# Transmembrane Serine Protease and Anti-Androgen Receptor Activity of *Artemisia annua*: another therapeutic option for COVID-19

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## Abstract

As early as 2011 for SARS-CoV-2003, it was noted that when a target cell expresses two receptors on its surface (such as type II pneumocytes), on the one hand the angiotensin converting enzyme type 2 (ACE2) receptor, a zinc metalloprotease, and on the other hand the transmembrane serine protease TMPRSS2 (TMPRSS2), it is more likely to be infected. Membrane expression of TMPRSS2 is known in the literature to be stimulated by androgen receptor (AR) expression on the cell surface. AR are expressed by the stimulation of androgens (A). For SARS-CoV-2, it has been shown that its cellular entry is blocked by a specific TMPRSS2 protease inhibitor. The first step for cell entry is therefore the AR activation of TMPRSS2 expression on the membrane surface of target cells that may possess both ACE2 and TMPRSS2 receptors. The initial priming of the S protein, spike protein, of the SARS-CoV-2 virus, is made on the expressed protease. Then, once priming is complete, the serine protease can cause cleavage of the ACE2 receptor present and thus increase the entry of the virus into the cell. Androgen expression of AR and therefore TMPRSS2 is known to occur in cells of the lung, prostate, gastrointestinal tract and upper airways and in certain cancerous prostate and lung tissues. This activation chain is particularly evident in tumor cells and metastatic cancers that are resistant to hormone therapy. Artemisinin, its derivatives and *Artemisia annua* have demonstrated their efficacy in inhibiting the growth of tumor and metastatic prostate cancer cells in vitro, in vivo and clinically in humans. Their action involves a decrease in AR expression and

subsequently of TMPRSS2 involved in the diffusion of biochemical cellular messages stimulating the appearance of tumor and metastatic cells of the prostate. If artemisinin and its derivatives of *Artemisia annua* have this inhibitory capacity via AR on TMPRSS2 in prostate cancer, they could similarly have it on cells infected with SARS-CoV-2 and thus inhibit the initial priming of the viral S protein which is the first key to the stimulation of the ACE2 receptor and intracellular viral penetration.

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# I. Serine Protease and Androgen Receptors (AR) Definitions and functions

## 1.1 Transmembrane Serine Protease

Transmembrane serine protease is an enzyme encoded by the TMPRSS2 gene.

The TMPRSS2 gene encodes a protein of the serine protease family. This protein contains four domains:

- Type II transmembrane domain
- Receptor class A domain
- Scavenger receptor cysteine-rich domain
- Protease domain

Serine proteases are known to be involved in many physiological (iron homeostasis) and pathological processes e.g. idiopathic pulmonary fibrosis, prostate cancer, pulmonary adenocarcinoma, SARS-CoV-2003, COVID-19, Haemophilus influenzae infections.

In idiopathic pulmonary fibrosis, the damaged endothelium causes the production of fibronectin and other co-factors that stimulate fibroblast proliferation. Type II pneumocytes no longer produce surfactant nor maintain the permeable barrier with type I pneumocytes.

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**TMPRSS2 Is the Major Activating Protease of Influenza A Virus in Primary Human Airway Cells and Influenza B Virus in Human Type II Pneumocytes**
Journal of Virology, 2019 Oct 15;93(21). pp. 649-19. Print 2019 Nov 1

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Cordier JF.
**Fibroses pulmonaires**
Méd. et sciences, 1994, 10 (12) p 1223-33.

## 1.2 Androgen receptors

The androgen receptor is the receptor for the male sex hormones testosterone and dihydrotestosterone. The hormones are receptor agonists. Their expression is seen in the testes, prostate, lung, adrenal glands, kidneys, brain.

