

ASTAXANTHIN AFFECTS CANCER AND ELECTRO-CHEMICAL EFFECTS ON CELLULAR STRUCTURES

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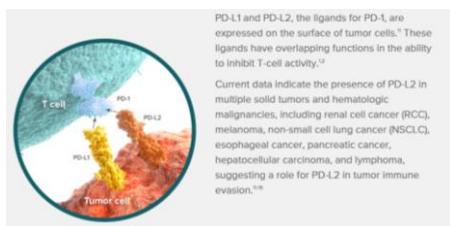
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Abstract #: MLT 17-1093

Published online 9/30/2017

ABSTRACT

The electronic roles of the cell membrane and the electrical charge potential across cell surfaces are extremely important in the health of all cells. All cells have an electrical charge potential across the exterior and interior of the cell membrane. This electrical potential is required in supporting the activation of the sodium (Na⁺)/potassium (K⁺) ion gradient of each cell. Research appears to indicate that the concentration gradients of intracellular sodium, low oxygen levels and potassium affects the electrical potential of a normal cell. These actions transform the cell from an aerobic function to an anoxic function. The biochemistry of anoxic cancer cells favors the expression of excess PD-L1 on the cell membrane. It also appears that the presence of excess H⁺ ions, as measured by a reduction in blood pH, and C Reactive Protein is a possible indicator of inflammation and the transformation of an aerobic normal cell (oxidative) into an anaerobic/anoxic cancerous cell (reductive).



The properties and carboxylic action of ASTAXANTHIN on the active sites on PD-L1, through Hydrogen bonding, have been shown to have a dramatic effect on correcting and maintaining cellular protein structure, cellular membrane electrical potential, inhibition and inactivation of PD-L1 sites and cellular metabolism.

SUMMARY

It is now understood that carotenoids, such as Astaxanthin, play a crucial role in establishing and maintaining the electrical potential across the phospholipid cell membrane through its ability to regulate and neutralize cations. It is this ability that terminates the reductive (anaerobic/anabolic) properties necessary to sustain cancer growth and metabolism.

Astaxanthin is a lipid-soluble keto-carotenoid having a deep red color. The high electronegativity of astaxanthin is a direct result of its hydroxyl and ketone functional groups (carboxylic acid groups) established on both ends of a carbon chain of alternating double bonds. This “dumbbell shaped” molecule consists of a central region of electrons that can be donated or adsorbed to reduce a reactive oxidizing molecule, such as PD-1, PD-L1 and PD-L2.

Astaxanthin, unlike several carotenes and one other known carotenoid, is not converted to vitamin A (retinol) in the human body. Like other carotenoids, astaxanthin has self-limited absorption orally and such low toxicity by mouth that no toxic side effect has been observed.

After vitamin D, astaxanthin is the second-most-important supplement you should be taking. Astaxanthin, as a free radical scavenger/antioxidant, is 400 times more reactive as an antioxidant than beta carotene. Astaxanthin is not only the world’s strongest natural antioxidant, it’s also a safe and natural anti-inflammatory.

Astaxanthin is incredibly potent and well-rounded in its antioxidant activity. As an example, in one very important antioxidant test called “singlet oxygen quenching,” astaxanthin has been shown to be 550 times stronger than vitamin E, 800 times stronger than CoQ10 and 6,000 times stronger than vitamin C. But it’s not only its supreme antioxidant power that makes astaxanthin the most favored antioxidant, it’s also how astaxanthin functions once it gets into the body.

In summary, improved cell membrane electrical potential and membrane capacitance and PD-L1 interference and inhibition, resulting from Astaxanthin use at 4mg/kg, has been shown to affect the following:

1. Conversion of anaerobic to aerobic mitochondrial production of ATP
2. Reduced Hydrogen and Cation concentration and increased cellular pH
3. Stabilized Sodium and Potassium ion concentration gradients
4. Altered cell membrane permeability of sodium and potassium ions
5. Returned cancer cell electrical potential (-18mv) to normal cell potential (-70mv)
6. Reduced inflammation response resulting from large sodium intracellular concentration resulting in water retention of the cell.
7. Maximized production, inhibition and expression of proteins and other macromolecules such as PD-1, PD-L1, PD-L2 sodium and potassium channel proteins and sodium/potassium pump proteins.
8. Improved the absorption of certain nutrients, including essential fatty acids, phospholipids, sterols and nutrients and mineral transporter ions such as sodium, potassium, magnesium and calcium that help normalize intracellular mineral concentration gradients in diseased cells.

The combination of cell membrane repair, inactivation of PD-L1 and PD-L2, increase cellular pH, correction of deficiencies of intracellular mineral concentrations primarily potassium, magnesium, zinc and calcium, correction of excessive intracellular levels of sodium resulted in the overall improvement of cell membrane capacitance and electrical polarization.

