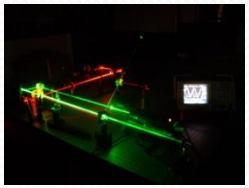

Photodynamic Therapy - Principles of PDT

Dr. Humberto Cabrera Morales

Instituto Venezolano de Investigaciones Científicas

The Abdus Salam International Centre for Theoretical Physics

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Outline

1. Introduction

2. Motivation

3. Photodynamic therapy.

4. Light-tissue interaction.

5. Conclusions

1. Introduction

Neoplasms: are defined as a new formation of cell clusters, which have lost their ability to control cell division.

Neoplasms can either be benign or malignant.

Benign tumors:

1. Differentiated.
2. Slow rate of proliferation.
3. Encapsulated.
4. Do not infiltrate surrounding tissue.
5. Usually do not result in patient death.

1. Introduction

Malignant tumors:

1. High rate of cell proliferation.
2. Loss of contact inhibition.
3. Lack of differentiation.
4. Grow by invading and infiltrating.
5. Loss of cohesiveness.
6. Resistance to apoptosis.

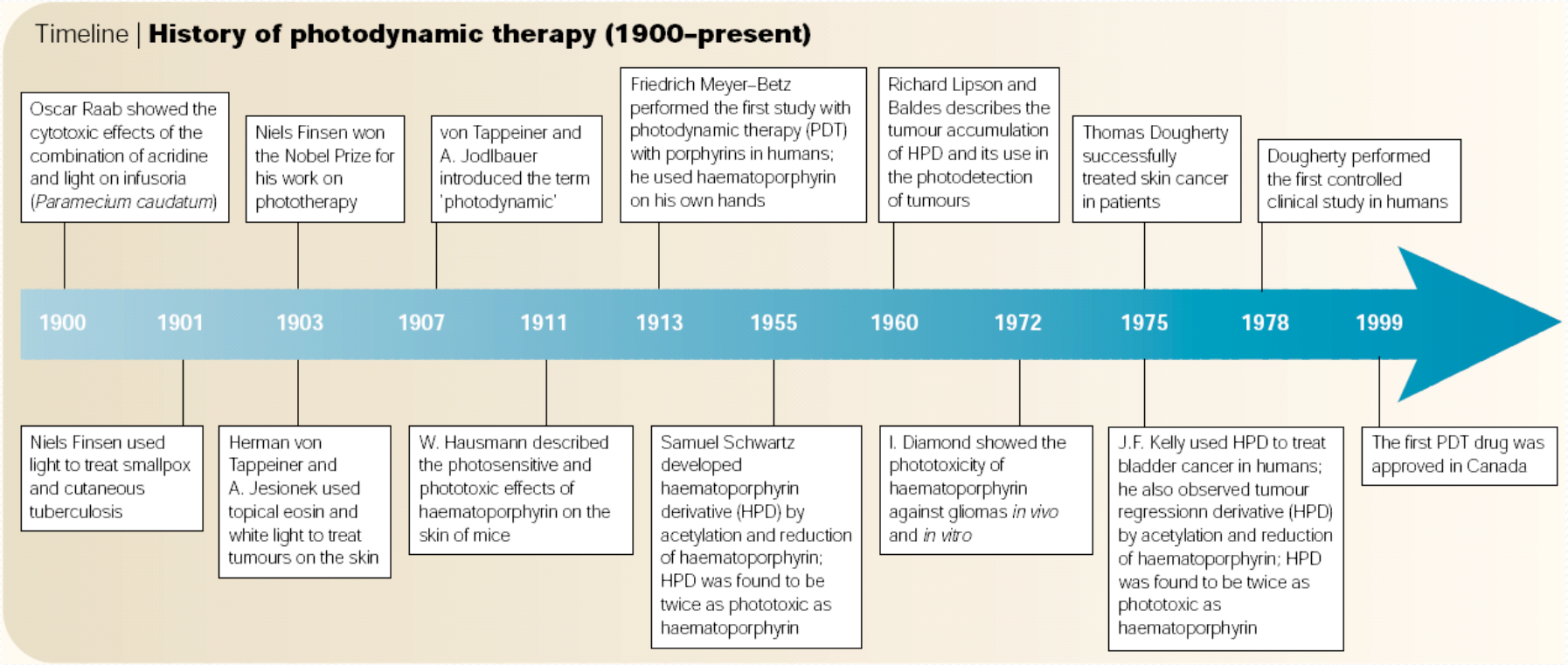
1. Introduction

- Cell proliferation is a tightly controlled process that ensures the accurate replication and transcription of genetic information.
- Genetic mutations can either be repaired, or result in the induction of apoptosis.
- Mutations in certain genes, called oncogenes, can result in dysregulation of the cell cycle, resistance to apoptosis, and in the development of cancer.
- A large number of today's most effective cancer treatments are cytotoxic agents that target the cell cycle.

