Medical Research Archives

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OPEN ACCESS

Published: July 10, 2023

Citation: Pak VN, 2023. Alpha-Fetoprotein: A Revolutionary Anti-Cancer Drug, Medical Research Archives, [online] 11(7). https://doi.org/10.18103/mra.v11i7.4125

DOI
https://doi.org/10.18103/mra.v11i7.4125

ISSN: 2375-1924

RESEARCH ARTICLE

Alpha-Fetoprotein: A Revolutionary Anti-Cancer Drug

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ABSTRACT

Alpha-fetoprotein is an oncofetal protein the embryo produces during fetal development. The protein serves two critical functions simultaneously: it delivers nutrients to growing embryo cells and immature myeloid-derived suppressor cells, so the mother's immune system doesn't attack the embryo. The protein is present in minuscule amounts in adults and elevated alpha-fetoprotein levels serve as pregnancy or tumor markers. Exogenous alpha-fetoprotein has a new application as an immunotherapy drug. It can deliver drugs in a natural shuttle manner to myeloid-derived suppressor cells and stimulate them to calm the hyperactive immune response during many physiological and pathological conditions. On the other hand, alpha-fetoprotein loaded with toxins kills myeloid-derived suppressor cells and unleashes natural killer cells and cytotoxic lymphocytes to erase cancer. Most cancers have cells that specifically bind alpha-fetoprotein, and this protein targets chemotherapy to them also. So, alpha-fetoprotein with toxins combines both potent cancer immunotherapy and targeted chemotherapy activities. Alpha-fetoprotein can be chemically conjugated with or bind toxins non-covalently. Both preparations have demonstrated superior efficacy and safety compared to chemotherapy alone. Alpha-fetoprotein-toxin immuno/chemotherapy is not personalized. There is no need to preselect patients for cancer treatments as they have elevated myeloid-derived suppressor cell levels. The anti-cancer efficacy of porcine alpha-fetoprotein non-covalent complexes with selected toxins administered orally is a remarkable discovery that needs research. Cancer treatment and prevention are different issues, and they could need different approaches. Alpha-fetoprotein administration with drugs or toxins could be as effective in early cancer and metastasis prevention as mifepristone pills in pregnancy prevention.

Keywords: alpha-fetoprotein, MDSC, pregnancy, embryo toxin, cancer, immunotherapy, targeted chemotherapy, autoimmune diseases, COVID-19, FcRn, mifepristone.

Introduction

Alpha-fetoprotein (AFP) is a major plasma protein produced by the yolk sac and the liver during embryo and fetal life. The protein is thought to be the fetal counterpart of serum albumin, and the AFP and albumin genes are present in tandem in the same transcriptional orientation on chromosome 4. AFP stimulates cell divisions and differentiation by transporting molecules in a shuttle manner intracellularly via the AFP receptor (AFPR). AFP controls and enhances the genetic program realization by up-regulating the expression of the proteins in the AFP-binding cells and dramatically influences their functional activity and metabolism.[1–3] AFPR is a glycosylated protein, it is maximally expressed in immature, undifferentiated fetal cells and tissues, as well as in most cancer cells.[4] A putative 65 kDa AFPR was isolated[5], although it remains uncharacterized. The AFP theme is well-covered in the literature.[6–10]

Mothers' myeloid-derived suppressor cells (MDSCs) are AFPR-positive. MDSCs are small heterogeneous cell populations of immature myeloid cells that profoundly suppress natural killer (NK) cell– and T cell–mediated antitumor immunity and exert robust immunosuppressive functions. MDSCs consist of two major subsets: monocytic MDSCs (M-MDSCs), and granulocytic MDSCs (G-MDSCs).[11] MDSCs in pregnancy play a critical role in a balanced immune system at the feto-maternal interface.[12] They facilitate maternal-fetal immune tolerance.[13] Regulatory T cells (Tregs) also have a tolerogenic function in pregnancy and cancer.[14] Nevertheless, Tregs are subordinated to monocytes/macrophages, which are more potent than lymphocytes in immune suppression.

MDSCs play an orchestrating role in pregnancy, cancer as well as in disease settings such as autoimmunity, transplantation, bacterial, viral, and parasitic infections, sepsis, obesity, trauma, stress, vaccination, and aging which elevate MDSCs levels.[15,16]

The immune response can be adjusted by three major players: MDSCs, AFP, and AFP-binding drugs. AFP with an anti-inflammatory drug is beneficial in settings where cellular immunity is hyperactive. On the other hand, AFP-toxin complexes or AFP-toxin conjugates serve as targeted chemotherapy and targeting MDSCs - as cancer immunotherapy. Cancer immunotherapy harnesses the power of the immune system to recognize and attack cancer cells. Immunotherapy is better tolerated than chemotherapy and radiation therapy. It provides long-term protection due to the immune system memory that allows it to recognize and attack metastasis.

MDSCs are naturally generated in the bone marrow from hematopoietic stem cells or can be of exogenous origin. They are being exploited as therapeutic agents to reduce damaging cellular immunity. Potent AFP-binding drugs are available. Recombinant AFP is going to be a revolutionary immunotherapy drug registered soon. All can be used for the treatment of autoimmune diseases (ADs) and cancer.[17]

Alpha-fetoprotein

With a minimal amount (< 0.2 μg/mL), AFP normalizes immune system responses so the mother's immune cells don't attack the embryo. It is most surprising when the embryo is an alien to the surrogate mother. The power of the immune response is regulated by monocytes/macrophages. AFP did selectively induce a rapid downregulation of the MHC class II antigens of monocytes.[2] AFP suppresses immune responses via intrinsic factors (e.g., the abundance of different isoforms, glycosylation patterns, etc.), as well as extrinsic factors such as binding ligands (e.g., various hormones, prostaglandins, fatty acids).[18]

Nutrient delivery from the mother to the embryo is another AFP critical function.

AFP has several glycosylated isoforms. In contrast, porcine AFP (pAFP), which is close to but not identical to human AFP with a high homology of the amino acid structure and similar immunologic properties, has no micro-heterogeneity.[19] Mono-type glycosylated pAFP has both immunosuppressive and nutrient delivery activity. AFP has at least two faces combining immunosuppressive and delivery functions (Fig. 1).

