Evaluation of photodynamic treatment efficiency on glioblastoma cells ex vivo – initial studies

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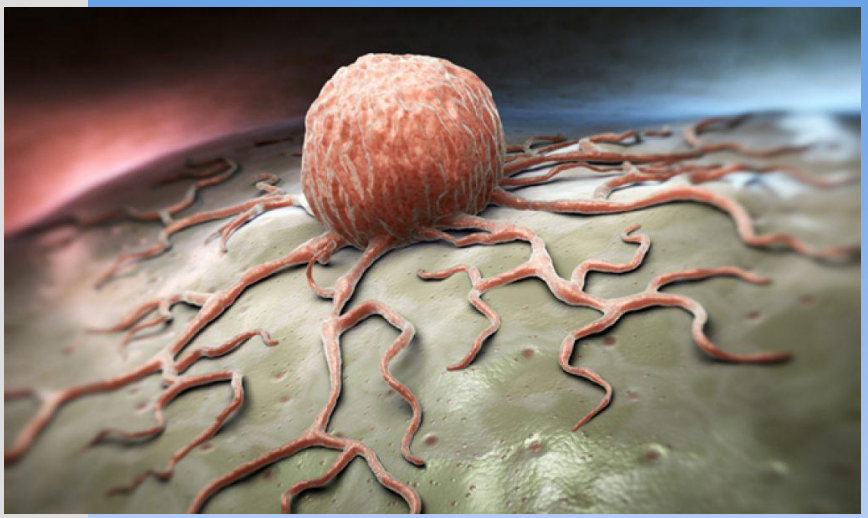
Glioblastoma multiforme (GBM) tumours



GBM is aggressively cancerous as the cells reproduce rapidly and spread extensively in the brain, and is also highly resistant to conventional therapy. This makes treatment exceptionally tough and challenging. GBM patients usually survive less than 12-15 months after diagnosis.

Signs and symptoms of glioblastoma are non-specific.

They may include:

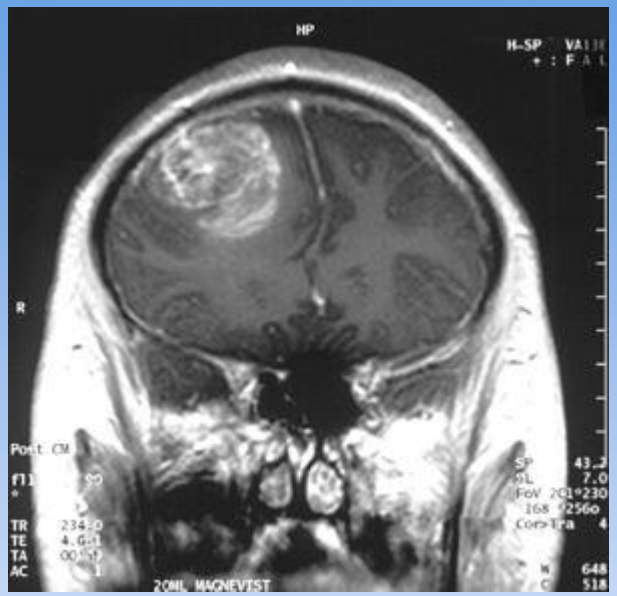
- headaches;
- personality changes;
- nausea;
- symptoms, similar to a stroke;

Worsening of symptoms often is rapid. This may progress to unconsciousness.

https://www.asianscientist.com/2017/06/in-the-lab/bcl6-gliobastoma-multiforme-biomarker/

Conventional treatment of GBM

- It is very difficult to treat glioblastoma due to several complicating factors:
  - The tumor cells are very resistant to conventional therapies.
  - The brain is susceptible to damage due to conventional therapy.
  - The brain has a very limited capacity to repair itself.
  - Many drugs cannot cross the blood–brain barrier to act on the tumor.