2. Motivation

Traditional cancer treatments (radiation, surgery and/or chemotherapy) have deleterious side effects. As an alternative to these treatments PDT offers a more targeted and less invasive treatment regimen.

3. Photodynamic therapy



[1]. **Nature** 2003, 3, 380.

3. Photodynamic therapy

The very first attempts to apply PDT to treatment of tumors and other skin diseases, such as lupus of the skin and chondylomata of the female genitalia, were performed by the group of von Tappeiner in 1903-1905 [1]

The modern era of PDT was founded in the 1970s with the pioneering work of Dougherty and co-workers at the Roswell Park Memorial Cancer Institute in Buffalo who used HPD Photofrin [2]

[2] H. von Tappeiner, A. Jesionek (1903). Therapeutische Versuche mit fluorescierenden Stoffen. *Munch. Med. Wochenschr.,* **47,** 2042-2044.

[3] T.J. Dougherty, J. *Clin. Laser Med. Surg.* (1 996), **14**, 219-221.

3. Photodynamic therapy

What is Photodynamic Therapy ?

Photosensitizer (retained in tumors characterizing mainly by neovascularization)

+

Visible light - wavelength to activate phosensitizer

↓

Singlet oxygen and/or ROS (electron and/or H transfer,

free radicals)

↓

Tumors cell death

by

1. Necrosis and/or apoptosis

2. Direct destruction of tumor vasculature

3. An acute inflammatory response that attracts leukocytes such as dendritic cells and neutrophils (antitumor immunity)

3. Photodynamic therapy

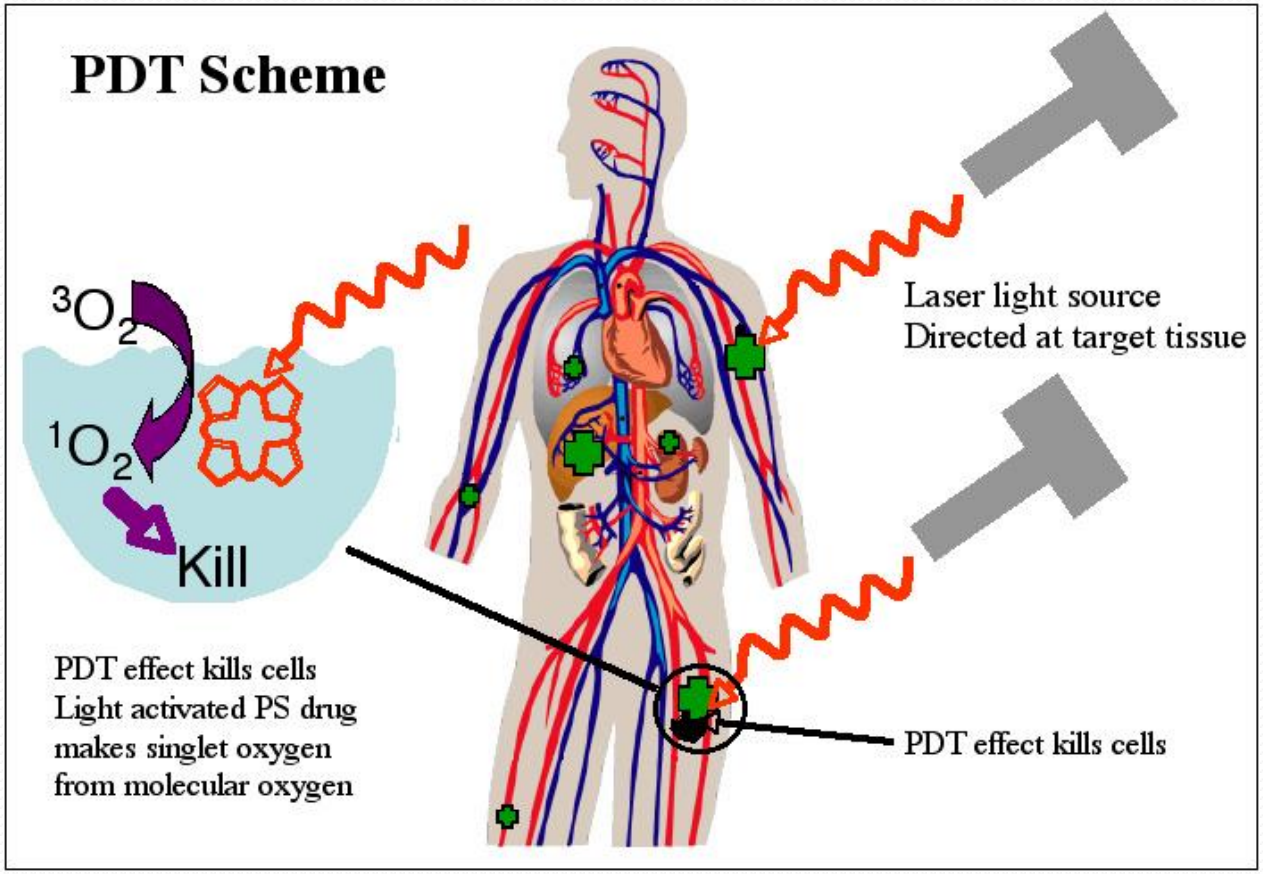


Figure 1. PDT scheme

3. Photodynamic therapy

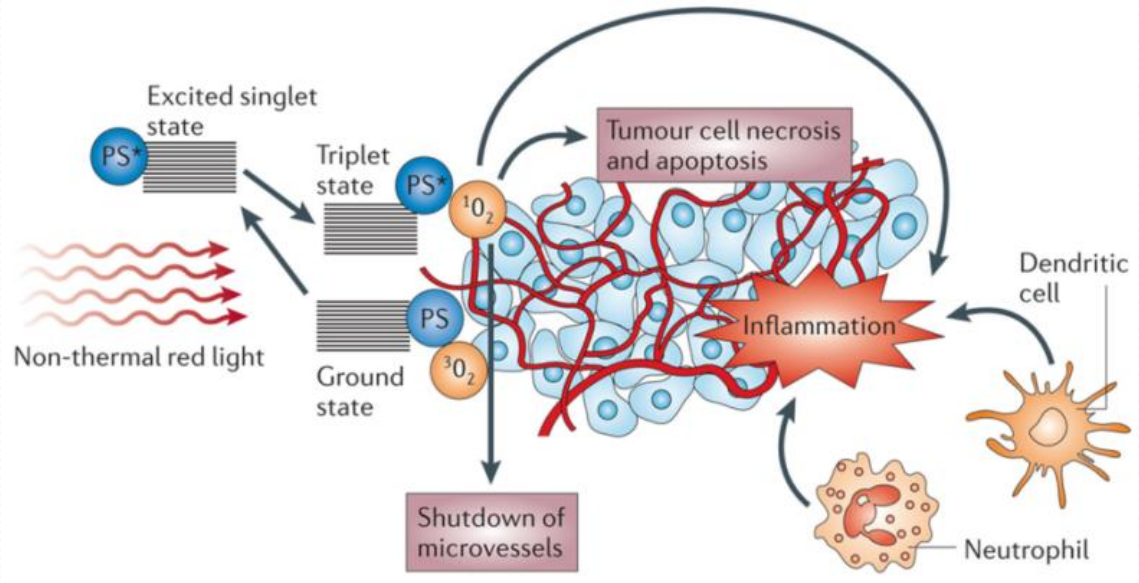