Figure 1. Yanus statue with two faces (Wikipedia).

Though AFP has intrinsic immunosuppressive activity, the numerous ligands, rather than the AFP itself, suppress immune system activity in pigs and humans.

An AFP-mediated supply of essential nutrients elevates the activity of MDSCs. For

example, the administration of polyunsaturated fatty acids (PUFAs) strikingly enhances both the expansion and suppressive activity of mouse G-MDSCs.[20] AFP binds PUFAs metabolites like steroid hormones, leukotrienes, prostaglandins, etc., while prostaglandin E2, a principal mediator of inflammation, is implicated in the promotion of many types of cancer.

Myeloid cells represent the dominant driver of response or resistance to cancer therapy. MDSCs stand out as promising targets for the development of novel immunotherapeutic regimens with superior efficacy. They prevent both innate and adaptive immunity to erase cancer. It is important to find the most efficacious treatment regimens and their combinations.[21-23] Myeloid cell targeting can become a key foundational approach to an overall strategy for improving tumor responses to immunotherapy.[24]

Adjusting the MDSCs activity in cancer and other immune disorders/diseases without unduly comprising any normal physiological function can be done with AFP or AFP with AFP-binding drugs.

AFP as a shuttle

AFP enters the maternal bloodstream and picks from albumin essential nutrients needed by a rapidly growing embryo. AFP (69 kDa) shuttles dozens of ligands (<2 kDa) during its five days of half-life and an hour to unload a ligand in the cell.[25] Monocytes[26] and cancer cells can secrete and absorb AFP-ligand complexes.[27] AFP found in monomeric as well as dimeric and trimeric forms. There are three separate and distinct binding regions for a) retinoids, b) PUFAs and estrogens, and c) dyes, metals, and tryptophan methyl esters.[28] AFP binds a compendium of different ligands by these sites.[29]

Albumin is in a massive excess in the mother's blood (35–55 mg/mL) compared to AFP (<200 ng/mL), and both delivery proteins compete for the surface-bound ligands. The PUFA-binding site is a hydrophobic cavity (Fig. 2) that serves as a natural nano-container hiding inside 1-2 hydrophobic ligands. Albumin cannot extract the ligand from the hydrophobic cavity because of the neutral pH in the blood. Unlike natural estradiol and estrone, AFP strongly binds the synthetic estrogen diethylstilbestrol (DES), which fits the AFP hydrophobic cavity.[30] DES with AFP can cross the placenta and become an embryo toxin/teratogen. Cortisol and dehydroepiandrosterone may bind AFP and adjust the activity of monocytes/ macrophages.[31]

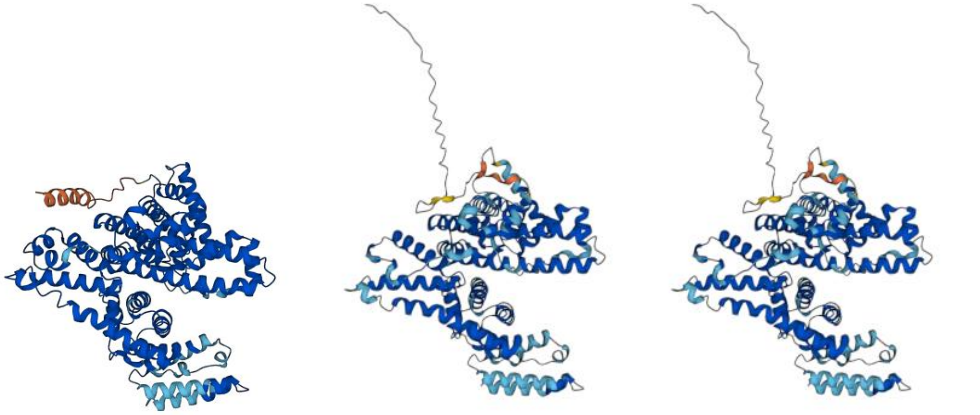

Figure 2. Serum albumin (A), human (B), and porcine (C) AFP 3D structures.[32] The hydrophobic cavity is in the middle of the AFP protein.

Like oxygen binding changes the hemoglobin conformation, PUFAs change AFP conformation[33] and isoelectric point from 5.3 to 4.7 leading to higher complex stability.[34,35] The outside-binding metals can additionally stabilize AFP-ligand complex.[36] However, zinc-binding does not lead to global changes in the AFP structure and stability.[37]

Like hemoglobin, AFP releases the payload inside the cell compartment with an acidic pH and returns for the next delivery round.

AFP bound with a PUFA's metabolite (N-AAP) in a reversible non-covalent complex affected the immune response to sheep erythrocytes in mice. Neither N-AAP itself nor AFP has no such effect.[38]

AFP elevated levels

An AFP level between 0 ng/mL to 40 ng/mL is considered normal for adults. Any level above 40 is considered high, and any level above 400 is considered extremely high, which increases the likelihood of a cancer diagnosis

(hepatocarcinoma, teratoma, and gastric cancer).[39] Some other cancers such as lymphoma, and renal cell carcinoma might also result in high AFP levels.

AFP is intrinsically involved in the overall process of cancer progression from carcinogenesis to metastasis. A single AFP injection into intact mice activated MDSCs and has led to a 20% reduction of NK cells activity. MDSC activity increased when tumor cells were inoculated three days after an AFP injection. In the AFP-treated mice, the tumor mass at day 14 was 60% larger than in the untreated mice.[40]

Recombinant AFP (ACT-101, Alpha Cancer Technologies Inc.) injections did not accelerate tumor growth in immune-compromised animals, but they slightly reduced survival compared to control.[41]

Hepatocellular carcinoma (HCC) is a prevalent disease with a progression that is modulated by the immune system.[42] The 5-year HCC-specific survival of patients not receiving surgery was 14.7% for AFP-negative patients versus 6.1% for AFP-positive patients.[43]

AFP is not just a biomarker for HCC, but also an ardent promoter of liver cancer growth and progression. AFP promotes HCC cell invasion and metastasis via up-regulating the expression of metastasis-related proteins. A high serum concentration of AFP correlated with the metastasis of HCC cells in clinical patients.[44] Elevated AFP levels significantly worse prognosis in fibrolamellar carcinoma patients.[45]