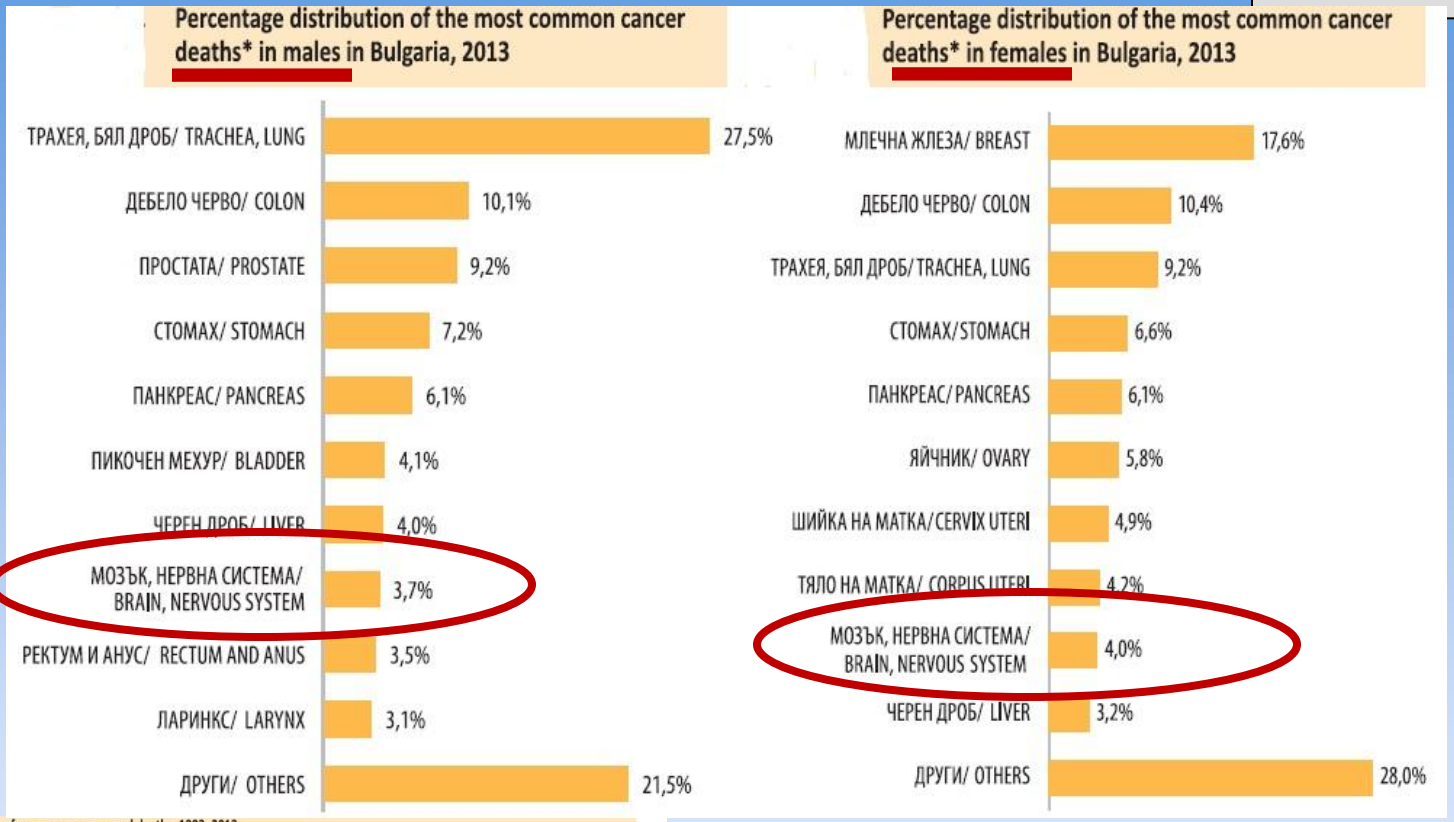
Coronal MRI with contrast of a glioblastoma WHO grade IV in a **15-year-old** male*

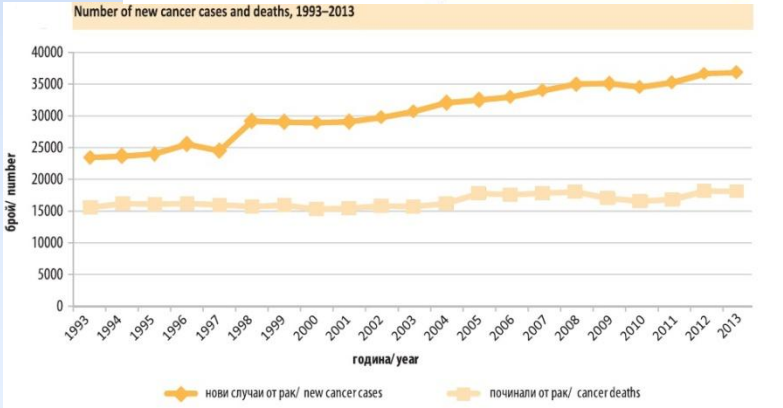
*https://en.wikipedia.org/wiki/Glioblastoma