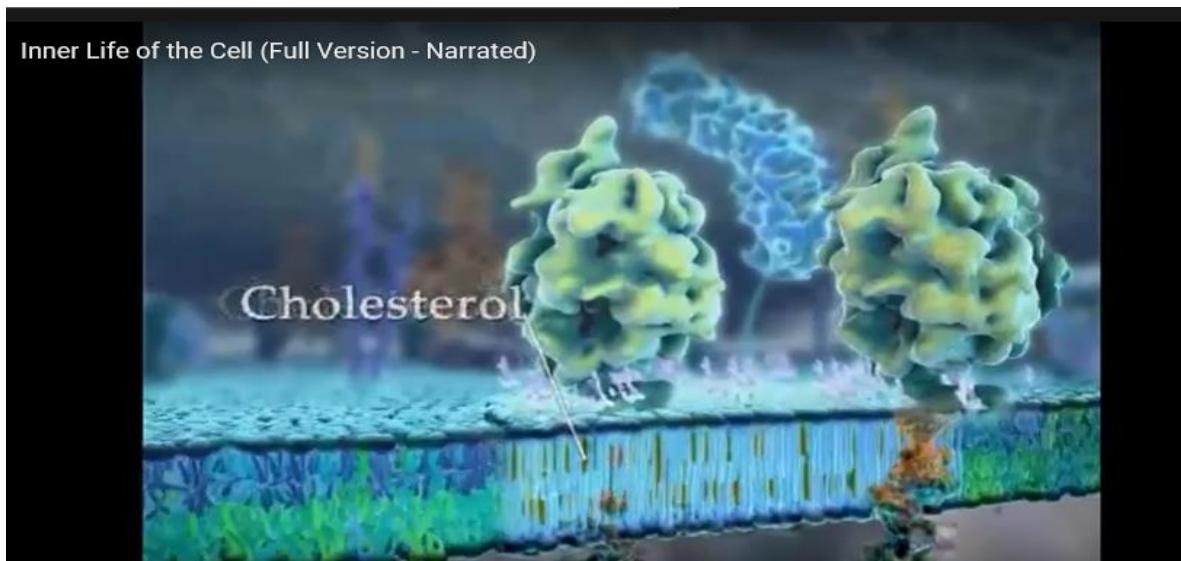
CONCLUSIONS:

1. Increasing Astaxanthin levels in diet is highly recommended as a precancer, cancer and anti-inflammatory prophylactic at levels up to 400mg/day.
2. No discernable side effects of Astaxanthin have been observed at dose levels up to 400 mg/day.
3. Some decolorization of feces was observed (red to orange) due to pass thru of unabsorbed astaxanthin. At first notice, dose levels were reduced by 20% every 3 days until decolorization disappeared.
4. Anti-inflammatory and pain reduction associated with arthritis have been noted within 4 weeks of treatment at >50mg/day.
5. No side effects or skin color changes were observed.

6. High intracellular sodium and low potassium levels were observed in cancerous cells.

INTRODUCTION

Cell membranes are composed of a bilayer of highly mobile lipid molecules that electrically act as an insulator (dielectric).



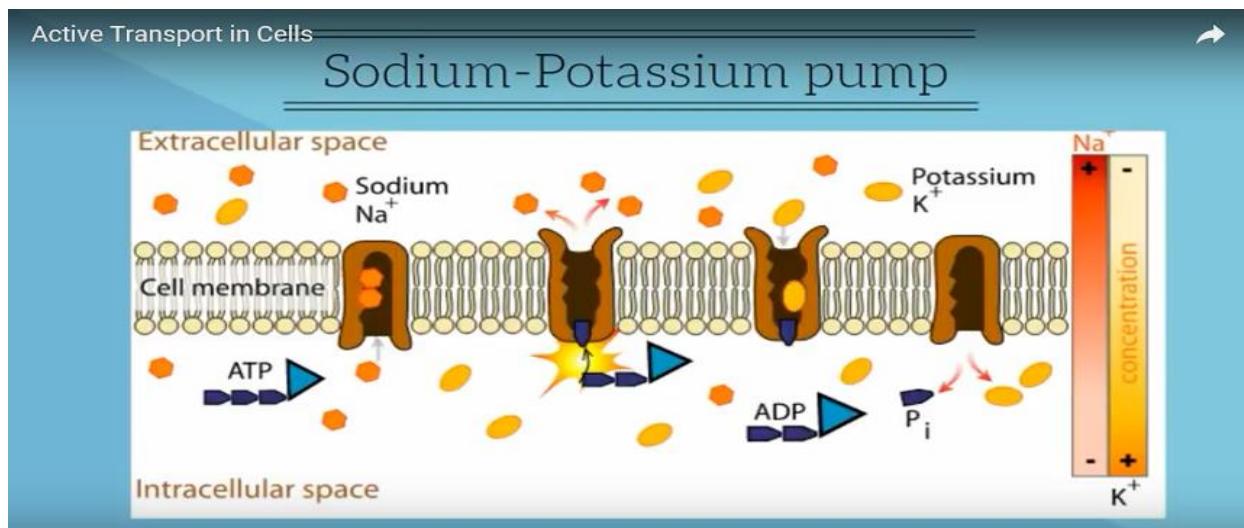
The insulating properties of the cell membrane lipids also act to restrict the movement of charged ions and electrons across the membrane except through specialized protein "gates" known as ion channels (1). Since the cell membrane is selectively permeable to sodium and potassium ions, a different concentration of these and other charged mineral ions will build up on either side of the membrane. The different concentrations and electronegativities of these charged molecules cause the outer membrane surface to have a relatively higher positive charge than the inner membrane surface thus creating an electrical potential across the membrane (2).



