Our investigation on melanoma cell lines isolated from the less or highly invasive primary melanomas, as well as from the lung metastases showed that cells are likely to be reprogrammed by hyperpolarized light and the 3HWC substance (hyper-harmonized hydroxylated fullerene), the newly patented fullerene derivative.

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Mallignant cell REPROGRAMMING

All biological processes, from basic communications at the molecular level to the most complex functions in the body, possess a dual nature. Coherence and inseparability of the phenomena of decomposition and creation, mutually opposed but unbreakable manifestations of cell physiology, are the best illustration of the duality principle. Every day, in our body a number of cells die, and a certain number are born. Perfect coordination of these events makes us healthy, which represents proper interactions within us and between us and the environment. The diseases that are the most difficult to deal with are those resulting from the loss of this balance and are often related to uncontrolled division or uncontrolled death of cells. The most striking example of the former is malignant tumors.

Due to an intensive, uncontrolled cell division that progressively disturbs tissue architecture, and consequently, its function, tumor tissue acquires chaotic behavior, implicating a lack of any strategy in progression. In line with this, the starting points in the treatment of malignant diseases were set up in accordance with the idea of a tumor mass as a random, rather than organized group of highly dividing transformed cells, not taking into account the complexity of intercellular communication in the tumor tissue. In summary, all processes involved in the initiation, development, progression,

and dissemination used to be characterized as random.Abnormal tissue and blood vessel architecture at the later stages of the disease were often used as a proof of this hypothesis.

Starting from the middle of the previous century, a wide spectrum of naturally occurring or synthesized chemotherapeutic agents have been used, whose anti-tumor activity is realized through blockage of cell division and/or activation of programmed cell death as an intrinsic software present in the genome of each cell in the body. The most common form of the programmed cell death they provoke is apoptosis. Since its discovery, apoptosis has been investigated as a main route of elimination of abnormal, intoxicated, or sufficient cells.

What has been overlooked, though, is how dying cells balance events in their environment, transmitting information (signals) crucial for maintaining the tissue homeostasis, and thus the entire organism. In a cascade of biochemical and biophysical events that lead to the disruption of the cell nucleus and cytoplasm, the cell in which this program has been launched activates a number of molecules whose role is more complex than it was believed before. They are not involved in the realization of the death signal only – an apoptotic cell organizes its own removal and takes care of all the consequences that its death will bring to the tissue it belongs to, thus showing altruistic behavior, contrary to its prior egoistic one. Immediately upon the activation of the death program, a cell first organizes both the cleaning of its own residues in advance, and the production of protective molecules preventing further destruction of the surrounding tissue.

Then, owing to the activity of the same enzyme crucial for the last steps in the degradation of genetic material, a dying cell initiates cell division of the neighboring cells to compensate for the loss and to maintain the tissue balance. All these aspects of a cell's social life and its commitment to the whole entity as opposed to its egoistic behavior, were completely neglected when the induction of apoptosis became a key criterion for the selection of effective chemotherapy. The cause of poor efficacy of regular chemotherapeutic protocols in aggressive tumor treatment lies in this cell behavior. While in less aggressive forms the goal of the therapy is to cure, in highly invasive tumors the aim of the treatment is to turn the disease into a chronic state, therefore prolonging life. In these cases, healing, if it occurs at all, is a personal achievement.

What distinguishes invasive/metastatic clones from less aggressive ones, making them resistant to killing-based therapies? It was long believed that genetic changes alone are the cause of malignant transformations and disease progression drivers. Today we know that the presence of genetic abnormalities makes these cells visible to our immune system. The problem arises from the fact that transformed cells look and behave like their healthy equivalents, rendering impossible their recognition as dangerous. This allows their uninterrupted and even supported existence in the tissue, organs, and organism of the host.

With regard to the scientific and technological development in the field of molecular biology, altered cell specificity and its malignant potential can be described using several parameters, ranging from cell morphology to intracellular protein and gene signature. The most fascinating fact connected to the malignant alteration is that this process presents a reverse pathway that the cells passed through in the process of embryonic development, which can be described as particular regression (figure 1).