Therapy of GBM lesions

- **Symptomatic therapy** - supportive treatment focuses on relieving symptoms and improving the patient's neurologic function. The primary supportive agents are anticonvulsants and corticosteroids;
- **Palliative therapy** – this treatment usually is conducted to improve quality of life and to achieve a longer survival time. It includes surgery, radiation therapy, and chemotherapy;
- **Surgery** - this is the first stage of treatment of glioblastoma. Removal of 98% or more of the tumor has been associated with a significantly longer life expectancy(up to 24 months);
- **Radiotherapy** - Subsequent to surgery, radiotherapy becomes the mainstay of treatment for people with GBM. A pivotal clinical trial carried out in the early 1970s showed that among 300 GBM patients randomized to radiation or non-radiation therapy, those who received radiation had a median survival more than double;
- **Chemotherapy** - Most of the studies show no benefit from the addition of chemotherapy for this type of tumour.
- ***Other therapies – PDT, BNCT, etc.***

GBM statistics





Nevertheless that GBM is not in “top ten” incidence rate for the tumours, it appears in the statistics of “top ten” cancer deaths incidence for male and female population.

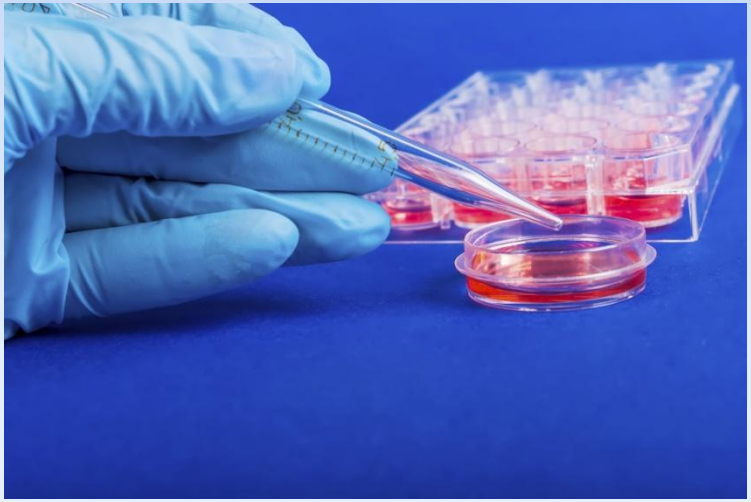
National Cancer Registry of Bulgaria, 2015

Summary

- Photodynamic therapy is well-established and extensively used method in treatment of different cancer types. This research reveals its potential in the treatment of cultivated human glioblastoma cells with adherent morphology.
- As the blood-brain barrier (BBB) permeability of the drugs is a significant problem that could not be solved for large biomolecules, we search for an appropriate low-molecular weight photosensitizer that could be applied for photodynamic treatment of glioblastoma cells.
- Such promising compound is a delta-aminolevulinic acid (5-ALA), which could pass BBB and plays the role of precursor of a protoporphyrin IX (PpIX) – photosensitizer that is accumulated selectively in the tumour cells and could be a proper tool in PDT of human glioblastoma.
- ***This initial study investigates effect of using different fluence rates and light doses, and aims to establish the most efficient values for further clinical application. A hypothesis about the personal response to the PDT for different patients is developed by the clinicians, and a procedure for personalization of the PDT procedure for each patient is under development.***