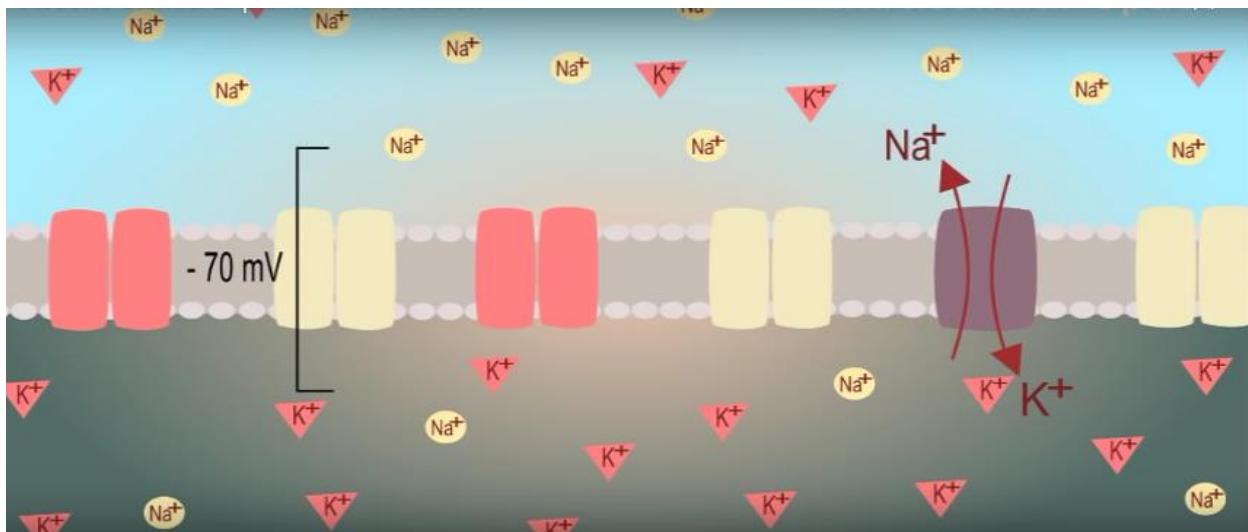
All cells have an imbalance in electrical charge between the inside of the cell and the outside of the cell. The difference is known as the membrane potential. This membrane electrical potential is created by the difference in the concentration of ions (Na, K, Mg, etc.) inside and outside the cell. This electrical potential creates an electrochemical force across the cell membrane. “Electrochemical forces across the membrane regulate chemical exchange across the cell (3).” The cell membrane potential helps control cell membrane transport of a variety of nutrients and is essential in the metabolism and conversion of ADP into ATP.

DISCUSSION

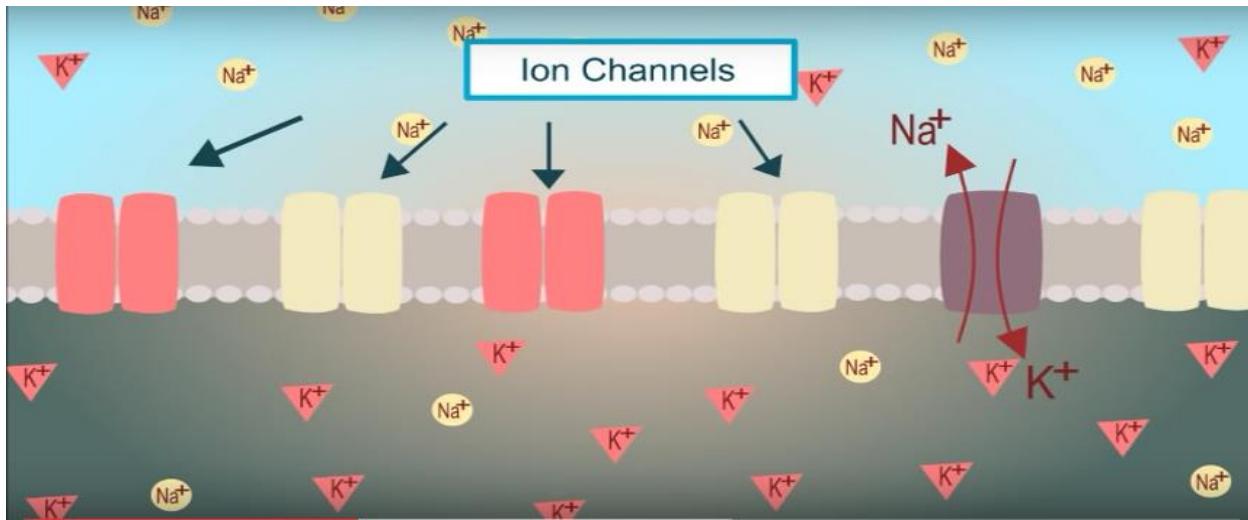
All healthy living cells have a membrane potential of -60 to -100mV. The negative sign of the membrane potential indicates that the inside surface of the cell membrane is relatively more negative than the outside of the cell membrane due to the differing electronegativities of potassium ions and organic proteins.