As far as the cell is altered in its morphology and function typical of the tissue to which it belongs and closer to its embryonic form, its plasticity and freedom in shape, intracellular features, and communication become higher, resulting in an increased power of transformation and the more aggressive phenotype. As the cell maturation from the embryonic to adult tissue is deter-

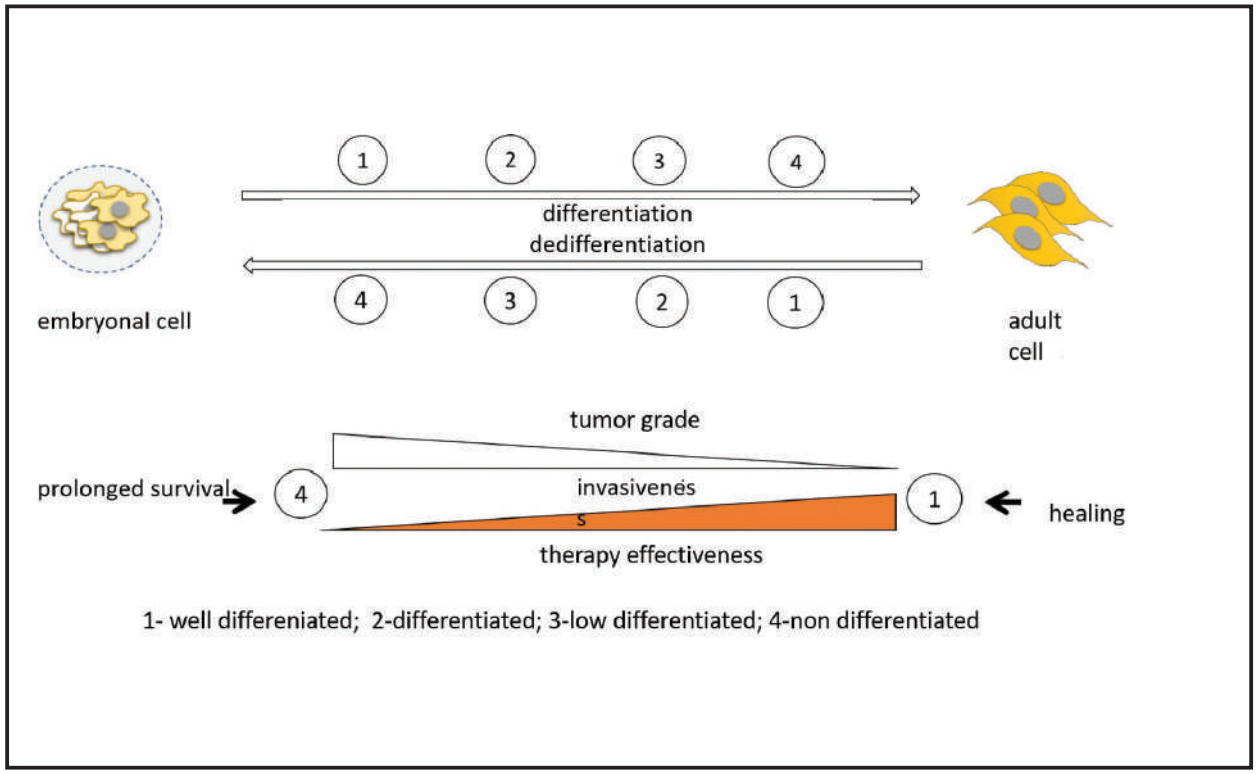


Figure 1

mined by differentiation, the process of malignant transformation is marked as dedifferentiation and serves as a scale for defining the so-called tumor grade in the diagnosis. On that scale, tumors of the lowest grade contain cells that have made minor progress in the process of dedifferentiation and still possess the characteristics of the tissue to which they belong. Their response to therapy is good, and the goal of the treatment is healing. Conversely, high-grade tumors possess undifferentiated cell fraction. These cells are similar to their embryonic ancestors and their morphology and characteristics are far more different from those typical of their home tissue. The presence of low/nondifferentiated cells in the tumor is considered a bad prognostic sign, and the therapy in these cases is tailored with an aim to extend and improve the quality of life, but without healing.