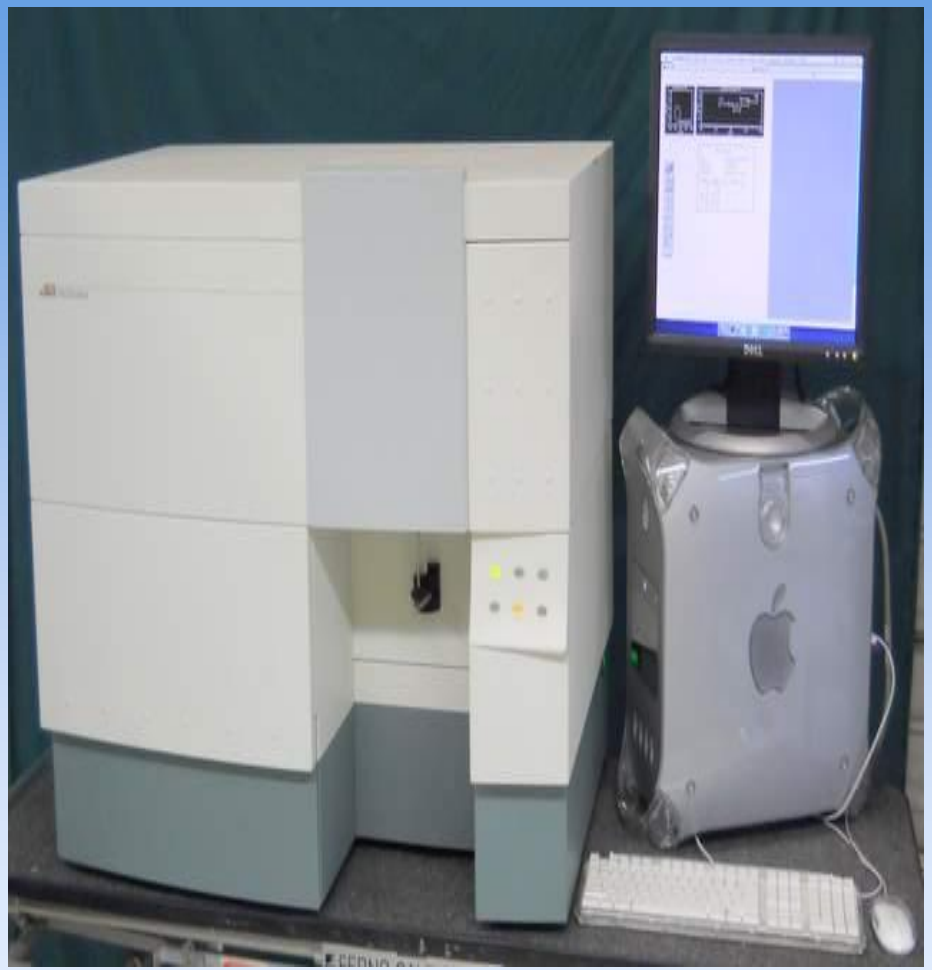
Materials and methods – GBM samples

- After surgical removal of glioblastoma tumours from patients, cells with adherent morphology were grown and prepared for photodynamic treatment. Four hours prior to every experiment, glioblastoma cells were cultivated with 5-ALA (100 μg/L), at Laboratory of Immunology, University Hospital “St. Ivan Rilski”, in order to be produced and accumulated a proper concentration of PpIX in the cells. Next step includes delivering of selected light dose to prepared sample and forward assessment of apoptosis levels using flowcytometry. The idea of the experiment is to be established the most efficient light parameters and to achieve this in our research were used different light doses: 12,5 J/cm^2, 25 J/cm^2, 40 J/cm^2, and the same fluence rate: 5 mW/cm^2.