Figure 2. The mechanism of action on tumours in photodynamic therapy.

The photosensitizer (PS) absorbs light and an electron moves to the first short-lived excited singlet state. This is followed by intersystem crossing, in which the excited electron changes its spin and produces a longer-lived triplet state. The PS triplet transfers energy to ground-state triplet oxygen, which produces reactive singlet oxygen (1O2). 1O2 can directly kill tumour cells by the induction of necrosis and/or apoptosis, can cause destruction of tumour vasculature and produces an acute inflammatory response that attracts leukocytes such as dendritic cells and neutrophils.

3. Photodynamic therapy

Why do some photosensitizers localize selectively in tumors?

The selective tumor uptake is probably because of the differences in the physiology between tumors and normal tissues:
(1) tumors have a larger interstitial volume than normal tissues,
(2) tumors often contain a larger fraction of macrophages than normal tissues,
(3) tumors have a leaky microvasculature,
(4) tumors have poor lymphatic drainage,
(5) the extracellular pH is low in tumors,
(6) tumors contain a relatively large amount of newly synthesized collagen and
(7) tumor tissue contains many receptors for lipoproteins.

[4] T. Patrice, Photodynamic therapy, COMPREHENSIVE SERIES IN PHOTOCHEMISTRY & PHOTOBIOLOGY European Society for Photobiology (2003), **Chapter 1**, 8.

3. Photodynamic therapy

Another cause of selectivity is related with the fact that tumors (for example basal cell carcinomas bcc) has a higher microvessel density, as compared to surrounding normal tissue [5].