ACE2 receptor:

The ACE2 receptor is a zinc metalloprotease capable of hydrolysing angiotensin-1 to angiotensin-2. It is an enzyme bound to the outer surface of the plasma membranes of cells in the lung, arteries, heart, kidney and digestive system. It plays an important role in the renin-angiotensin-aldosterone system (RAAS), which regulates hydro-sodium homeostasis and blood pressure. ACE2 is the point of entry into human cells of certain coronaviruses, including human coronavirus NL63 (HCoV-NL63), SARS-CoV, the coronavirus that causes SARS, and SARS-CoV-2, the coronavirus that causes COVID-19.


Mohammed A. R. Chamsi-Pasha, Zhili Shao et W. H. Wilson Tang
**Angiotensin-Converting Enzyme 2 as a Therapeutic Target for Heart Failure**
Current Heart Failure Reports, vol. 11, no 1, mars 2014, p. 58-63

Annamaria Mascolo, Konrad Urbanek, Antonella De Angelis, Maurizio Sessa & al
**Angiotensin II and angiotensin 1–7: which is their role in atrial fibrillation?**
Heart Failure Reviews, vol. 25, no 2, mars 2020, p. 367-380

Anthony R. Fehr et Stanley Perlman,
**Coronaviruses: An Overview of Their Replication and Pathogenesis**
Coronaviruses, vol. 1282, 12 février 2015, p. 1-23

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**A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury**
Nature Medicine, vol. 11, no 8, 10 juillet 2005, p. 875-879

Peng Zhou, Xing-Lou Yang, Xian-Guang Wang, Ben Hu, Lei Zhang, & al
**A pneumonia outbreak associated with a new coronavirus of probable bat origin**
Nature, vol. 579, no 7798, 3 février 2020, p. 270-273


# II. Serine protease, ACE2 receptor : two keys to be activated to enter the target cell of the virus and link with coronavirus

It was demonstrated in 2011 for SARS-CoV-2003, which is genetically very similar to SARS-CoV2, that when an S protein (spike protein of SARS-CoV) binds to the ACE2 receptor of its host cell, the virus-cell complex was proteolytically treated with the transmembrane serine protease type 2 TMPRSS2, leading to cleavage of ACE2 and activation of the viral S-spiral protein, in a process similar to that observed for influenza or human metapneumovirus (MPVH). This process facilitates the penetration of the virus into its target cell.

ACE2 is currently known to be one of the recognized COVID-19 receptors. A hypothesis put forward in 2011 stated that when a cell had both ACE2 and TMPRSS2 receptors on its surface together, such as type II pneumocytes, it was more likely to be infected with SARS-CoV. For SARS-CoV-2 in 2019 it was established that its entry into the cell is blocked by a specific protease inhibitor. The virus uses the serine protease TMPRSS2 to prime its S protein and then the ACE2 receptor, cleaved by TMPRSS2 for entry into the target cell.

Androgens stimulate the expression of androgen receptors and androgen receptors (AR) are currently the only known stimulators of the expression of the TMPRSS2 human cell membrane serine protease gene. AR are present in many organs (lungs, upper airways, intestine, prostate, testicles and kidneys). In addition, TPMRSS2 protease expression is found in humans in prostate cells and is expressed in vivo in A549 pulmonary adenocarcinoma cells injected into mice stimulated by AR.

It is now established that for SARS-CoV-2 (COVID-19) the first step for cell penetration to occur is activation by androgen receptors (AR) of the expression of the TMPRSS2 gene in the cell membrane. It is on this protease that the S protein of the virus is primed.