Limited effects of therapy in malignant diseases of highly invasive and metastatic character are still attributed to the initial or acquired resistance of cancer cells to the toxic effects of therapeutics, but also to the fact that the use of these drugs multiply diminish the body power, affecting the healthy cells vitality. Causes of drug resistance are determined by a number of signals and mechanisms developed by the transformed cell to block death pathways, amplify division, and/or remove the drug from the intracellular compartment. To overcome this, protocols that include the combination of drugs with different modes of actions have been established.

Malignant cells have shown a remarkable capacity to overcome such an attack, but not only at an individual level as previously believed, but rather at a collective level, acting as a self-governing multicellular entity with a heterogeneous cell structure, complex intercellular interactions, and indisputably "intelligent" communication with the host organism using their skills for self-progression. More than 40 years ago, scientists noticed a "paradoxical" pattern – radiotherapy potentates the division of cancer cells after a short-term improvement (Figure 2).

Analyses of high-grade tumor tissue samples showed that the presence of cells in apoptosis correlated with a worse outcome. This

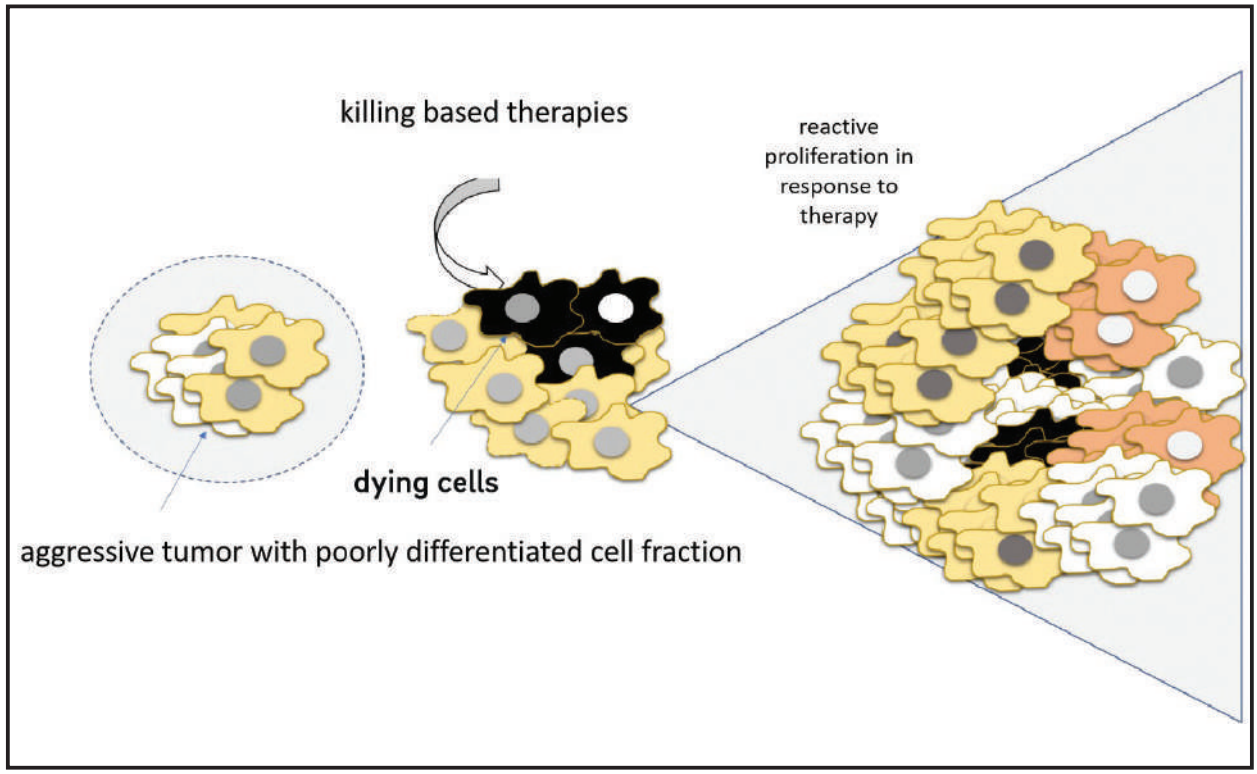


Figure 2

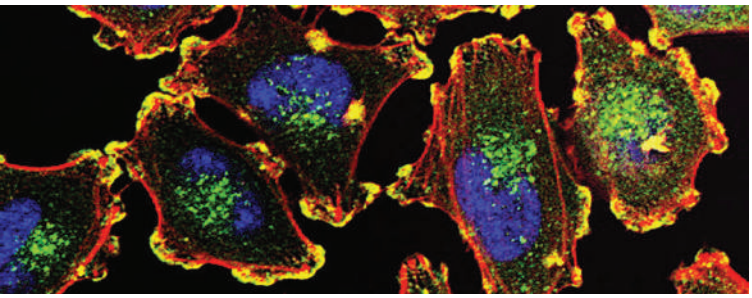
observation opposes the claim that the cause of tumor resistance to therapy lies in immortal cell phenotype. Another almost absurd finding of better survival among patients whose tumors key death program executor molecule caspase 3 didn't express unequivocally points to the fact that aggressive therapy based on cell death induction can initiate more aggressive division of cancer cells and tumor expansion.

This is precisely because of the fact that a malignant tumor is not a simple cluster of clones of transformed cells, but a complex multi-cellular community consisting of heterogeneous cells with clearly defined roles, fascinating plasticity, and complex communication within the commune and, most probably, with distant sites in the body as well, enabling not only their survival, but also dissemination. The intensive cell division often happens upon the exposure to chemo- or radiotherapy and is ascribed to the resistance of the tumor cells to death signals, leaving them to divide unhindered. However, there is a growing amount of data showing that tumor expansion upon therapy represents a reactive response of the tumor tissue to the signals released from the damage site caused by the treatment. It is precisely the aspect that distinguishes high-grade tumors from less aggressive forms, while understanding this distinction should serve as the starting point in setting up a platform that should offer a solution to limiting tumor growth without killing the cells.