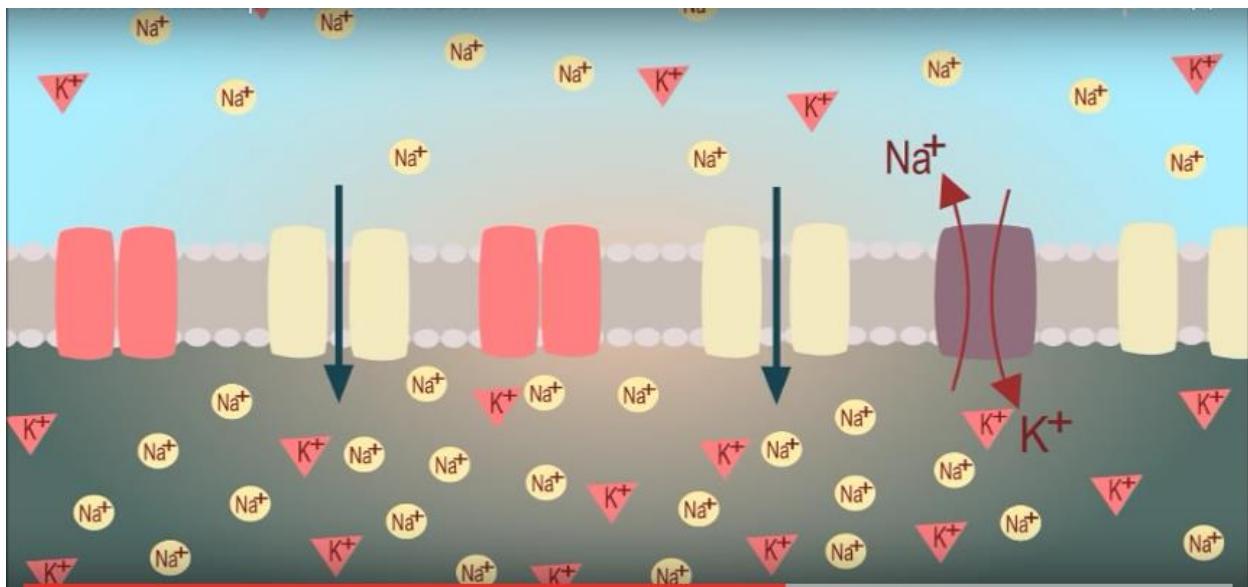
In a healthy normal cell, there are low concentrations of PD-L1/PD-L2 expression, higher concentrations of K⁺ ions and negatively charged proteins on the inside surface of the cell membrane. Therefore, since a high concentration of positively charged sodium ions (Na⁺) are on the outside of the cell, the cell interior is slightly more negative than the outside cell membrane, creating an electrical potential across the cell membrane. This large electrical potential across the cell membrane has been measured at up to -18,000,000 to -23,000,000 volts/meter in healthy cells and 0 to -16,000,000 volts/meter in cancerous / damaged cells.



Healthy cells maintain a high concentration of potassium and a low concentration of sodium inside the cell membrane. What is known is that in cancer, changes in cell membrane structure, changes in membrane function, changes in cell concentrations of minerals, changes in cell membrane electrical potential, changes in the electrical connections within the cells and between cells, and changes in cellular energy production all occur.

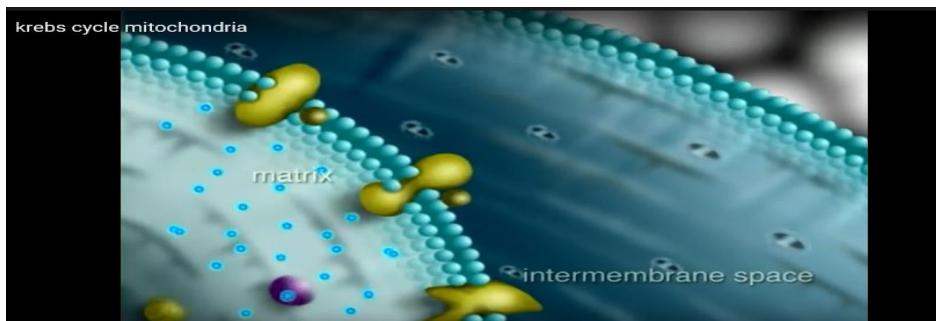


But when cells are injured or cancerous, or during hypoxia, or more sodium leak channels exist or more leak potassium channels are present, or the sodium/potassium pump is ineffective, or the dielectric properties of the phospholipid membrane is compromised, then, when activated, water, hydrogen ions and excess sodium ions flow into the cells and potassium, magnesium, calcium and zinc are lost from the cellular interior and the cell membrane potential falls.



This collapse in electrical potential will convert the cells metabolism from that of oxidative to a reductive metabolism and the cellular pH decreases from 7.35 to as low as 5.8. A reductive anaerobic/anabolic metabolism favors abnormal

cancerous cell growth and cellular inflammation and increased expression of PD-L1/PD-L2 onto the cancerous cell membrane.