AFP inhibits the apoptosis of AFP-binding tumors, macrophages, and other cells.[46] AFP <0.2 μM concentrations are observed physiologically, while 5–7 μM can induce the apoptosis of cancer cells *in vitro*.[47]

Hereditary persistence of AFP may be found in individuals with no obvious pathology. Elevated AFP levels were stable without liver injury or cancer development. Thus, asymptomatic healthy adults with elevated serum AFP levels (>7 ng/mL) may play a role in expressing a protective phenotype against hepatic steatosis, myosteatosis, and sarcopenia.[48]

During pregnancy, AFP in the mother's blood does not cause cancer, and the cancer risk in pregnant and non-pregnant women is the same. Moreover, in one anecdotal case, early-stage cancer growing in a lady's womb disappeared due to her pregnancy. The pregnancy hormones were inferred to cause this miracle and made the tumor disintegrate.[49] Nevertheless, it could be the synergy action of AFP, AFP-binding pregnancy prevention drugs, or moderate toxins from the food/spices the lady took at that time.

Natural AFP as a drug

Symptoms of ADs: rheumatoid arthritis (RA), inflammatory bowel disease (IBD), Hashimoto disease, multiple sclerosis (MS), myasthenia gravis, and others go into remission during pregnancy. It correlates very well with the rise and fall of the AFP.

AFP purified from the umbilical cord and abortion blood was registered and used as an immune modulation drug in the Russian Federation until it was withdrawn from the market for ethical reasons. Injections of 75±15 μg AFP[50] imitate its blood concentration during pregnancy (75 μg/5 liters of blood = 15 ng/mL). Clinical applications in hundreds of patients demonstrated excellent drug safety without serious adverse events. AFP was used to treat chronic obstructive pulmonary disease, IBD, Hashimoto's disease, hepatitis, and others. Although AFP injections lead to considerable relief from ADs symptoms as in pregnancy, the treatments are conducted in conjunction with other drugs, medications, or supplements. Thus, the doses of corticosteroids were reduced several times, pointing to a positive drug interaction. Or AFP could considerably increase muscle strength in mice due to interaction with steroids naturally existing in the blood. AFP is acclaimed not as an immune suppressor but as an immune regulator.[8] AFP can normalize immune system parameters through M-MDSCs[51] and effector T cells subpopulations ratio.[52]

AFP with rimantadine (173.9 Da) was used to treat patients with hepatitis C.[53] The AFP-rimantadine complex could decrease MDSCs levels which are elevated in chronic hepatitis C virus patients.[54] AFP synergy with interferons was beneficial in hepatitis A[55], B, C[56], D[57], and AIDS treatments.[56]

58 patients with stage III-IV malignancies of different localizations were treated with AFP (4 μg/kg/day, IV injections) for 4-8 weeks. No effect on neoplastic processes was registered in poorly differentiated cell tumors, nor was any antitumor activation of the immune system. On the other hand, in moderately- and high-differentiated cell tumors, several foci of acute immune inflammation were induced. AFP was assumed to induce tumor cell "apoptosis by freeing antigenic determinants from shielding antibodies" via the elimination of immunological enhancement of tumor growth.[58]

There is an alternative to tumor cells' apoptosis explanation for their elimination. AFP monotherapy could only worsen the disease. For the cancer patients' benefit, the treatments conducted with chemo- and other therapies. The high response in patients with high-differentiated cancer cells is a result of AFP synergy with the chemotherapy used in complex therapy. Chemotherapy has a direct cytotoxic effect on cancer cells, and AFP-binding

chemotherapy depletes MDSCs, unleashing both innate and adaptive immunity to erase cancer cells.

The low differentiated tumor cells can secrete/absorb tumor AFP (tAFP), which prevails over injected AFP. Both glycoproteins require PUFAs to potentiate the immunosuppression of monocytes and dendritic cells (DCs). Besides, tAFP serves as a shuttle for immunosuppressive *hydrophilic* low molecular weight (<3 kDa) ligands and directly drives NK cell death.[59] In patients with low differentiated tumor cells, the poor outcome of the treatment determined by MDSCs, tAFP, AFP, and their hydrophobic and hydrophilic ligands and drugs in the tumor microenvironment (TME).

Recombinant AFP as a drug

AFP is a glycosylated protein with 16 disulfide bridges required to make it functional. MM-093 is a non-glycosylated, recombinant version of human AFP that differs from naturally occurring human AFP only in one amino acid substitution at position 233 (glutamine for asparagine). MM-093 (now ACT-101) is simple in design, biodegradable, not immunogenic in humans, and has shown an excellent safety profile at doses much higher than the AFP concentrations in serum during pregnancy. Like the natural AFP, it binds 1-2 hydrophobic molecules and delivers them into the AFPR-positive cells. The recombinant protein bioequivalence allows its use instead of the natural AFP to treat the same diseases.[60]

AFP[61] and MDSCs[62] are crucial players in ADs and have therapeutic potential in those and other therapies. RA tends to remit during pregnancy, with more patients achieving remission in the third trimester, coinciding with an increase in levels of AFP. During the treatment of patients with MM-093 in several Phase I and II clinical trials (RA, psoriasis, and uveitis) no safety-related issues have been observed. 12 patients with RA, who had active disease and were on stable doses of methotrexate, received subcutaneous injections of placebo or 21 mg/week of MM-093 (serum levels 1.3 µg/mL) for 12 weeks and were followed for an additional four weeks.[63] Unfortunately, MM-093 did not show any efficacy in Phase II clinical trials for RA. One of the reasons can be the wrong supporting drug. The patients received methotrexate, which depletes MDSCs[64], while to suppress RA MDSCs should be stimulated. MDSCs reciprocally regulate Th17/Tregs and attenuate inflammatory arthritis via interleukin-10 (IL-10) in mice. MDSCs might be promising therapeutics for ADs including RA.[65] Increasing the population of MDSCs and manipulating their plasticity with microenvironment ligands can become a therapeutic approach for RA treatment.[66] Like in cases with natural AFP, AFP-binding drugs indomethacin or glucocorticoids (dexamethasone, prednisolone) could be used instead of methotrexate. AFP binds indomethacin potentiating its anti-inflammatory activity.[67] Amelioration of RA during pregnancy has a strong hormonal basis.[68] Dexamethasone with its anti-inflammatory and immunosuppressive effects, potentiates MDSCs to achieve immune tolerance in organ transplantation.[69] MDSC numbers were positively correlated with serum IL-6 levels and the glucocorticoid administration index. IL-6 and methylprednisolone enhanced the differentiation of bone marrow cells to MDSCs *in vitro*, and MDSCs may regulate acute transplant rejection.[70] Glucocorticoids promote MDSCs expansion induced by trauma in the spleen, peripheral blood, and bone marrow in a murine trauma model, and MDSCs may be beneficial for the trauma host.[71]