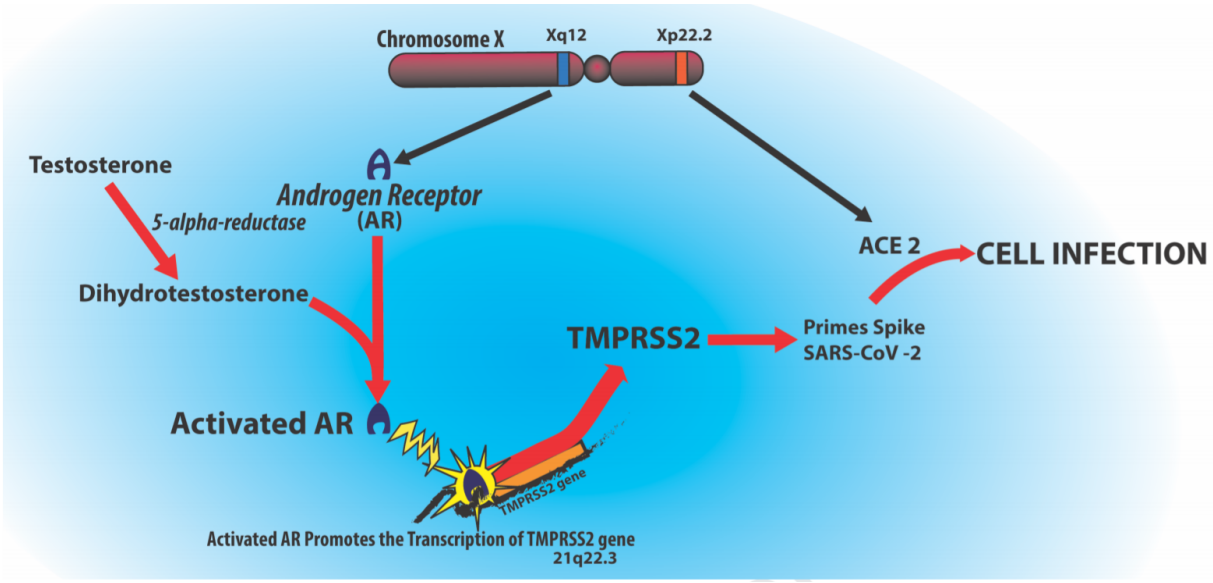


**Figure 1.** The androgen-activated androgen receptor (AR), secondarily stimulates transcription of the serine protease gene TMPRSS2. Excerpt from from Carlos Gustavo Wambier, MD, phD, Andy Goren, MD. **« SARS-CoV2 infection is likely to be androgen mediated ».** *Journal of the American academy of dermatology* (2020) Https://doi.org/10.1016/J.jaad.2020.04.032

Activation of androgen receptors by androgens is necessary for transcription of the TMPRSS2 gene. Subsequently, once the serine TMPRSS2 protease has been expressed, the priming of the SARS-CoV-2 S protein takes place on this cellular receptor (e.g. type II pneumocyte or intestinal cells). Finally, serine protease can cause cleavage of ACE2 to increase virus entry into the cell.