Cells have many discrete electrical zones. A cell appears to contain four electrified zones.

1. The central zone contains negatively charged organic molecules and maintains a steady bulk negativity.
2. An inner positive zone exists between the inner aspect of the cell membrane and the central negative zone.
3. The inner positive zone is composed of a thin layer of freely mobile mineral cations particularly potassium and a small amount of calcium as well.
4. The outer positive zone exists around the outer surface of the cell membrane and consists of a denser zone of mobile cations composed mostly of sodium, calcium and a small amount of potassium. Because the concentration of positive charges is larger on the outer surface of the cell membrane than the concentration of positive charges on the inner surface of the cell membrane, an electrical potential exists across the cell membrane.

How can the surface of cells be electrically negative if a shell of positively charged mineral ions surrounds the exterior surface of the cell membrane? The answer lies in the existence of an outer electrically negative zone composed of the glycocalyx. The outermost electrically negative zone is composed of negatively charged sialic acid molecules that cap the tips of glycoproteins and glycolipids that extend outward from the cell membrane like tree branches. The

outermost negative zone is separated from the positive cell membrane surface by about 20 micrometers. According to Charmin, “It is this outermost calyx zone of steady negativity that makes each cell act as a negatively charged body; every cell creates a negatively charged field around itself that influences any other charged body close to it.”

It is the negatively charged sialic acid residues of the cell coat (glycocalyx) that gives each cell its zeta potential. Since the negatively charged electric field around cells are created by sialic acid residues, any factor that increases or decreases the number of sialic acid residues will change the degree of surface negativity a cell exhibits.

Some of the characteristic features of cancerous cells that affect their electrical activity are:

- 1. Cancer cells are less efficient in their production of cellular energy (ATP); metabolically favoring anaerobic processes (without oxygen) over aerobic processes (with oxygen)**
- 2. Cancer cells have cell membranes that exhibit different electrochemical properties and a different distribution of electrical charges than normal tissues with 100 to 1000 times the levels of PD-L1 or PD-L2 expression at the cell membrane**
- 3. Cancer cells also have different lipid and sterol content than normal cells.**
- 4. Cancer cells have altered membrane composition and membrane permeability, which results in the movement of potassium, magnesium and calcium out of the cell and the accumulation of sodium and water into the cell, causing tumor growth.**

The characteristics listed above have one thing in common; they are all the result of an altered membrane electrical charge and increased levels of PD-L1 expression. It is these expressive proteins that appear to suppress T and B cell attack on the cancer cell. Astaxanthin, due to its high electronegativity, and reactive carboxylic sites, inactivates the PD-L1 sites. This inactivation allows for immediate cancer cell lysis from the T cell attack. Due to the elevated level

of expressed PD-L1 sites relative to normal cells, normal cells remain unaffected.

PD-L1 Expression in Cancer

PD-L1 is often expressed within the microenvironment of various tumor types.^{3,10}

Percent of Tumor Samples Expressing PD-L1

| Tumor Type | PD-L1-Positive |
|---|----------------|
| Breast ³ | 31%–34% |
| Colorectal ^{3,10} | 40%–53% |
| Gastric ^{3,10} | 42%–72% |
| Head and neck squamous cell carcinoma (HNSCC) ¹¹ | 46%–100% |
| Hepatocellular carcinoma (HCC) ^{3,10} | 25%–93% |
| Melanoma ^{12,13} | 24%–81% |
| Non-small cell lung cancer (NSCLC) ^{3,14} | 24%–95% |
| Ovarian ³ | 33%–80% |
| Pancreatic ^{15,16} | 15%–39% |
| Renal cell carcinoma (RCC) ^{3,14} | 10%–24% |
| Urothelial carcinoma ^{17,18} | 32%–67% |

It is believed that these cancer cell characteristics can be modified using carotenoid type molecules that increases the pH from <7.0 to 7.2-7.4, eliminates the effects of PD-L1 and reduces the anaerobic metabolism of cancer cells. The most effective carotenoid is Astaxanthin.

Astaxanthin therapy is beneficial in cancer treatment due to its extreme effect on the electrical charge exhibited by the PD-L1, on the cell membrane and, due to the high electronegativity of Astaxanthin, sialic acid residues from cancer cells are removed or reduced due to the Astaxanthin/ sialic acid reactions without any obvious negative side effects to normal cells. This causes the cancer to lose its metabolic functions. This elimination of the electrical charge on the PD-L1 protein and the loss of metabolic function results in apoptosis (cell death) and cell lysis resulting from macrophage and T and B cell specific attack on the cancer cell.

Additionally, Astaxanthin, being electrically negative, will preferentially adsorb or neutralize the cations of hydrogen and sodium inside the cell and increase the concentration of sodium on the outside cell membrane. This appears to return the cell to a more favorable electrical potential which favors apoptosis of the cancer cell. This also makes the cancer cell more vulnerable to macrophage attack due to its altered state from anaerobic to an aerobic state.