MDSCs play a positive and negative role in regulating the progression of RA, MS, IBD, and systemic lupus erythematosus. In certain pathological conditions, MDSCs act as a "double-edged sword", either favoring disease outcome or exacerbating disease progression. MDSCs promote T cells proliferation and increase the number of Th17 cells, eventually leading to immune imbalance. On the other hand, MDSCs increase the number of Tregs and B cells, thus maintaining immune tolerance.[72] The desired balance can be adjusted by drug delivery to MDSCs by AFP. AFP with glucocorticoids or indomethacin stimulate MDSCs' activity. That establishes MDSC as a potential therapeutic target for immune suppression during ADs, transplantation, trauma, septic shock[73], and other conditions.

AFP or AFP with drugs can normalize the immune response through MDSCs which are "here, there, and everywhere". For example, MDSCs can be pro- and anti-inflammatory in COVID-19. The immunomodulatory activities of MDSCs are governed by the ongoing inflammatory process, and while early MDSCs are pro-inflammatory, late MDSCs may exert tolerogenic effects and contribute to the reduction of inflammation.[74] The good and the paradox of MDSCs[75] activities during COVID-19 could be adjusted by AFP and glucocorticoids or AFP-toxins depending on the stage and severity of the disease.

AFP has a spectrum of activities and a multifactorial mechanism of action. Strong efficacy signals were observed in IBD in animal models as well as in a placebo-controlled Phase II study with AFP.[76] The additional feeding of mice with PUFAs did not increase AFP efficacy. The potent anti-inflammatory drug could be used instead of PUFAs. AFP was proposed as a novel therapeutic agent for IBD.[77]

AFP is the third ligand to the neonatal Fc receptor (FcRn)[78], the key regulator of IgG levels.[79,80] AFP has the potential to interfere with the IgG binding to this receptor, but this is possibly not the major effect because of the IgG and albumin excesses over ACT-101 in the blood (8-18 mg/mL, 35–55 mg/mL, and 1.3 μg/mL, respectively).

AFP should be combined with other therapies as it is safer than standard immunosuppressive therapies and has many mechanisms of action. It can help where steroid-sparing therapy is desired.

So, AFP or AFP-drug is a new potent immunotherapy.

The ^{125}I - labeled AFP is absorbed by tumors in a specific way and can reach 6% of the inoculated amount per 1 g of tumor.[81] AFP conjugates with radioactive isotopes could be helpful in cancer diagnosis (^{89}Zr-ACT-101) and treatment (^{177}Lu-ACT-101), respectively.[60]

AFP-toxin covalent conjugates in cancer treatment

Apoptosis is the first defense mechanism against cancer cells, but it is damaged in cancer patients' cells. To restore the programmed death, AFP can deliver apoptosis inducers inside the cancer cells. AFPR is re-expressed on over 80% of cancers (including colorectal, ovarian, breast, prostate, lung, lymphoma, melanoma, etc.). Differentiated cells have no AFPR. Hence, it is the perfect target for AFP-toxin preparations.

The AFP-toxin drug overcomes multiple drug resistance of cancer cells. While albumin brings the payload to the lysosome for degradation, AFP-toxin is transported directly to the cell's perinuclear compartment, allowing toxins loaded into AFP to bypass the pumps that can move substrates out of cells. This way, AFP-doxorubicin (Dox) conjugate overcomes the multiple drug resistance of cancer cells.[82]

The AFP-toxin conjugates targeted chemotherapy is well-covered in the literature.[83-87]

The immune system is external to the cancer cells defense mechanism. MDSCs are "more equal than others" as they orchestrate innate and adaptive immune systems cells. They are the "diamonds" of cancer therapy.[88] Myeloid cells are compromised in cancer patients and should be eliminated.[89,90]

MDSCs are recruited by solid tumors to shield them from recognition and attack by the immune system like they protect the embryo during pregnancy. The depletion of MDSCs may cause pregnancy loss via upregulating the cytotoxicity of decidual NK cells.[91] Similarly, MDCSs' depletion can erase cancer.

MDSCs are the major tumor-induced negative regulators of cancer immunity.[92] AFP-daunorubicin conjugate has shown a 50% reduction of MDSCs *in vitro*. The conjugate also selectively eliminates MDSCs and inhibits tumor growth in mice model.[93] Depleting MDSCs activates the immune system and increases the efficacy of targeted chemotherapy. Thus, the depletion of MDSCs and tumor cells for cancer chemoimmunotherapy[94] overcomes Tregs-depleting for cancer immunotherapy because it not only causes apoptosis of cancer cells but also regulates the TME to ultimately enhance the antitumor effect of cytotoxic lymphocytes (CTLs) through MDSCs depletion.[95] AFP-toxin selectively destroys MDSCs and cancer cells while sparing normal cells. It is the perfect combination of the most potent cancer immunotherapy with the best-targeted chemotherapy.[96] The result is a dual-pronged therapy with targeted lethality.

Immunotherapy overcomes targeted chemotherapy in efficacy. Thus, vaccination with AFP and other oncofetal proteins demonstrated a 77.1% 5-year and a 65.4% 10-year survival rate in cancer patients.[97] While targeted chemotherapy is supposed to kill 100% of cancer cells, depletion of only a fraction of MDSCs unleashes numerous NK cells and CTLs that effectively erase cancer cells and generate memory cells.