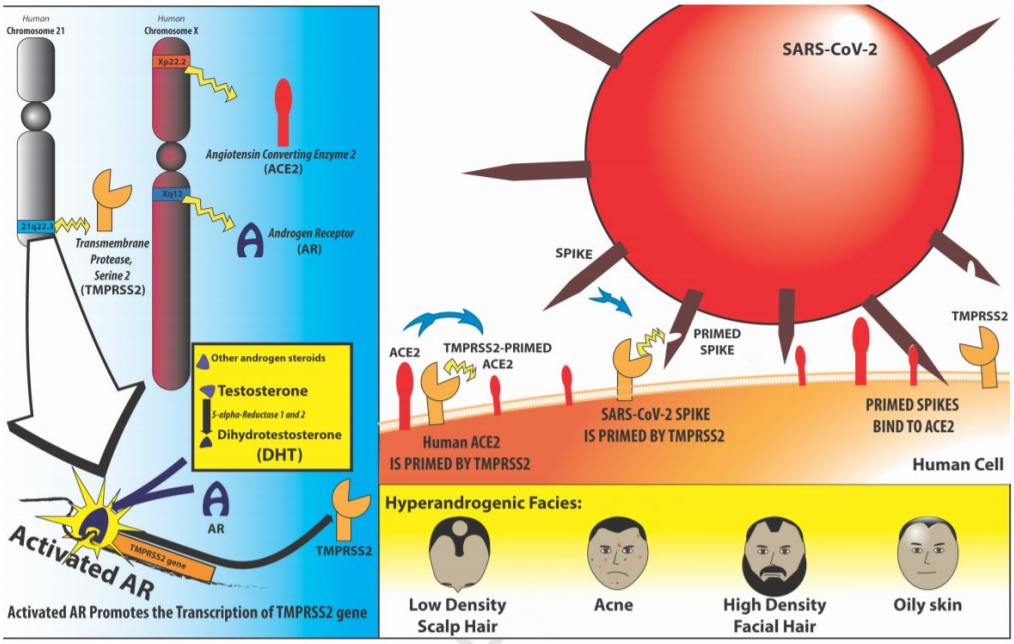


**Figure 2 :** Priming of the SARS-CoV-2 virus S protein by expressed serine protease and secondary cleavage of the ACE2 receptor of the COVID-19 infected cell. Excerpt from Carlos Gustavo Wambier, Andy Goren, (op. cit.)

This involvement of androgen receptors in the pathophysiology of COVID-19 would explain the current epidemiological and clinical data concerning the predominant incidence of COVID-19 in men and the severity of their pathology. In addition, there are large inter-individual and sex-related variabilities in the expression of androgen receptors in their tissue distribution.

These mechanisms provide an understanding of clinically observed target organ damage related to the presence of both receptors together. Viral entry via the cells of the nasal cavities and upper airways, olfactory and gustatory damage and secondary damage to the central nervous system (clinical and CT scan), acute respiratory failure and fibrosing interstitial pneumonia, diarrhoea, renal failure, heart failure.

Carlos Gustavo Wambier, MD, phD, Andy Goren, MD.
**SARS-CoV2 infection is likely to be androgen mediated**
Journal of the American Academy of Dermatology (2020) Https://doi.org/10.1016/J.jaad.2020.04.032
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Jing Qi, PHD, yang Zhou PHD, Jiao Hua, Liying Zhang & al.
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**Androgen receptor and androgen-dependent gene expression in lung**
Molecular and cellular endocrinology, volume 317, issue 1-2,;14-24 (2010)

Heurich A, Hoffman Winkler, h Gierer S, Liepold T, Jahn O, Pohlman S
**TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments**

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Journal of Virology, 2014 Jan; 88(2): 1293–1307

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Cancer Discovery 2014 Nov; 4(11): 1310-25.

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**Prostate-localized and androgen-regulated expression of the membrane-bound serine protease TMPRSS2**
Cancer research, vol 59, 17, September 1999, p 4180 -4184.

Markku H. Vaarala; Katja S. Porvari et al.
**Expression of transmembrane serine protease TMPRSS2 in mouse and human tissues**
Journal of Pathology, vol 193, 1 Janvier 2001, p 134 140.

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**SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor**
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**Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response**
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Heurich A, Hoffman Winkler, h Gierer S, Liepold T, Jahn O, Pohlman S
**TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein**
Journal of Virology, 2014 Jan; 88(2): 1293–1307

Rafal Butowt and Katarzyna Bilinska
**SARS-CoV-2: Olfaction, Brain Infection, and the Urgent Need for Clinical Samples Allowing Earlier Virus Detection**
ACS Chemical Neuroscience, April 13, 2020: https://dx.doi.org/10.1021/acschemneuro.0c00172

Sungnak, W., Huang, N., Bécavin, C. et al.
**SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes**
Nature Medecine (2020). https://doi.org/10.1038/s41591-020-0868-6

# III Serine protease TMPRSS2 in healthy and cancerous lung and prostate tissue

The TMPRSS2 serine protease gene is located on chromosome 21. It is widely expressed on the surface of cells of the prostate epithelium compared to its expression in other human tissues (type II pneumocyte, intestinal cells, upper airway cells, bladder and kidney).