Dr. Cure has proposed that the reverse polarization from -70mv to greater than -18mv will depolarize normal cell membranes. He postulates that the

depolarization (fall in membrane potential) of the cell membrane due to the accumulation of excess negative surface charges may precede and create the reduction in intracellular potassium and the rise in the intracellular sodium launching the cell into a carcinogenic state (Cure, 1991). Therefore, it is crucial that the electrical potential be reestablished. The reestablishment of the electrical potential across the cancer cell is achieved by using the high electronegativity of carotenoids such as Astaxanthin and vitamins such as vitamin C and D. The Astaxanthin increases the polarization of the charge of the cell, thereby reducing the electrical abnormalities associated with the cancer cell.

It also means that therapeutic methods including the use of free radical scavengers, such as vitamin C and Astaxanthin will alter the electrical charge of cell membranes, the composition of cell membranes and the content of intracellular minerals resulting in alterations in the metabolic activity of cancer cells and improve the lymphocyte identification and destruction of the cancer cell through hydrogenation or cell apoptosis.

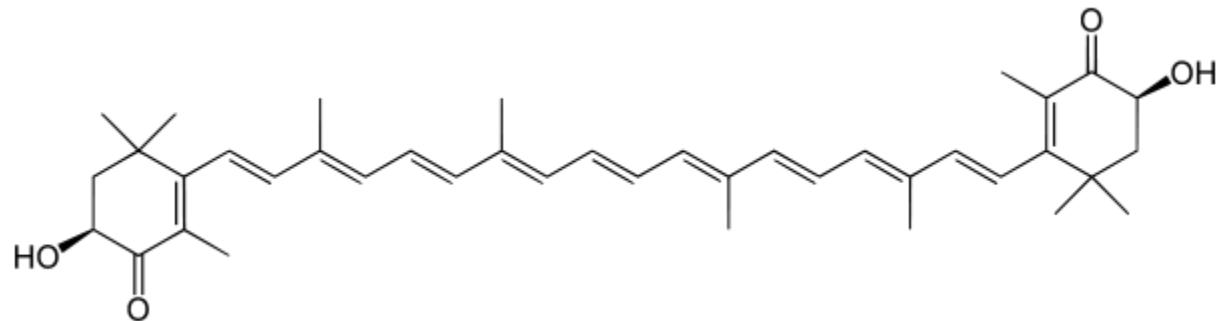
Therefore, the electrical properties of biological tissues are dependent on all the physical and electrical mechanisms which control the mobility and availability of the relevant ions such as expressive proteins, sodium, chloride, potassium, magnesium and calcium (Scharfetter, 1999). The electrical charges associated with semiconducting proteins and extracellular matrix proteoglycans also contribute to the conductivity of a tissue. So, the electrical properties of tissues also relate to electron availability, which can be affected by such factors as the degree of tissue acidity, the degree of tissue hypoxia, the degree that water is structured, and the availability of electron donors such as vitamin C and Astaxanthin, and the presence of electrophilic compounds on the cell membrane and in the extracellular matrix (ECM).

According to Dr. Robert Pekar, "Every biological process is also an electric process" and "health and sickness are related to the bio-electric currents in our body (Pekar, 1997)."

Recognizing that cancer cells have high concentrations of expressed PD-L1 and altered electrical properties also leads to strategies toward correcting these properties.

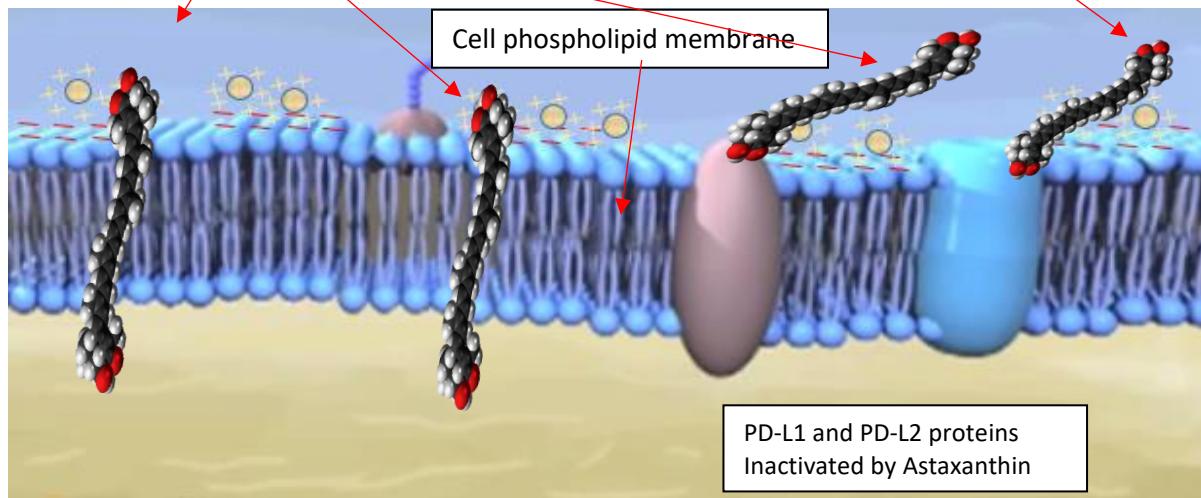
ASTAXANTHIN BIOCHEMICAL PROPERTIES:

The chemical structure of Astaxanthin is:



The ends of the molecule are comprised of OH- and C=O (hydroxide/ketone) functional groups connected by highly conjugated double bonded carbon chain, creating a reactive, electronegative charged molecule, capable of donating electrons and hydrogen bonding. The connecting structure of the lipid soluble molecule is essentially the same length as the width of the phospholipid cellular membrane, thereby residing within the structure of the cellular membrane.