AFP-maytansine (1:5.96) conjugate (ACT-903) demonstrated 100% survival in immune-deficient NCr-nu/nu mice tumor xenograft models. A significant reduction of tumor burden compared to control was achieved in the 40 and 50 mg/kg dose groups. Maytansine is 1000-fold more potent than Dox and has been clinically validated, including the approved antibody-drug conjugate Kadcyla®. The conjugate is stable in circulation with no signs of toxicity.[41] Observed efficacy and excellent tolerability of ACT-903 in the ovarian xenograft models, consistent with prior research using colorectal xenograft models, support advancing its development toward clinical use. The NCr-nu/nu mice have no adaptive immune system. In non-immunocompromised cancer patients, the treatment efficacy is expected to be even more impressive with 3-10 times lower doses of ACT-903.

On the other hand, immunotherapy with AFP-toxin should be cautious of agranulocytosis.

AFP-toxin non-covalent complexes in cancer treatment

AFP could worsen cancer, so conjugates of AFP and its derivatives with toxins are assumed to be preferable in cancer therapy.[98] Nevertheless, the delivery of drugs in a way that is right for the patient—safe, painless, reliable, targeted,

efficient, and cost-effective—can be completed with a full-length AFP. AFP can serve as a natural shuttle delivery vehicle.[99]

The MCF-7 human breast cancer cells have 2,000 AFPRs with high binding affinity and 135,000 with low binding on their surface. The AFP binding was inhibited by 50% in the presence of a 5,000-fold excess of albumin. Competition by other serum proteins was not significant. At 37°C, AFP was endocytosed, and the uptake curve reached a plateau after 3-4 hours of incubation.[100]

The AFP's natural ability to shuttle PUFAs to AFPR-positive cells has been used to treat hepatoma-bearing rats. Conjugated with daunomycin (daunorubicin), PUFA retains a strong AFP-binding ability. Like Dox, daunomycin does not bind AFP, so it was first conjugated with PUFA. Mice with AFP-producing hepatoma cells were injected with PUFA-daunomycin conjugate. AFP bound conjugate in the blood, delivered it to hepatoma cells, and demonstrated a high anti-cancer activity.[101]

AFP binds streptomycin and phenytoin and does not bind acetazolamide, tetracycline, and amethopterin.[102] Cyclophosphamide, Dox, 5-FU, bleomycin, vincristine, and etoposide do not bind AFP and may be given safely to a woman in need during any trimester of pregnancy, as they do not hurt the child or the mother.[103] On the other hand, prescribed drugs with embryo-toxic or teratogenic properties can be repurposed for cancer treatments. A registered drug-data package can shortcut the clinical trials of AFP with those drugs and accelerate regulatory approval.[104]

The drugs be preferably potent toxins with known mechanisms of action, as well as non-mutagenic, non-carcinogenic, chemically stable, with analytical assay developed, and cheap, etc. DES or dioxin[105] are mutagens and carcinogens; hence, they are not recommended.

The HPLC of the AFP-thapsigargin (TG) (1:2) complex and the elution times of some of the drugs, toxins, and PUFAs are in Fig. 3 (unpublished).

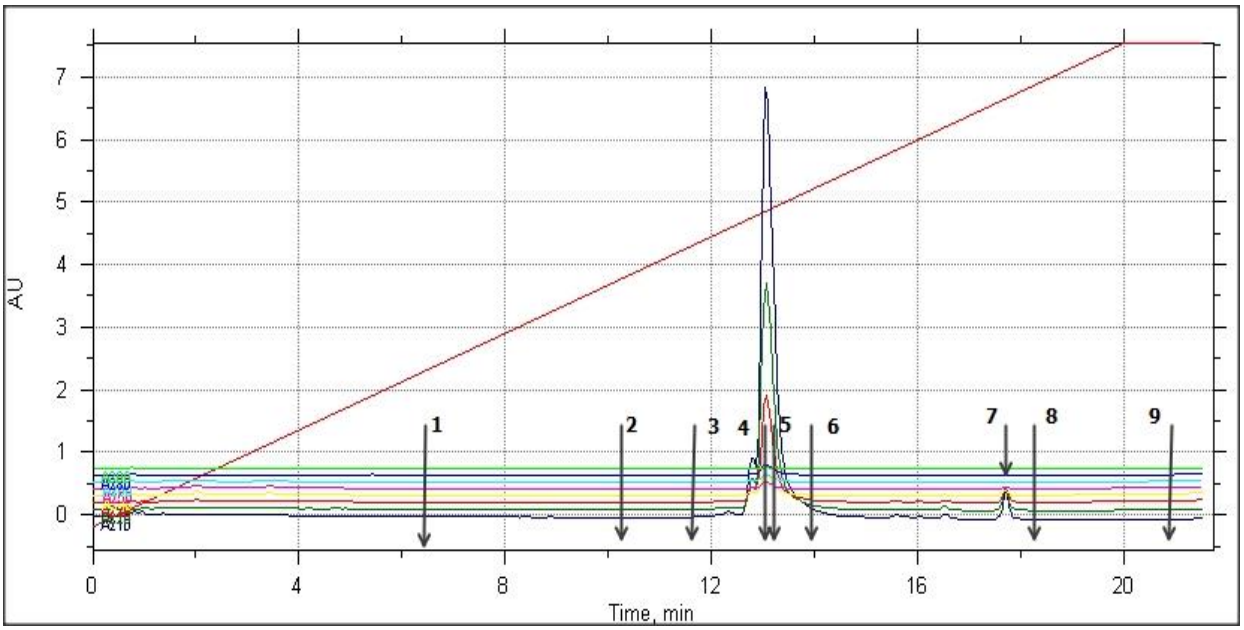

Figure 3. Reverse-phase HPLC of the AFP-thapsigargin (1:2) complex (peaks 4 and 7, respectively). The numbers mark the positions of hydrophobic agents in similar HPLC conditions[106]: 1) atractyloside, 2) ajoene, 3) amphotericin B, 5) paclitaxel, 6) rotenone, 8) betulinic acid, 9) PUFAs (docosahexaenoic acid, eicosapentaenoic acid).