How to change the malignant character of the cell? Not by conflict! Aggressiveness almost always promotes more aggression, especially having in mind the fact that the malignant transformation arises from the previously compromised function of certain cells in the tissue. Can we remind these cells about their lineage by forcing them to enter a more mature state similar to that they achieved by embryogenesis? Such reprogramming was seen many times in experimental practice and has been used as an approach in the treatment of some hematological malignancies since the 1980s, yielding remarkable results. The treatment of patients suffering from a therapy resistant form of acute promyelocytic leukemia with cis-retinoic acid led to a complete remission in 95.8% of patients.

A similar approach of inducing cancer cell phenotype conversion into a more differentiated state in the treatment of solid tumors is still disputable. There are sporadic examples of a successful reprogramming of liposarcoma and anaplastic thyroid tumors into less aggressive forms in clinical practice. In preclinical investigations, however, there are numerous examples of inducing cell differentiation in extremely aggressive tumors, such as neuroblastoma, glioma, melanoma, as well as breast, colon, pancreas, and lung cancers upon exposure to naturally occurring or synthetic compounds.

Our investigation on melanoma cell lines isolated from the less or highly invasive primary melanomas, as well as from the lung metastases showed that cells are likely to be reprogrammed by hyperpolarized light and the 3HWC substance (hyper-harmonized hydroxylated fullerene), the newly patented fullerene derivative.
Fullerene is the third pure crystal form of carbon, alongside graphite and diamond.
All three forms belong to the point symmetry crystals, with graphite and diamond crystallizing in 32 crystallographic groups (basic crystallographic aspects replicate into crystal formations of different sizes), and fullerene crystallizes into the icosahedral symmetry formation (a molecular crystal that has only one size). Whereas in graphite and diamond, as in all other crystals in nature, the products of symmetrical elements and their own energies are integers ...-2, -1, 0, 1, 2..., in fullerene (molecule C_{60}), in addition to the integers, there are also non-integer values, like ±1/2(1+Ö5) = ± 1.61803.... ±1/2(1-Ö5) = ± 0.61803. Fullerene was discovered by chance in 1985 by Harold Croto, Robert Kerl, and Richard Smally when they attempted to simulate cosmic dust around carbon-rich red giant stars using laser. They won the Nobel Prize for this discovery in 1996.