In prostate cancer, the TMPRSS2 serine protease anchored in the cell membrane stimulates a proteolytic cascade that acts, via an endopeptidase, as a mediator of cancer cell invasion, growth and tumor metastasis. Matriptase is the substrate for TMPRSS2. Activation of matriptase is induced by androgens, via the expression of androgen receptors (AR), which increase the expression of TMPRSS2.

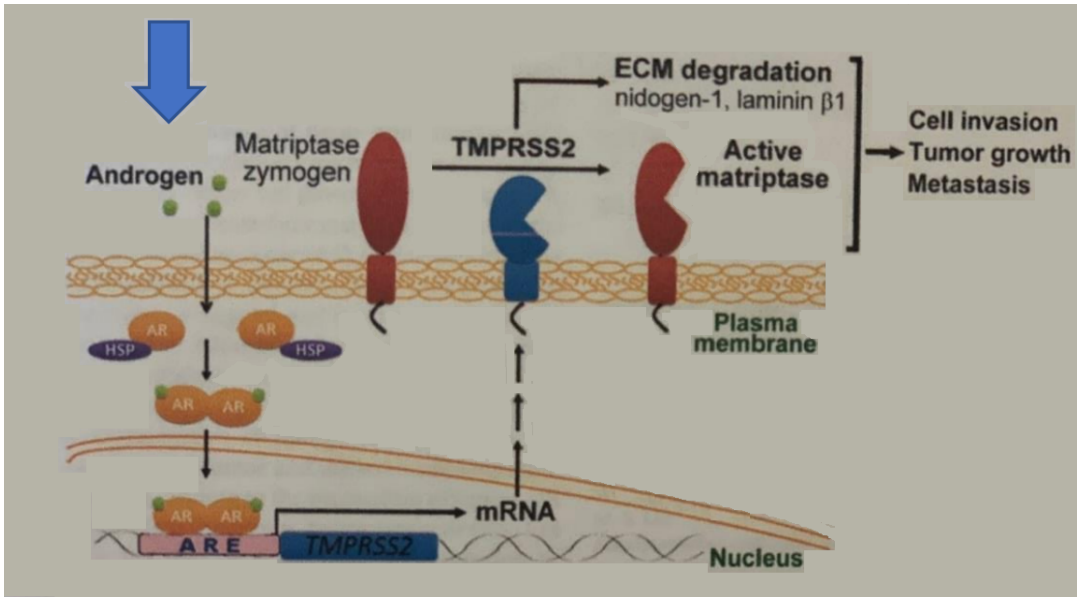


**Figure 3** : Gene expression of the serine protease TMPRSS2 on the cell membrane of the prostate cancer cell via stimulation of androgen receptors (AR). Secondary activation of matriptase that stimulates metastatic cancer cell production and tumor growth.

Ko Chung-Jung, Huang Cheng-Chung, Lin Hsin-ying et al.
**Androgen-Induced TMPRSS2 Activates Matriptase and Promotes Extracellular Matrix Degradation, Prostate Cancer Cell Invasion, Tumor Growth, and Metastasis**
Cancer Res. 2015 Jul 15;75(14):2949-60. doi: 10.1158/0008-5472.CAN-14-3297. Epub 2015 May 27

Daniel E. H. Afar, Igor Vivanco, Rene S. Hubert, James Kuo, Emily Chen, Douglas C. Saffran, Arthur B. Raitano and Aya Jakobovits
**Catalytic cleavage of the androgen-regulated TMPRSS2 protease results in its secretion by prostate and prostate cancer epithelia**
Cancer Research, 1686–1692, February 15, 2001

Bioyang Lin, Camari Ferguson, James T, White et al. et al.
**Prostate-localized and Androgen-regulated Expression of the Membrane-bound Serine Protease TMPRSS2**
Cancer research, 59, 4180–4184, September 1, 1999

Type II pneumocytes line the pulmonary alveoli and secrete the surfactant. They are the stem cells of type I pneumocytes and allow the renewal and scarring repair of the pulmonary epithelium.