An anti-fungal polyene antibiotic amphotericin B can bind AFP before and after the injection. It has a half-life time of 48 h in the blood, and it was injected in excess to be shuttled by AFP during cancer treatments. 1–2 μg/kg AFP and <15 mg of amphotericin B in a course of ten infusions (one in three days) lead to the tumor and metastases mass decrease rate that significantly exceeded the known effects of conventional poly-chemotherapy. Infusions were accompanied by a chill and a fever, which were counteracted by medication in thirty minutes. AFP and amphotericin B infusions demonstrate response in six out of eight cancer patients and increase the quality of life for patients with a distributed tumor process.[107] AFP-amphotericin B could not have directly affected a lot of cancer cells. First, because of the low AFP

dose, and second, poor toxin (IC_{50} = 1 mM) amphotericin B.

AFP-toxin plays a dual role in cancer treatment: first and mainly, as MDSC-depleting immunotherapy and, second, as cancer cell-targeted chemotherapy. The supporting facts are a) the treatment with AFP-amphotericin B decreased the blood population of monocytes, which was restored with GM-CSF injections later; b) a chill and a fever are the results of the cytokine-release syndrome. The cytokines release enables the immune system to fight cancer[108]; c) the response lasts up to three months after one month of treatment, which is the continuing activity of the immune system.

Paclitaxel in low non-cytotoxic concentrations (1 mg/kg weekly × 3) decreased the accumulation and immunosuppressive activities of MDSCs in mice. It has also reversed immunosuppression and chronic inflammation.[109] In the complex with AFP, paclitaxel becomes soluble. It also obtains a longer lifetime in blood circulation and can be delivered mainly to AFP-binding cells.[110] AFP with paclitaxel has higher anti-cancer activity than paclitaxel alone.

Relatively low doses of chemotherapy induce the depletion of MDSCs.[111] These chemotherapies diminish MDSC-related immune suppression and promote the efficacy of other therapies. Cancer immunotherapy with paclitaxel, 5-FU, tadalafil, gemcitabine, cisplatin, and other substances have been proposed in clinics.[112] Water-soluble methotrexate, 5-FU[113], and Dox[114] can deplete MDSC. They do not bind, but in synergy with AFP demonstrate anti-cancer activity. Thus, a single injection of AFP and Dox demonstrated a significant enhancement of the survival rate and effectiveness in mice, resulting in complete remission in >40% of animals compared to monotherapy with Dox.[115]

An AFP-toxin complex (1:2) or AFP can be injected, while AFP-binding toxins can be administered through injection, orally, or by other methods. AFP delivers 1-2 hydrophobic drugs first and as an "oncoshuttle"[116] - dozens next. In summary, AFP will deliver more than 1-6 toxins carried by AFP conjugate.

The lack of overall toxicity, immunity depression, hemopoiesis suppression, and fast tumor and metastases reduction indicated good perspectives for AFP with AFP-binding toxins therapy.

AFP-toxin non-covalent complexes versus AFP-toxin conjugates

AFP is a low immunogenic protein, especially in physiological concentrations. The immunogenicity of the AFP-toxin complex remains as low as that of the AFP itself. A hydrophobic drug makes the tertiary structure of AFP rigid and stabilizes their complex in the bloodstream. AFP positively alters the pharmacokinetic profile of a drug which does not change the natural pharmacokinetic profile of AFP. Complex hide 1-2 drugs in the hydrophobic cavity, making drugs invisible to the immune system while conjugate exposes drugs on the AFP surface. Conjugate delivers a maximum of 6 drugs/run, while AFP can shuttle dozens of them, like PUFAs in pregnancy. Complex does not need sophisticated linkers, making manufacturing cheap and straightforward.

On the other hand, a conjugate is stable all the way, while complex can dissociate in the acidic TME. Nevertheless, complex depletes MDSCs in the blood and can prevent early cancers and metastases where TME is not established yet.

Oral porcine AFP-toxin complexes

Oral drug administration has advantages over injectables, but it is not often feasible because the bioavailability of protein pharmaceuticals is very low.[117] AFP is resistant to blood proteases, and it is somewhat resistant to trypsin proteolysis. AFP was manufactured in pills[118], but without supporting drugs any effects are questionable.

Porcine protein is more affordable than human one. PAFP is a better delivery protein than AFP as it delivers nutrients through six cell layers of the porcine epitheliochorial placenta. AFP must cross only three cell layers (trophoblast, embryonic connective tissue, and embryonic capillary endothelium) in the human hemochorial placenta. Interestingly, the AFP-prostaglandin E2 complex with a compact structure moves faster than smaller albumin (66.5 kDa) during electrophoresis in 12% PAAG.

Natural products isolated from plants are an important source of chemotherapeutics against cancer. However, natural substances cannot be used directly as drugs, either because they have low solubility or fast metabolism. Genistein, curcumin, artemisinin, and resveratrol administered in oil forms for better absorption had a stronger anti-cancer potency.[119] Optimization of the properties of natural phytochemicals can be done by binding them with AFP. Thus, injections with curcumin and genistein have demonstrated elevated anti-cancer properties after binding with AFP.[115] AFP potentiates the anti-cancer effect of acetoxychavicol acetate.[120] The excess of agents could enhance the anti-cancer effect safely.

Oral pAFP-toxins were used in cancer treatments instead of injections. Mice with tumor xenografts were gavage with pAFP bound in a 1:2

molar ratio with atractyloside (ATR), TG, betulinic acid, rotenone, ajoene, tocotrienol, cholecalciferol, isotretinoin, resveratrol, or PAC-1.[121]

Zinc-finger proteins are involved in all the principal pathways of cancer progression, from carcinogenesis to metastasis formation.[122] PAC-1 (EC_{50} = 0.22 μM) grabs zinc ions from caspase 3 and induces apoptosis.[123] On the opposite, AFP with fifteen suitable binding sites for zinc ions can bring them into the cell, prevent caspase 3-dependent apoptosis induced by PAC-1, and stimulate zinc-finger proteins. Possibly that is why the pAFP-PAC-1 complex did not demonstrate anti-cancer activity in mice.

Mitochondria-hitting drugs activate apoptosis. For example, chemically modified lonidamine mitigates lung tumorigenesis and brain metastasis.[124] An alternative way is to bind hydrophobic lonidamine with pAFP.