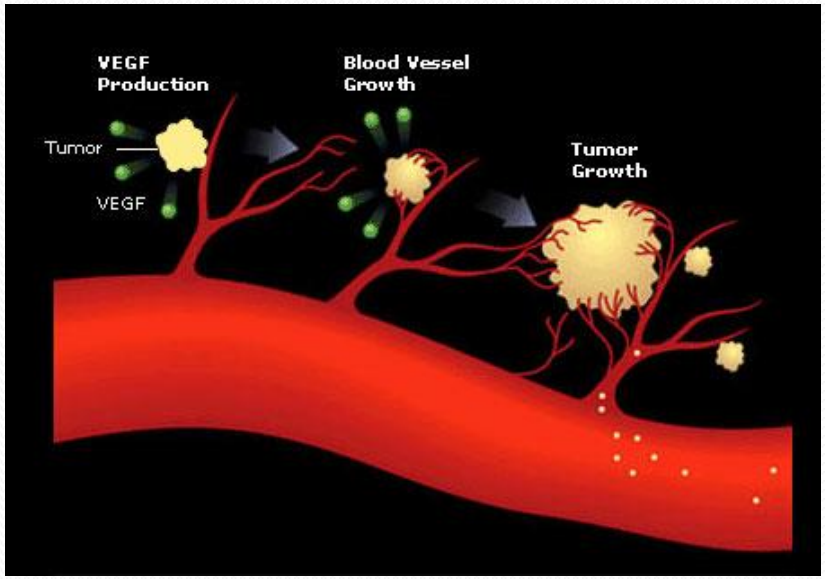


Figure 3. Higher microvessel density in bcc.

[5]. Chu et al. Stromal-cell-derived factor-1α (SDF-α/CXCL12)-enhanced angiogenesis of human basal cell carcinoma cells involves ERK1/2-NF-κ /interleukin-6 pathway. **Carcinogenesis** 2009; 30 (2): 205-213

3. Photodynamic therapy

Reaction Mechanisms

- Type 1:
  - Direct reaction with substrate (cell membrane or molecule)
  - Transfer of H atom to form radicals
  - Radicals react with O_2 to form oxygenated products
  - Half-life seconds
  - Radius affected mm, cm
- Type 2:
  - Transfer of energy to O_2 to form $^1O_2$
  - Half-life of $^1O_2$ < 0.04 ms
  - Radius affected < 0.02 mm

3. Photodynamic therapy

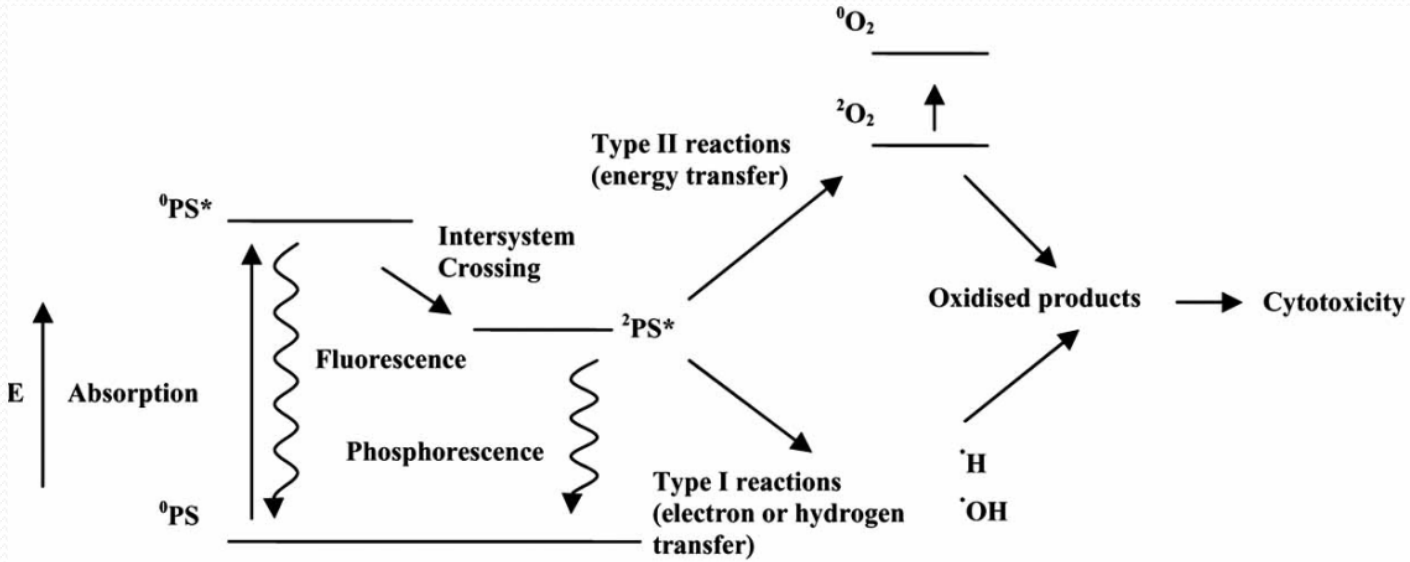


Figure 4. Type I and type II mechanisms.