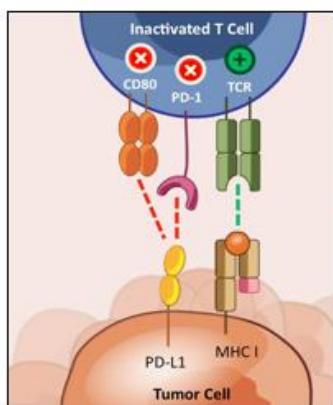
Astaxanthin molecule with high negative charge attracts Na^+ ions and inactivates PD-L1 and PD-L2 ligands



This configuration creates a polarization of the cancer cell, thereby increasing the electrical potential across the cell membrane. This increase in electrical potential causes Na^+ ions to accumulate on the exterior cell membrane, reduces the Na^+ concentration on the inside of the cell and inhibits the functionality of the PD pathway. This returns the cell to normal cell function, decreases intracellular water content, and/or inhibits the T cell inactivation resulting in cell volume reduction and T cell attack on the cancerous cell.

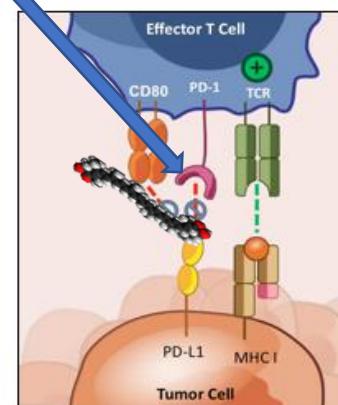
ASTAXANTHIN INTERFERENCE WITH PD-L1

Some Tumor Cells Upregulate PD-L1 to Evasive the Antitumor Response^{1,4,8}



Tumor antigens bound to major histocompatibility complex (MHC) on tumor cells are recognized by T-cell receptor (TCR), which activates T cells.⁹ Binding of PD-L1 to PD-1 receptors on T cells inactivates T cells, which may allow tumor cells to evade the immune response.^{4,8}