Melanoma metastasis (NIC)

The structure of the most famous fullerene, C_{60}, is an icosahedron consisting of 20 hexagons and 12 pentagonal rings joined into a spherical soccer ball-like shape. The number of pentagons is always constant, 12, while the number of hexagons can vary. Thus, the fullerene family consists of different molecules: C_{60}, C_{70}, C_{82} etc.

Accordingly, its structure is always spherical, resembling a cage.

Despite its extraordinary stability, the C_{60} molecule is very reactive, so there are numerous derivatives of this molecule. Functionalization of fullerenes increased their solubility and improved different chemical and physical properties, allowing their application in the treatment of numerous pathological conditions (Figure 3).

To improve solubility, multiple chemical modifications of fullerene have been created, and one of them is based on hydroxyl group addition. This modification results in the formation of water-soluble fullerols. Biological properties of fullerene and fullerol were initially tested jointly by physicists, chemists, biologists, physicians, and biomedical engineering experts more than 15 years ago.

Back in 1993, Wudl and coworkers showed that water-soluble derivatives of fullerene inhibit the activity of HIV protease, an enzyme necessary for the formation of an infective viral particle. Although the mechanism has not been fully clarified, it is speculated that fullerols block HIV protease activity through interaction with an enzyme-binding site.

Another important property of fullerene and its derivatives is the capacity to pre-