Rotenone (IC_{50} = 0.8-4.0 nM) acts on mitochondrion and induces oxidative stress and apoptosis.[125] In addition, rotenone, like paclitaxel, inhibits microtubule assembly by binding to tubulin leading to the inhibition of cell proliferation. Rotenone is an AFP-binding teratogen that has shown anti-carcinogenic activity in several studies.[126] Mice gavage with pAFP-rotenone complex (1:2) inhibited tumor growth. The treatment, combined with injections of cisplatin, increased mortality in mice.

TG is a potent cytotoxin isolated from traditional medicine *Thapsia garganica* over forty years ago. TG induces apoptosis in a proliferation-independent manner by releasing Ca2+ from the ER stores into the cytoplasm. In the NCI 60 Cancer Cell Line screen, TG has a $GI_{50} = 10^{-10}$ M, beating paclitaxel (10^{-8} M) and Dox (10^{-7} M) in this assay. A barrier preventing the direct usage of TG as an anticancer agent is its lack of selectivity since TG kills not only cancer but also normal cells. Several TG conjugates through chemical modifications became an anti-cancer drug.[127] An alternative approach is AFP-TG (1:2) non-covalent complex (ACT-902).

ACT-902 is more stable than AFP-paclitaxel complex due to TG's higher hydrophobicity (Fig. 3). ACT-902 depletes MDSCs and tumor-associated macrophages *in vitro*. 5 out of 6 tumors treated with ACT-902 show complete regression of tumors by day 7 of treatment. The ACT-902 injections (0.15 mg/kg) demonstrated superior efficacy and safety compared to chemotherapy alone.[60]

PAFP-TG (1:2) non-covalent complex administered orally have demonstrated tumor inhibition in mice[121] and humans (unpublished). So, the outcome of the ACT-902 treatment could be enhanced by an additional TG or other toxins for AFP shuttling. The daily oral dose of TG in mice, which at 30 ng is a fraction of the reported oral (in PCT WO2003/049717) or parenteral doses given to mice, was used for the treatment of virus infection without the inherent problem of drug resistance.[128]

The efficacy of the tumor growth inhibition with AFP-toxin (1:2) gavage in mice models correlated with the dose and the toxin potency: TG, ATR, rotenone>betulinic acid, ajoene>others. The addition of betulinic acid or ajoene leads to stronger tumor growth inhibition.[121]

An oral traditional medicine Impila contains a potent mitochondrion toxin and apoptosis inducer ATR.[129] Sixteen patients with colon, stomach, breast, and liver tumors took two capsules/day of 0.3 mg pAFP-0.006 mg ATR for one month. At the end of their treatment, the Karnofsky Index (improved quality of life, pain reduction, increased mobility, and stopped weight loss) rose on average to 20%. No significant side effects were registered.[121]

Twelve patients with liver metastatic colorectal cancer (mCRC) received two capsules (0.3 mg pAFP-0.006 mg ATR)/day as a monotherapy. CT scans before and after eight weeks of treatments with suboptimal doses of pAFP-ATR showed a response to the treatment in six patients. Two of these six patients had a full reduction of small metastases, one patient had a 73% reduction in the size of his metastases, and three patients stabilized. Two patients have been alive for more than five years after treatment, while the median survival rate for mCRC patients is nine months. PAFP-ATR improves the quality and the longevity of lives of cancer patients.[130]

An ovarian stage IV cancer woman took daily capsules with 6.0 mg pAFP-0.12 mg ATR in several courses and survived for over 15 years.

Gavage with pAFP-isotretinoin has shown uncertain anti-cancer activity, and pAFP with methotrexate leads to visible tumor growth inhibition in mice but also increased mortality.

Oral administration dilutes and prolongs pAFP-toxin absorption resulting in mild consequent reactions. Nevertheless, an investigation conducted has shown the pAFP or pAFP-rotenone (1:2) absence in both the free form and in the complex with a toxin in the blood after mice gavage.[132] Rotenone is believed to be moderately toxic to humans with an oral lethal dose estimated from 300 to 500 mg/kg. In safe doses, it could be possibly added to pAFP-rotenone (1:2) oral administration.

Unlike injectable, the oral pAFP-drugs administration cannot provide a direct cytotoxic effect, either on MDSCs or cancer cells. Besides,

MDSCs are rare in the intestinal lymphatic system compared to blood.[133]

Some stomach, colon cancers, and gastric carcinomas produce AFP[134], while a high level of AFPR has been detected in gastric cancers.[135,136] Therefore, oral AFP-toxin formulations can treat at least AFPR-positive cancers in the gut.

FcRn is also a promising target for the oral delivery of CRC therapeutics.[137] An adult human gut contains FcRn-positive enterocytes which specifically bind three delivery proteins: IgG, albumin, and AFP. AFP has a higher than albumin binding affinity to PUFAs[138] and FcRn. IgG-antigen, albumin-nutrient, and possibly AFP-toxin complexes could cross the intestines without dissociation due to FcRn-mediated transcytosis. This way, AFP with toxins can be delivered to monocytes/macrophages in the lymph nodes.

In peripheral lymphoid organs, M-MDSCs differentiate into macrophages and dendritic cells (DCs).[139] FcRn is mainly present in DCs but expressed by monocytes, macrophages, and neutrophils.[140,141] Due to immature cell plasticity, they can also transform into each other. Like MDSCs depletion in the blood, AFP-toxin can deplete FcRn-positive DCs and macrophages in the lymph nodes. It works as an immunotherapy, eventually leading to the destruction of distant cancer. The efficacy of porcine alpha-fetoprotein with selected toxins administered orally is a remarkable discovery that needs research. Interestingly, FcRn is elevated in MDSCs, monocyte, and DCs in pancreatic cancer ductal adenocarcinoma.[142]

Cancer prophylactics can be as simple as pregnancy prevention.

Cancer treatment and prevention are different issues, and they could need different approaches. A healthy lifestyle can prevent cancer incidence partly only, while cancer prevention needs everyone. Early cancer prevention could not need early cancer diagnostics.

Cancer can be viewed as a reversal to an embryonic state. In ancient Rome and Greece, women used an oral contraceptive called silphium to prevent pregnancy. This valuable herb is seen on a coin with a crab that once was a cancer disease name (Fig. 4). It speculated that silphium prevented or treated cancer also.[143]

Figure. 4. A coin of Magas of Cyrene c. 300–282/75 BC. Reverse: silphium and small crab symbols.