Androgen receptors have been highlighted in human lung cancer cell lines derived from human type II pneumocytes (A549 adenocarcinoma-derived cell lines). Following exposure of these cells to androgens, the androgen receptor level increases and upregulates the expression of the serine protease gene TMPRSS2.

Laura Mikkonen, Pâivi Pihlajamaa, Biswajyoti Sahu, et al
**Androgen receptor and androgen-dependent gene expression in lung**
Molecular and cellular endocrinology, volume 317, issue 1-2,;14-24 (2010)

# IV. Artemisinin and artesunate in clinical, in vivo and in vitro treatment of prostate cancer and in vivo treatment of pulmonary adenocarcinoma (type II pneumocyte)

In 2010, the effects of dihydroartemisinin and two artemisinin dimers on apoptosis and proliferation of prostate cancer cells were studied in vitro. All the molecules studied showed an increase in cell apoptosis and a cessation of growth of metastatic cells and original tumor cells. Cell treatment with one of the artemisinin derivatives decreased androgen receptors and was associated with a concomitant loss of the cell cycle regulator of the cyclin D1 and c-Myc proteins. The study demonstrated the potential of artemisinin derivatives in the treatment of prostate cancer and its metastasis, particularly in patients with resistance to hormone therapy.

In 2017, another study looked into the treatment of metastatic prostate cancer that is resistant to anti-androgenic treatments. In a PCA 22 RVL cell model, in vitro and in vivo, artesunate in two daily doses shows a regression of tumor cells and a significant decrease in bone and lung metastasis cells. Androgen receptors are significantly decreased. It is also found that artesunate suppresses the growth of cancerous and metastatic prostate cancer cells by inhibiting the expression of androgen receptors and reducing the secondary expression of the serine protease TMPRSS2.

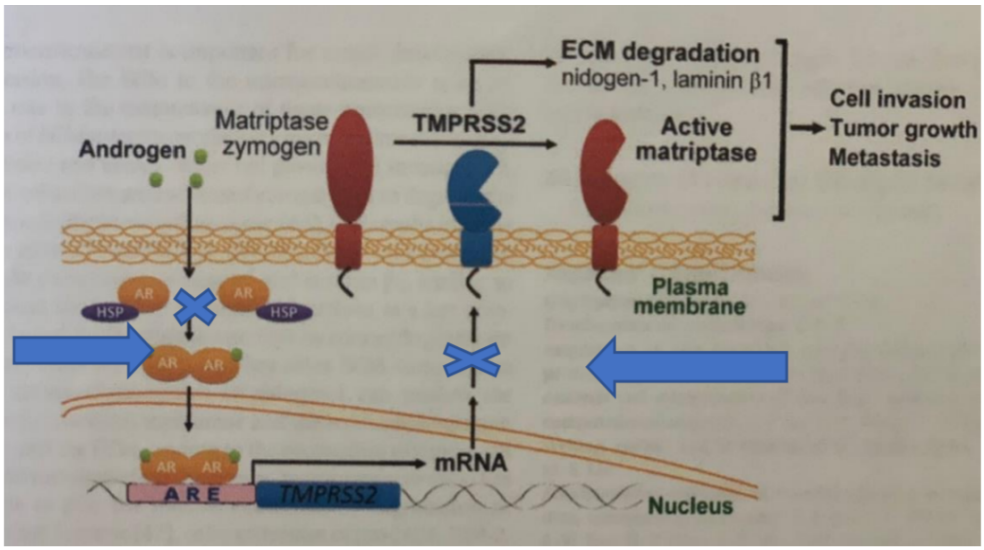


Figure 4 : action of artesunate (blue arrow) : inhibition of the expression of the serine protease gene TMPRSS2 via its inhibitory action on androgen receptors (AR)

Zhenzhong Wang, Chao Wang, Ziyu WU, JUN XUE, Baixin Shen, Wei Zuo
**Artesunate Suppresses the Growth of Prostatic Cancer Cells through Inhibiting Androgen Receptor**
Biological and Pharmaceutical Bulletin, 40, 479-485 (2017)