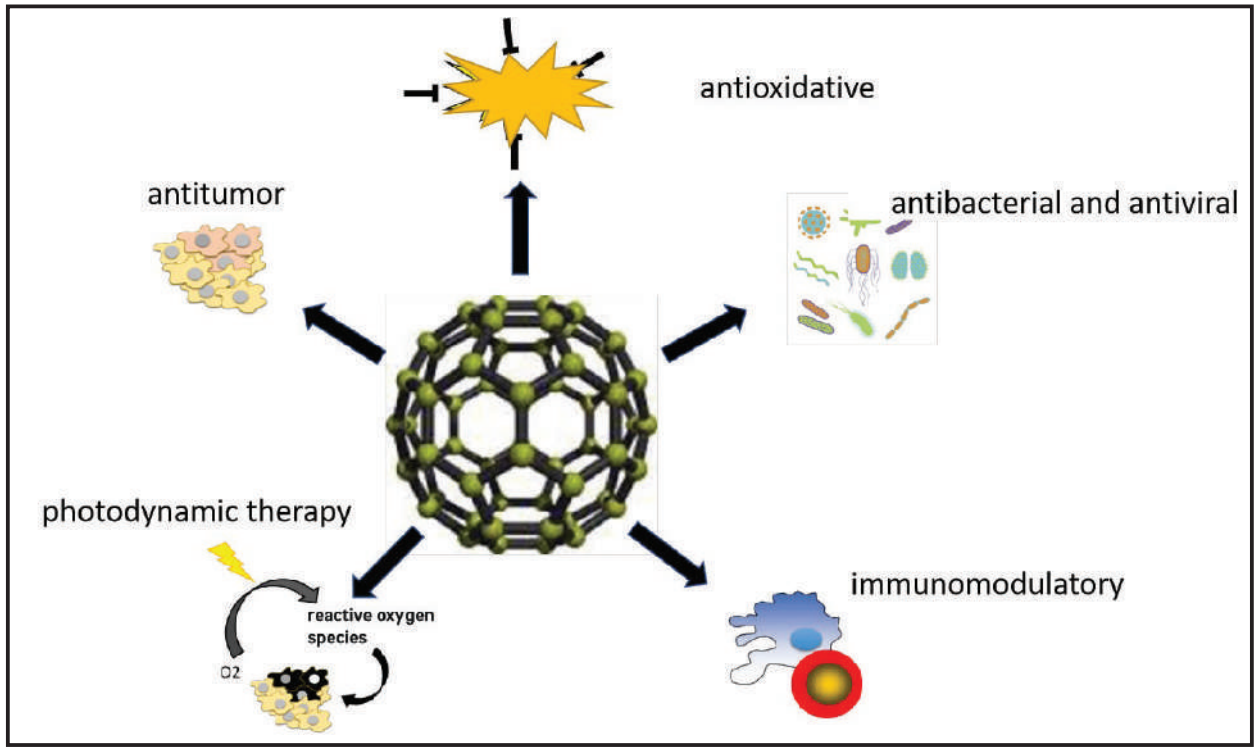


Figure 3

vent the bacteria-host cells contact through interaction with adhesive molecules. There are also a lot of data about their interplay with the immune system and cancer cells. It was found that these nanostructures can suppress the growth of a certain type of cancer cells in experimental conditions, as well as modulate cytotoxic drugs activity even.

Perhaps the most important characteristic of fullerene derivatives is their antioxidant potential. They act as sponges that scavenge free radicals, and consequently protect cells from their toxic effects. Conversely, there are also data showing high levels of oxygen radicals produced by fullerenes after their exposure to light of different wavelengths. In that case, liberated reactive molecules damage the cell membrane, interacting with lipids and proteins. This property serves as the basis in photodynamic therapy, in which fullerene derivatives are used as sensitive substances.

Our research, conducted at the request of the Scientific Board of ZEPTER INTERNATIONAL, led by Prof. Ljubiša Rakić, PhD, full member of Serbian Academy of Sciences and Arts, showed that modified fullerol (3H-WC substance) in interaction with hyperpolarized light (whose angular photon organization is icosahedral symmetry) suppresses the growth of melanoma cells (malignant skin cancer) in laboratory conditions (Figure 4).

Most importantly, the administered treatment was equally effective in cell lines originating from primary tumors of different grades as well as in those originating from metastases. The reduced number of melanoma cells in the culture was not caused by the induction of cell death. In fact, the treatment provokes cell differentiation accompanied by the downregulation of dividing potential and increased production of the pigment melanin. Ultra-structural analyses confirmed the increased number of melanosomes – organelles which produce melanin – but they also revealed a series of changes that indicated a substantial alteration in the cell's metabolic status. Accordingly, a certain number of cells were brought into a state of senescence, or cellular dormancy. Senescent cells remain vital but do not re-

