T, et al. Expression profiling of epithelial plasticity in tumor progression. *Oncogene* 2003;22(46):7155-69. doi:10.1038/sj.onc.1206887
13. Derksen PW, Liu X, Saridin F, van der Gulden H, Zevenhoven J, Evers B, et al. Somatic inactivation of E-cadherin and p53 in mice leads to metastatic lobular mammary carcinoma through induction of anoikis resistance and angiogenesis. *Cancer Cell* 2006;10(5):437-49. doi:10.1016/j.ccr.2006.09.013
14. Perl AK, Wilgenbus P, Dahl U, Semb H, Christofori G. A causal role for E-cadherin in the transition from adenoma to carcinoma. *Nature* 1998;392(6672):190-3. doi:10.1038/32433
15. Hazan RB, Phillips GR, Qiao RF, Norton L, Aaronson SA. Exogenous expression of N-cadherin in breast cancer cells induces cell migration, invasion, and metastasis. *J Cell Biol* 2000;148(4):779-90. doi:10.1083/jcb.148.4.779
16. Nieman MT, Prudoff RS, Johnson KR, Wheelock MJ. N-cadherin promotes motility in human breast cancer cells regardless of their E-cadherin expression. *J Cell Biol* 1999;147(3):631-44. doi:10.1083/jcb.147.3.631

17. Sobczyńska-Rak A, Żylińska B, Nowicka B, Rak E, Rzepka T. Role and mechanisms of angiogenesis in tumours. *Biology (Basel)* 2025;14(7):756. doi:10.3390/biology14070756
18. Palmer BF, Clegg DJ. Oxygen sensing and metabolic homeostasis. *Mol Cell Endocrinol* 2014;397(1-2):51-8. doi:10.1016/j.mce.2014.08.001
19. Alavian F, Ghiasvand S, Javidi MA. Investigation of the effects of previous smallpox vaccination against monkeypox disease: a systemic review. *Journal of Applied Biotechnology Reports* 2023; 10: 1140-1155. doi: 10.30491/jabr.2023.379139.1590
20. Deldadeh N, Shahbazi S, Ghiasvand S, Shahriari F, Javidi MA. COVID-19 vaccination anti-cancer impact on the PI3K/AKT signaling pathway in MC4L2 mice models. *Microb Pathog* 2024;196:106955. doi:10.1016/j.micpath.2024.106955
21. Deldadeh N, Haghighat S, Omid Z, Sarraimi-Foroushani R, Madjid Ansari A, Sanati H, et al. Anti-cancer effect of COVID-19 vaccines in mice models. *Life Sci* 2023;325:121569. doi:10.1016/j.lfs.2023.121569
22. Faria NR, Quick J, Claro IM, Thézé J, de Jesus JG, Giovanetti M, et al. Establishment and cryptic transmission of Zika virus in Brazil and the Americas. *Nature* 2017;546(7658):406-10. doi:10.1038/nature22401
23. Mentzer AJ, Brenner N, Allen N, Littlejohns TJ, Chong AY, Cortes A, et al. Identification of host-pathogen-disease relationships using a scalable multiplex serology platform in UK Biobank. *Nat Commun* 2022;13(1):1818. doi:10.1038/s41467-022-29307-3
24. Corti C, Crimini E, Tarantino P, Pravettoni G, Eggermont AM, Delaloge S, et al. SARS-CoV-2 vaccines for cancer patients: a call to action. *Eur J Cancer* 2021;148:316-27. doi:10.1016/j.ejca.2021.01.046
25. So AC, McGrath H, Ting J, Srikandarajah K, Germanou S, Moss C, et al. COVID-19 vaccine safety in cancer patients: a single centre experience. *Cancers (Basel)* 2021;13(14):3573. doi:10.3390/cancers13143573
26. Fanciullino R, Ciccolini J, Milano G. COVID-19 vaccine race: watch your step for cancer patients. *Br J Cancer* 2021;124(5):860-1. doi:10.1038/s41416-020-01219-3
27. Gialeli C, Theocharis AD, Karamanos NK. Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. *FEBS J* 2011;278(1):16-27. doi:10.1111/j.1742-4658.2010.07919.x
28. Jiang H, Li H. Prognostic values of tumoral MMP2 and MMP9 overexpression in breast cancer: a systematic review and meta-analysis. *BMC Cancer* 2021;21(1):149. doi:10.1186/s12885-021-07860-2
29. Dai L, Gao GF. Viral targets for vaccines against COVID-19. *Nat Rev Immunol* 2021;21(2):73-82. doi:10.1038/s41577-020-00480-0
30. Dang T, Palacio N, Sanchez S, Park M, Class J, Visvabharathy L, et al. Cross-protective immunity following coronavirus vaccination and coronavirus infection. *J Clin Invest* 2021;131(24): e151969. doi:10.1172/jci151969
31. Couzin-Frankel J. Breakthrough of the year 2013. Cancer immunotherapy. *Science* 2013;342(6165):1432-3. doi:10.1126/science.342.6165.1432
32. Lin MJ, Svensson-Arvelund J, Lubitz GS, Marabelle A, Melero I, Brown BD, et al. Cancer vaccines: the next immunotherapy frontier. *Nat Cancer* 2022;3(8):911-26. doi:10.1038/s43018-022-00418-6
33. Schuster M, Nechansky A, Kircheis R. Cancer immunotherapy. *Biotechnol J* 2006;1(2):138-47. doi:10.1002/biot.200500044
34. Farkona S, Diamandis EP, Blasutig IM. Cancer immunotherapy: the beginning of the end of cancer? *BMC Med* 2016;14:73. doi:10.1186/s12916-016-0623-5
35. Karayiannakis AJ, Syrigos KN, Polychronidis A, Simopoulos C. Expression patterns of alpha-, beta- and gamma-catenin in pancreatic cancer: correlation with E-cadherin expression, pathological features and prognosis. *Anticancer Res* 2001;21(6A):4127-34.
36. Joo YE, Rew JS, Park CS, Kim SJ. Expression of E-cadherin, alpha- and beta-catenins in patients with pancreatic adenocarcinoma. *Pancreatol* 2002;2(2):129-37. doi:10.1159/000055903
37. Nakajima S, Doi R, Toyoda E, Tsuji S, Wada M, Koizumi M, et al. N-cadherin expression and epithelial-mesenchymal transition in pancreatic carcinoma. *Clin Cancer Res* 2004;10(12):4125-33. doi:10.1158/1078-0432.CCR-0578-03
38. Goel HL, Mercurio AM. VEGF targets the tumour cell. *Nat Rev Cancer* 2013;13(12):871-82. doi:10.1038/nrc3627