Alternatively in the type I mechanism the excited photosensitizer can abstract H-atoms from the sugar backbone of the DNA molecule leading to a cascade of reactions ultimately decomposing the DNA

In the type I mechanism oxygen is not always necessary for the photodynamic action to take place, however, in the type II mechanism oxygen is essential

3. Photodynamic therapy

What Are Free Radicals and ROS?

A free radical is an atom, molecule, or compound that is highly unstable because of its atomic or molecular structure (i.e., the distribution of electrons within the molecule). As a result, free radicals are very reactive as they attempt to pair up with other molecules, atoms, or even individual electrons to create a stable compound. To achieve a more stable state, free radicals can “steal” a hydrogen atom from another molecule, bind to another molecule, or interact in various ways with other free radicals.

3. Photodynamic therapy

Reactions Involving Free Radicals

Hydrogen abstraction, in which a radical interacts with another molecule that has a free hydrogen atom (i.e., a hydrogen donor). As a result, the radical binds to the hydrogen atom and becomes stable, whereas the hydrogen donor is converted to a free radical.

Addition, in which the radical binds to another, originally stable molecule, converting the combined molecule into a radical.

Termination, in which two radicals react with each other to form a stable compound.

Disproportionation, in which two identical radicals react with each other, with one of the radicals donating an electron to the other so that two different molecules are formed, each of which is stable.

3. Photodynamic therapy

Singlet oxygen (or $^1\mathbf{O_2}$) is the common name used for the diamagnetic form of molecular oxygen (O_2), which is less stable than the normal triplet oxygen. Because of differences in their electron shells, singlet and triplet oxygen differ in their chemical properties. Singlet oxygen is in the same quantum state as most molecules and thus reacts readily with them, thus making singlet oxygen highly reactive.

Singlet oxygen is usually generated with a photosensitizer pigment. The damaging effects of sunlight on many organic materials (polymers, etc.) are often attributed to the effects of singlet oxygen. In photodynamic therapy, singlet oxygen is produced to kill cancer cells.

3. Photodynamic therapy

The action mechanisms of PDT at the cellular level

The mechanism of action is clearly linked to the intracellular localization of the sensitizers. However, other factors, such as cell line and PDT doses, play important roles as well.

Cationic sensitizers localize in both the nucleus and mitochondria, lipophilic ones tend to stick to membrane structures, and water-soluble drugs are often found in lysosomes.

Cells can react in different ways to PDT: upon lethal doses they can undergo either necrosis or apoptosis, and in the case of sublethal damage, the cells can elicit a rescue response

[4] T. Patrice, Photodynamic therapy, COMPREHENSIVE SERIES IN PHOTOCHEMISTRY & PHOTOBIOLOGY European Society for Photobiology (2003), **Chapter 1**, 9.

3. Photodynamic therapy

The action mechanisms of PDT at the cellular level

Necrosis

Cell death in a necrotic fashion can be induced following organelle damage, such as membrane lipid peroxidation, disruption of lysosomal membrane, membrane enzyme inhibition or damage to nuclear components. In contrast to apoptosis as described below, necrosis is a less controlled way of cell death, which does not seem to involve complex signaling cascades. In PDT using photosensitizers which localize in the lysosomes, cell death is possibly due to release of lysosomal enzymes and other toxic moieties

[4] T. Patrice, Photodynamic therapy, COMPREHENSIVE SERIES IN PHOTOCHEMISTRY & PHOTOBIOLOGY European Society for Photobiology (2003), **Chapter 2**, 32.

[6] G. Li, R. Pottier, M.R. Szewczuk, J.C. Kennedy (1999). *Photochem. Photobiol.,* 69, 23 1-235.

3. Photodynamic therapy

The action mechanisms of PDT at the cellular level

Apoptosis

Several studies have shown that photosensitizers which localize preferentially in mitochondria are very rapid inducers of apoptosis, in contrast to photosensitizers localized in lysosomes and plasma membranes. Apoptosis induction by mitochondrial based photosensitizers is an extremely rapid process: cells can enter the execution phase of apoptosis within 30 min after illumination. The release of cytochrome c from mitochondria as being a critical signal for the induction of apoptosis.

[7] J.C. Kennedy, R.H. Pottier, D.C. Pross (1990). *J. Photochem. Photobiol. B Biol.,* 6, 143-148.