Blocking PD-L1 May Promote the Antitumor Response^{4,8}

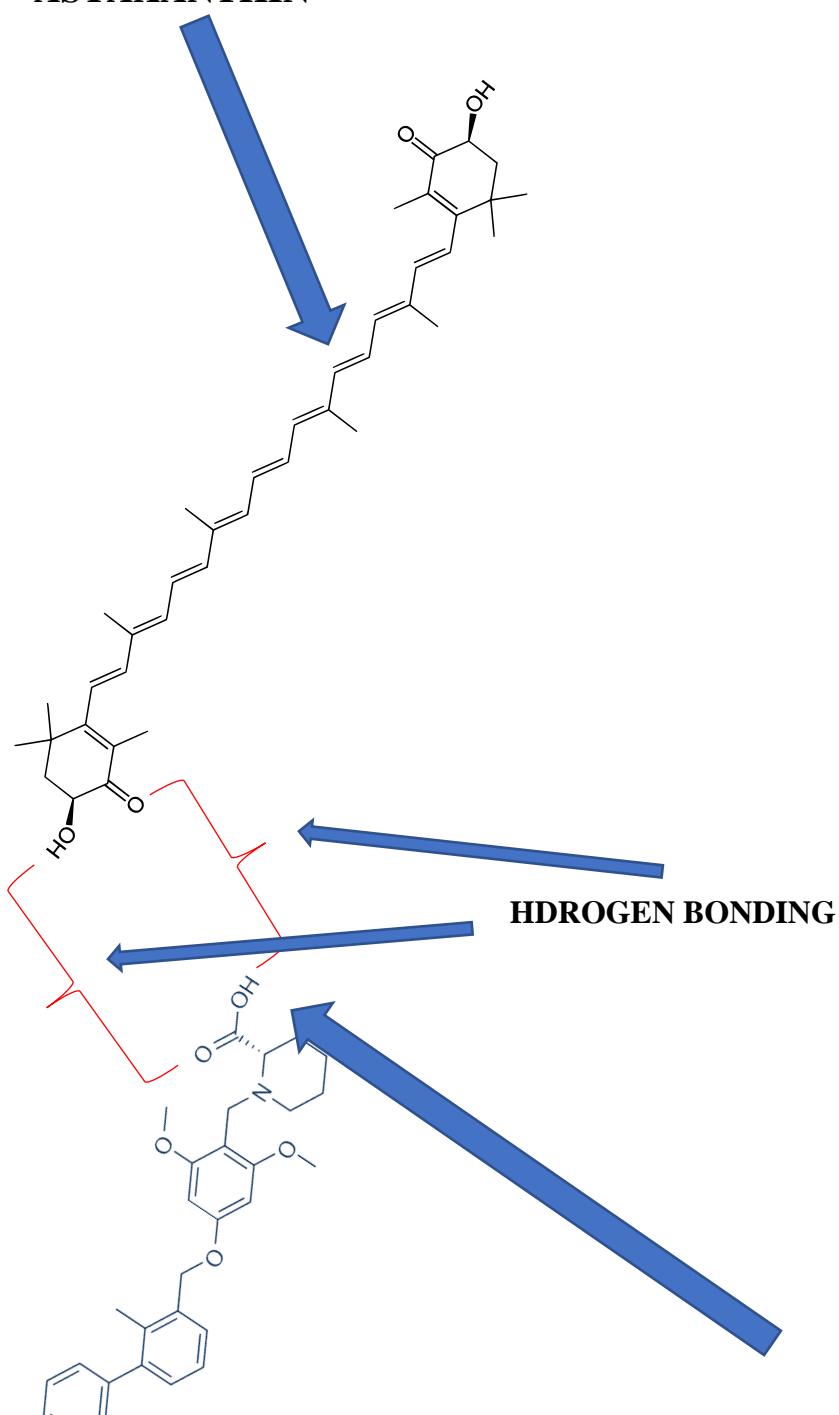


Antibodies that impair the ability of PD-L1 to interact with PD-1 and CD80 may prevent the ability of PD-L1 to suppress T-cell activation, helping to promote the antitumor response.^{1,3,4}

SCHEMATIC 1

ASTAXANTHIN BLOCK AND INHIBITION OF PD-L1

ASTAXANTHIN



SCHEMATIC 2

As indicated by the SCHEMATIC 1 above, the tumor cell expressed PD-L1 combines with the PD-1 site of the T cell, thereby inactivating the T cell. It is suspected that Astaxanthin inhibits the PD-L1 site through carboxylic acid reaction with the functional site of Astaxanthin. SCHEMATIC 2. This reaction stops the binding of PD-1 to PD-L1. Therefore, the T cell remains active to attack the tumor cell. Additionally, Astaxanthin imparts the following effects on the tumor cell:

1. Manipulation of fatty acids and sterols to address membrane composition.
2. Reduces intracellular sodium concentrations, since an intracellular excess of positively charged sodium ions reduces the negative interior potential of the inner membrane surface resulting in depolarization of the cell membrane potential.
3. Increases intracellular delivery of magnesium, potassium and calcium.
4. Removes the sialic acid and excessive negative charges from the external surface of cancer cells (glycocalyx) such as enzymes and electrical treatments. Since an excess of negative charges in the glycocalyx also can reduce the membrane potential of cancer cells.
5. Inhibits and alters electrical charge on PD-L1/PD-L2. More research is needed to determine if Astaxanthin may also play a role in inactivation of PD-1 sites on T cells and activation potential of NK cells.
6. Corrects intracellular, extracellular and membrane measures of the abnormal electrical properties of cancer cells, including a reduction of PD-L1 and PD-1 activity, resulting in the return of the cancer cell to normal cellular function or cellular apoptosis or cellular lysis.
7. Reduces intracellular water accumulation.

There are three distinct characteristics of astaxanthin:

1. Astaxanthin's unique molecular structure and lipid solubility allows for entire body transport and bioaccumulation throughout our bodies, including neurons and cellular structures within the brain, without side effects. Once in the cells, unlike other antioxidants, it protects the entire

cell; astaxanthin can have one end of its molecule on the outer surface of the cell, and the other end in the interior of the cell or can electrically attach to PD-L1/PD-L2 sites parallel or perpendicular to the cell membrane or interfere electrically with the programmed death characteristics of the cell. This full-coverage protection of crossing the phospholipid membrane allows for direct electrical connection between the inside and outside of the cell. This regulates and stabilizes the cellular electrical potential.

2. Astaxanthin crosses the blood-brain barrier, and once there it crosses the blood-retinal barrier. It also acts as a hyperactive anti-inflammatory like Non-Steroidal Anti-inflammatories such as aspirin and other NSAIDs.
3. Astaxanthin can never become a “pro-oxidant.” A lot of antioxidants, under certain conditions, can turn into pro-oxidants and start causing additional oxidation and damage in our bodies, but not astaxanthin. Astaxanthin acts as an anti-inflammatory that appears to reduce inflammation in the brain. This may have a dramatic effect on amyloidosis diseases, i.e. Alzheimer’s and Parkinson’s.

RECOMMENDATIONS TO REDUCE CANCER RISK

1. Reduce or eliminate Na⁺ intake by reducing extra-dietary sources of salt (NaCl).
2. Eliminate all sources of cellular hypoxia, such as smoking and dehydration. Blood oxygen levels, although a good indicator, may not be a good indicator of cellular hypoxia. The degree of cellular oxygenation is determined by the ability of the oxygen to be transported into the cell. This transport is determined by the solubility of O₂ in the interstitial fluid between the capillary and the cell membrane and the concentration gradient across the cell membrane.

- 3. Take astaxanthin as a checkpoint block on PD-L1 and PD-L2 sites on tumor cells. This allows the immune system to attack, lyse the tumor cell wall or cause apoptosis due to changes in electrical potential of the cell.**

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