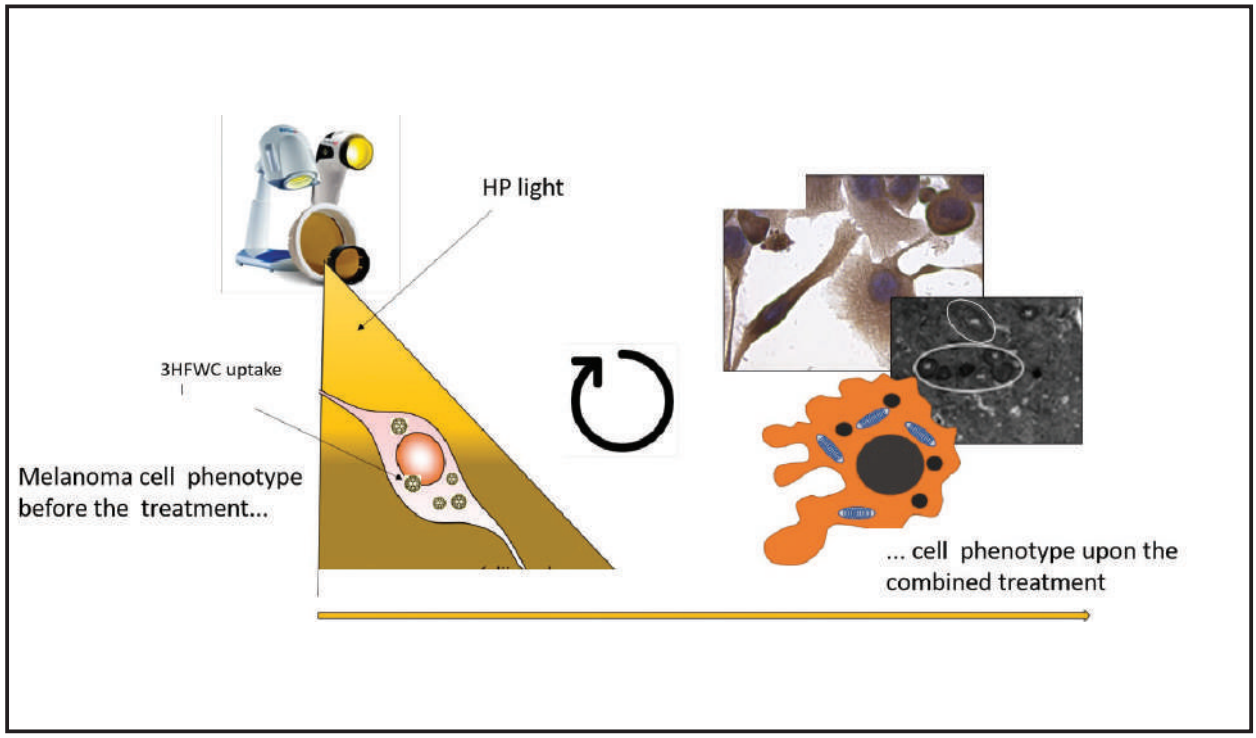
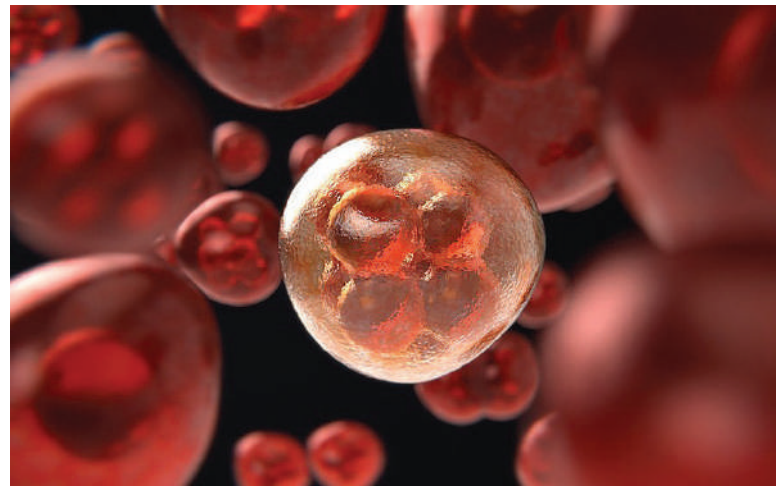


Figure 4

spond to growth and death stimuli and often spontaneously involve. Each of these effects represents a non-aggressive way to abrogate tumor progression, avoiding tumor repopulation in response to the damage triggered by standard treatments. Classical photodynamic therapy is based on photoexcitation of fullerene substances and consequential production of reactive oxygen species leading to cell toxicity. The application of hyperpolarized light in our research presumably favored the antioxidant potential of the 3HWC substance as yet unknown mechanisms of cell reprogram induction and establishment of phenotype close of healthy melanocyte. Quantum hyperpolarized light shows that the order of photons matches the energy structures of our tissue. Hyper-harmonized hydroxylated fullerene tends to harmonize biological processes. Following one of the basic postulates of quantum medicine that a harmonized structure conveys its energy/information to the structure of the broken record, can we expect that the exposure to hyperpolarized light together with hyper-harmonized hydroxylated fullerene could induce the malignant cells reprogramming?

This hypothesis has already a strong anchorage in the preliminary results of our research. According to the basic principle of "theory guides, experiment decides", introduced into science by Faraday, further experiments will continue. They will bring us closer to the secret of the black holes of this disease and to a better understanding of the rules and causes of its destructive potential. However, these are just the first steps on the path ahead of us, which will show whether it is possible to treat a tumor with a non-toxic approach.



Mallignant cells (Pixabay CC0 1.0)