3. Photodynamic therapy

The action mechanisms of PDT at the cellular level

Responses to sublethal PDT

Apart from a necrotic or apoptotic response, cells can also undergo a rescue response after PDT, dependent on PDT dose, cell type, and photosensitizer. Several stress proteins involved in cell rescue have been shown to be regulated upon PDT: members of the family of heat shock proteins, glucose regulated proteins and heme oxygenase . PDT, at sublethal doses, not only can induce a rescue response, but it also regulates gene and protein expression which is involved in other cellular functions.

3. Photodynamic therapy

The action mechanisms of PDT at the cellular level

Responses to sublethal PDT

Various transcription factors, such as AP-1 and NFKB, are activated by PDT. In turn, these transcription factors control, among other proteins, the expression of various cytokines, which indeed are induced by PDT. These cytokines play an important role by the induction of anti-tumor immunity. In addition to soluble mediators, PDT has also been shown to regulate adhesion molecules on cells, which may be relevant to long-term effects, such as tumor metastasis. Alterations in the expression of surface receptors such as MHC class **I** and have been reported and PDT-associated immune response has been attributed to these alterations

[8] Z. Malik, H. Lugaci (1987). *Br.* J. *Cuncer,* 56,589-595.

3. Photodynamic therapy

Mechanisms of PDT in vivo

PDT can generally induce tumor destruction in vivo in three different ways: vascular destruction, direct tumor cell destruction and elicitation of an anti-tumor immune response. The relative contribution of each depends on the localization of the photosensitizer within the tumor tissue, which is partly dependent on the time between photosensitizer administration and illumination and the properties of the tumor such as the degree of vascularity and its immune cell content

[4] T. Patrice, Photodynamic therapy, COMPREHENSIVE SERIES IN PHOTOCHEMISTRY & PHOTOBIOLOGY European Society for Photobiology (2003), **Chapter 2**, 37.