Like many traditional medicines, silphium could contain a toxin. More importantly, toxin bound in the blood with AFP is potentiated a hundred times and targeted to MDSCs and embryo cells. MDSCs depletion activates the immune system to prevent early implantation, reject the embryo, and kill embryo cells. Immunology of pregnancy and cancer is similar[97], and this mechanism involving MDSCs, AFP, and AFP-binding toxins can prevent early-stage cancer and metastases.[144]

Currently, mifepristone (RU486) pills substitute an extinct silphium in the early termination of pregnancy. The drug exerts the immunomodulatory, anti-glucocorticoid, and antiprogestin actions. Mifepristone may directly interfere with embryogenesis in addition to endometrial receptivity and embryonic implantation.[145]

Mifepristone regulates macrophage-mediated NK cell function in the decidua. The NK cells' cytotoxicity and migration ability significantly increased by macrophages pre-treated with mifepristone in a dose-dependent manner.[146] NK cells attack low-differentiated embryos, stem, cancer cells, and cancer stem cells.[147] These "spontaneous cytotoxic cells" can erase early cancers and metastases.

Low differentiated cancer stem cells which are close to embryo cells and are responsible for cancer recurrence and metastasis found in many cancers. Mifepristone decreases the growth of cancer stem cells by increasing the miRNA-153 levels.[148] Mifepristone decreases the level of anti-apoptotic protein Bcl-2 and increases the levels of pro-apoptotic protein Bax leading to apoptosis in high-grade gliomas.[149] Mifepristone therapy may provide a method to halt metastatic lung cancer positive for the PD-L1 marker when check-point inhibitors are no longer effective.[150]

Mifepristone can be repurposed to treat breast cancer[151], metastatic ovarian cancer[152],

uveal melanoma cells[153], prostate cancer[154], etc. It is considered a safe drug that, even with prolonged use, has relatively mild adverse effects. Mifepristone is recommended to be used together with other drugs, and AFP should be one of them. A hydrophobic mifepristone (429.6 Da) has a half-life of 18 hours, it binds albumin and should bind AFP/pAFP too. Mifepristone meets the requirements named earlier: it is non-mutagenic, non-carcinogenic, apoptosis inducer, have several mechanisms of action, chemically stable, with analytical assay developed, and cheap.

PAFP-ATR or pAFP-TG capsules were most effective in patients with small metastases. AFP being pre-bound, injected separately, or taken orally (pAFP) with mifepristone is expected to be as effective in early cancer and metastasis prevention as mifepristone pills in pregnancy prevention. PAFP does not need high purity making manufacturing cheap and pAFP-mifepristone capsules affordable for all in need. Early cancer and metastasis prophylactics with oral AFP/pAFP- drugs or toxins (mifepristone, TG, ATR, rotenone, etc.) could be elegantly simple and very close at hand.

Cancer patients have elevated MDSCs levels, and AFP-binding cells are present in most solid and liquid cancers. Hence, AFP-toxin immuno/chemotherapy is not personalized, and there is no need to preselect patients for cancer treatment or prophylactics.

Conclusions

AFP is an immune suppressor and delivery protein that embryo cells secrete to cancel the mother's immune attack. Delivering nutrients, AFP "corrupts" the top regulatory myeloid cells, which suppress both innate and adaptive immunity. This mechanism works not only during pregnancy, but also in cancer, and many other physiological and pathological conditions. For that reason, MDSC is the efficient target for different immunotherapies.

MDSCs play a positive and negative role in regulating the immune response. In certain pathological conditions, MDSCs act as a "double-edged sword", either favoring disease outcome or exacerbating disease progression. The activity or viability of MDSCs can be adjusted by drugs. AFP becomes a revolutionary immunotherapy drug by delivering definite drugs to MDSCs. Thus, AFP with AFP-binding anti-inflammatory drugs can suppress immune reactions in settings where they are hyperactive.

On the other hand, AFP with AFP-binding toxin can treat cancer (Fig. 5).

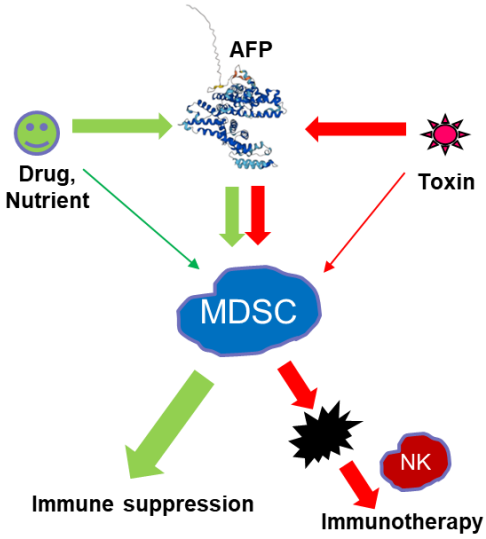

Figure. 5. AFP binds the drug or toxin and stimulate MDSCs for immune suppression or deplete them and unleash NK cells for cancer immunotherapy.

Cancer patients have elevated MDSCs levels, and AFP-binding cells are present in most solid and liquid cancers. For that reason, AFP-toxin is the powerful synergy of the potent cancer immunotherapy with the targeted chemotherapy. The result is a dual-pronged therapy with targeted lethality. AFP-toxin immuno/chemotherapy is not personalized, and there is no need to preselect patients for treatment.

AFP-toxin conjugates are promising anti-cancer drugs. They selectively destroy MDSCs and cancer cells while sparing normal cells.

The discovery of the anti-cancer mechanism of action of pAFP with toxins non-covalent complexes administered orally needs research. Immunotherapy action prevails over AFP-toxin chemotherapy one because the available targets for complexes are dendritic cells and macrophages in the lymph nodes, not distant cancer cells.

Cancer treatment and early cancer and metastasis prevention are different issues, and they could use different approaches. Cancer is a kind of reversal to an embryonic state with a similar immune system adjustment. Mifepristone pills are currently used to prevent early pregnancy. It is inferred that oral formulations of pAFP-mifepristone or mifepristone pills plus AFP injections could prevent early cancer and metastasis. Cancer prophylactics become available to everyone.

The author has no conflicts of interest to declare.

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