Nunes JJ, Pandey SK, Yadav A, Goel S, Ateeq B.
**Targeting NF-kappa B Signaling by Artesunate Restores Sensitivity of Castrate-Resistant Prostate Cancer Cells to Antiandrogens**
Neoplasia. 2017 April ; 19(4): 333-345

Colm Morrissey, Byron Gallis, Jeffrey W, Solazzi, Byung Ju Kim et al.
**Effect of artemisinin derivatives on apoptosis and cell cycle in prostate cancer cells**
Anti-cancers Drugs 2012 April; 21(4) : 223-232.

The anticancer activity of *Artemisia annua* is not limited to artemisinin but also extends to its derivatives such as artemisitene, arteannuin B, dihydroartemisinin, but also to other biomolecules such as scopoletins, 1,8-sineols, coumarins and flavonoids. It has been found that the resistance of cancer cells to one component of the plant does not imply resistance to another of its biomolecules.

Thomas Efferth, Florian Hermann, Ahmed Tahrani Michael Wink
**Cytotoxic activity of secondary metabolites derived from *Artemisia annua* L. towards cancer cells in comparison to its designated active constituent artemisinin**
Phytomedicine, 18 (2011) 959-969.

In humans, in view of the convincing results of in vivo and in vitro studies, there are a few reported cases of long-term treatment with *Artemisia annua* in capsule form in combination with short-term treatment with Bicalitumide. A very significant regression of metastases of prostatic carcinoma has been observed. No side effects were observed as with other chemotherapies. Other studies have been carried out on a small number of patients, always with very positive results.

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**Artemisinin and its synthetic derivatives as a possible therapy for cancer**
Medical Sciences 2018, 6, 19

Friedrich Wilhelm Michaelsen, Mohamed E.M. Seed et al.
**Activity of *Artemisia annua* and artemisinin derivatives, in prostate carcinoma** Phytomedecine 22, (2015) volume 22, issue 14 1223-1231

Dina J. Rassias, Pamela Weathers
**Dried leaf *Artemisia Annua* efficacy against non-small cell lung cancer**
Phytomedicine 52 (2019) 247-253

Laura Mikkonen, Pâivi Pihlajamaa, Biswajyoti Sahu, et al
**Androgen receptor and androgen-dependent gene expression in lung**
Molecular and cellular endocrinology, volume 317, issue 1-2,;14-24 (2010)

## Conclusion : *Artemisia annua* a plant to be tested in COVID-19 infection

If artemisinin, dihydroartemisinin and other sesquiterpene derivatives have anti-androgen receptor activities on prostate cancer cells, and inhibit the expression of the serine protease TMPRSS2 particularly in prostate cancer, they could also do so in COVID-19 infection on cells expressing this protease via their androgen receptors.

*Artemisia annua* has already been proven in vivo, in vitro and in clinical studies in prostate cancer, but also in vivo for pulmonary adenocarcinoma, two organs where cells with androgen receptors are found, by suppressing the expression of the membrane serine protease TMPRSS2. In the latter case, its in vitro activity is superior to that of artemisinin alone.

*Artemisia annua* would therefore be a therapeutic, accessible and inexpensive prospect for the management of COVID-19 infection where these same physiopathological mechanisms are found. Since the doses used in prostate cancer are high, it is justified to increase the doses of *Artemisia annua* in the herbal tea compared to the normal doses used in the treatment of malaria.

Furthermore, *Artemisia annua* is also rich in biomolecules that act powerfully on the body's adaptive immune response and would complement the inhibitory action of artemisinin and its derivatives on the COVID-19 virus receptor. Preventive therapy could down-regulate the expression of androgen receptors, thereby protecting patients during a pandemic. These data have yet to be confirmed by a properly conducted randomized clinical trial and biomolecular studies.