3. Photodynamic therapy

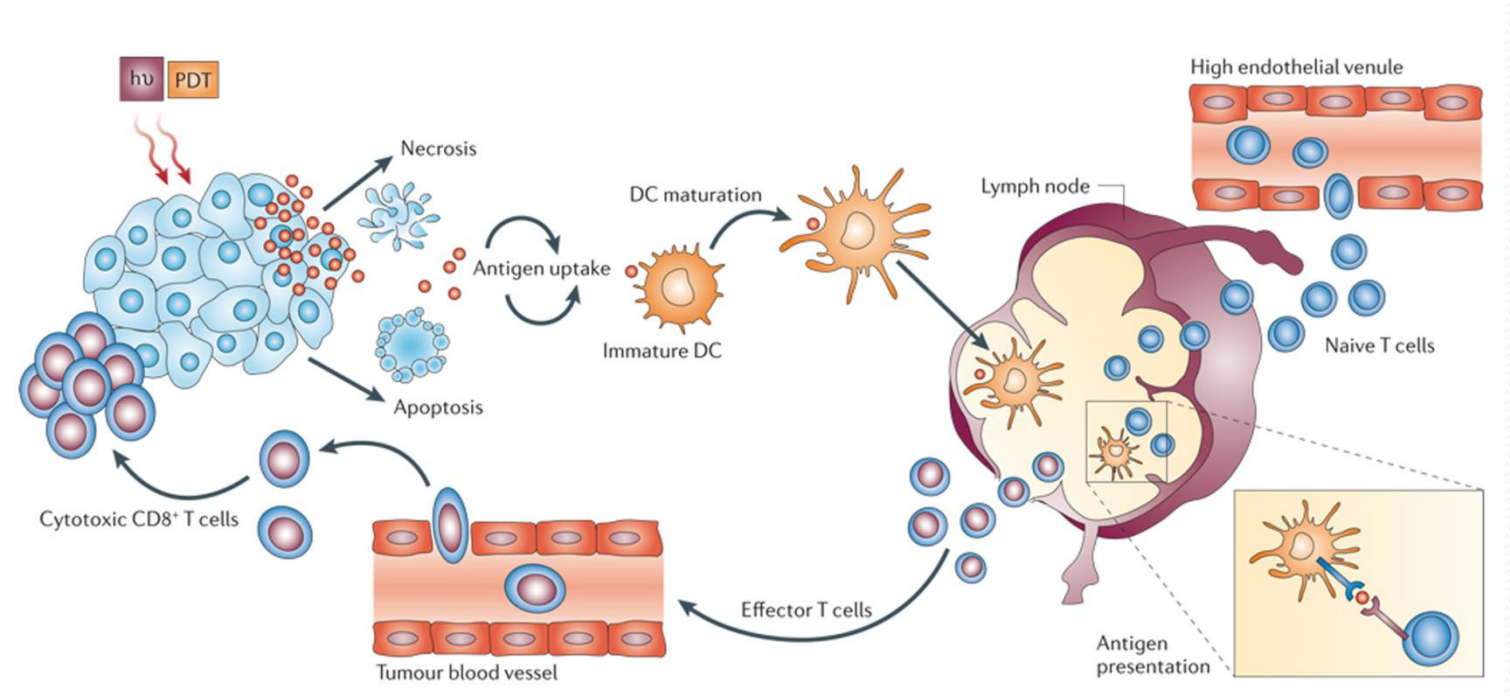


Fig. 5. Photodynamic therapy induces activation of antigen-specific T cells

When light (hv) is delivered to a photosensitizer (PS)-loaded tumour it induces both apoptotic and necrotic cell death. These cells are phagocytosed by dendritic cells (DCs) that have accumultated owing to the acute inflammatory response which is triggered by photodynamic therapy (PDT). DCs mature after stimulation by cytokines, which are released at the site of inflammation, and home to the regional lymph nodes where they present antigens to the T lymphocytes. Activated T lymphocytes become effector T cells and, attracted by chemokines, migrate to the tumour and kill the tumour cells

4. Light-tissue interaction

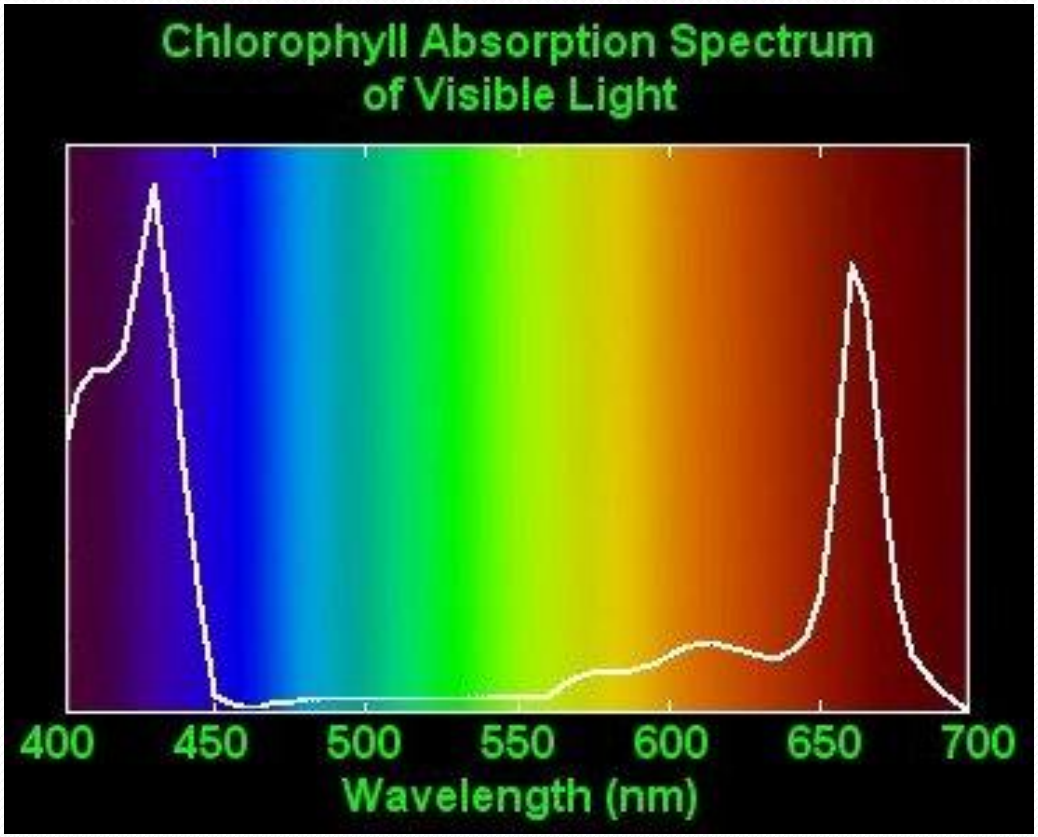


Fig. 6. Chlorophyll absorption spectrum

4. Light-tissue interaction

Photophysical characteristics of Chlorin derivatives

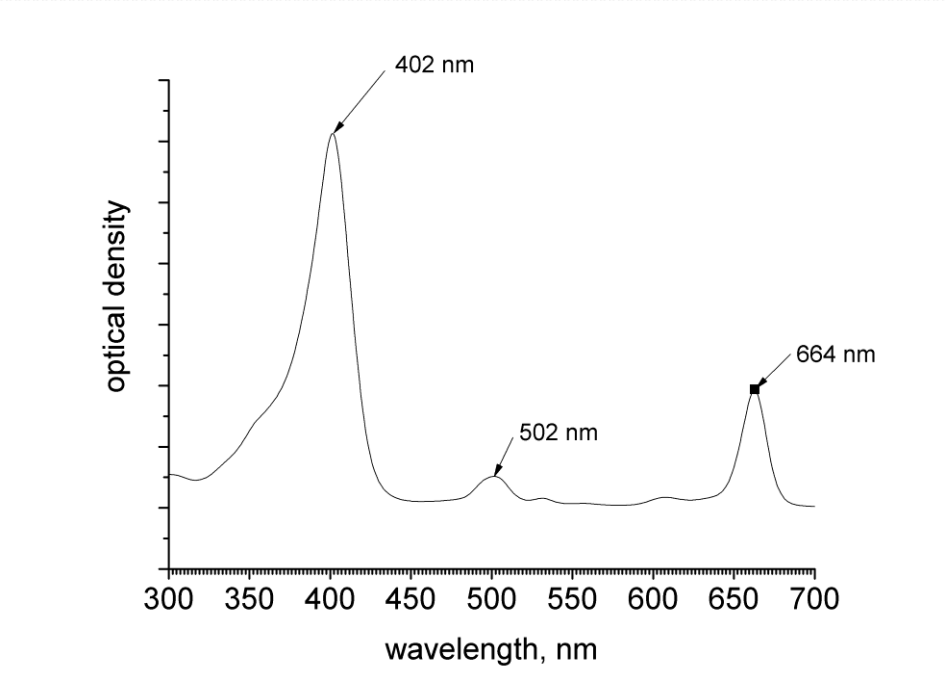


Figure 7. Typical absorption spectrum of Chlorin derivatives

4. Light-tissue interaction

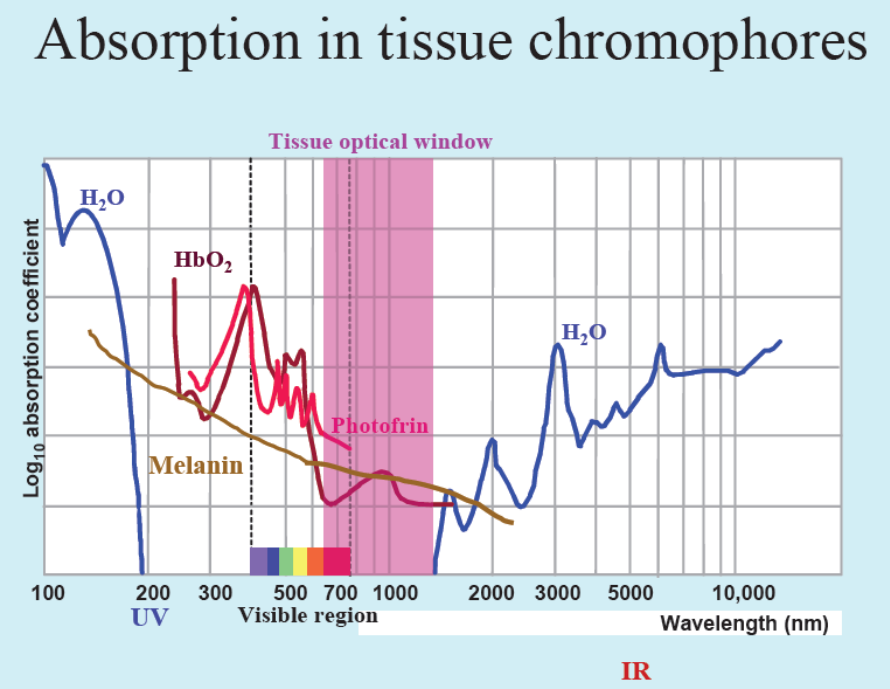


Figure 8. Absorption in tissue chromophores.

Longer wavelengths penetrate deeper

5. Conclusions

1. Relatively selective and less invasive treatment regimen

2. The mechanism of action is clearly linked to the intracellular localization of the sensitizers

3. PDT can generally induce tumor destruction in vivo in three different ways: vascular destruction, direct tumor cell destruction and elicitation of an anti-tumor immune response

4. Longer wavelengths penetrate deeper

5. Conclusions

LIMITATIONS:

Light needed to activate photosensitizer cannot penetrate more than 1cm of tissue depth using standard laser and low powered LED technology and hence is less effective in treatment of large tumors and metastasis. It may leave many people very sensitive to light post therapy and cannot be used in people allergic to porphyrins.

Thanks for your attention