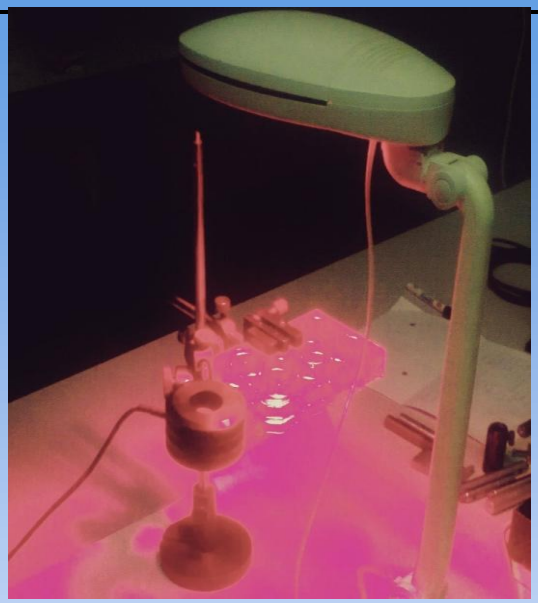
Flowcytometry – apoptosis evaluation

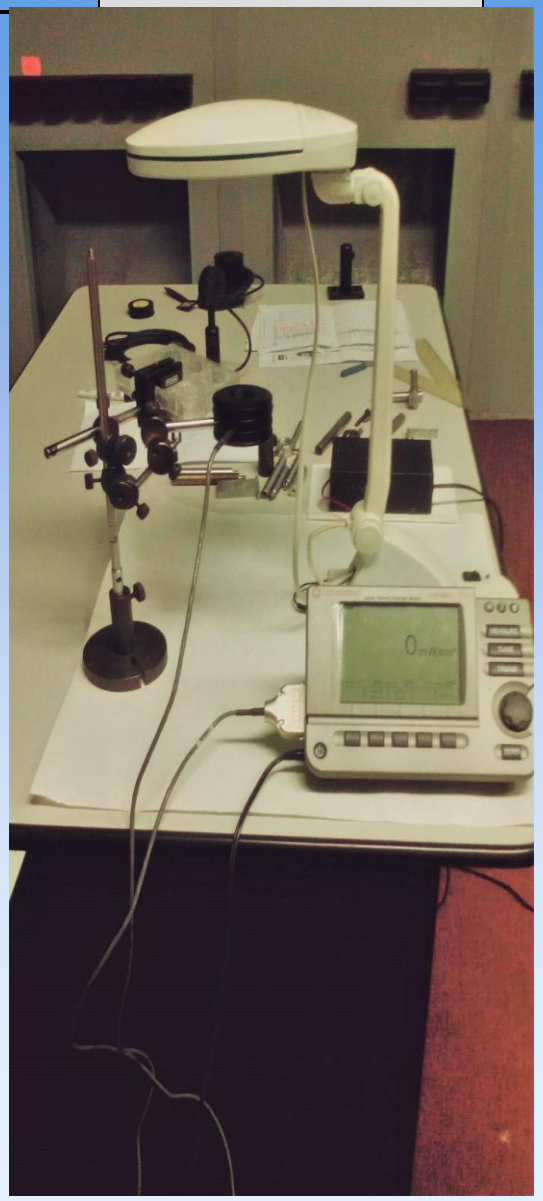
- By the means of FACSCalibur flow cytometer (Becton Dickinson, USA) and Cell Quest Software was made evaluation of PDT effect on used human glioblastoma cells.
- All conclusions are based on assessment of cellular apoptosis, known as well as programmed cell death, using flowcytometry.
- This evaluation of the photodynamic treatment effect was held in University Hospital “St. Ivan Rilski”, Sofia.



Flowcytometer – for evaluation of early and late apoptotic cells of GBM

PDT procedure - instrumentation





For the needs of PDT application a illumination device was developed in Laboratory of Biophotonics, BAS based on light-emitting diode (LED) matrix, as light sources for PDT therapeutic applications, emitting at 635 nm. The device is optimized for PDT in combination with 5-ALA/PpIX applied as photosensitizer.

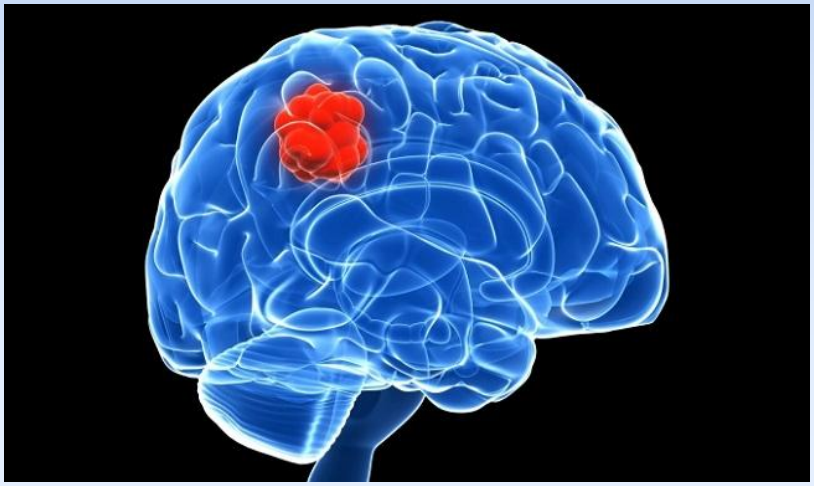
PDT irradiation source for GBM cells

Parameters	Specifications
Therapeutic field	40x50 mm
Therapeutic maximum of irradiation	630 nm
Power density	40 mW/cm^2
Diagnostic maximum of irradiation	405 nm
Object distance	5-8 cm
Standard irradiation time	15 - 20 min
Power supply	12 V
Weight	1 kg
Dimensions – Length X Width X Height - whole system - LEDs head	 25 x 15 x 50 cm 20 x 12 x 5 cm

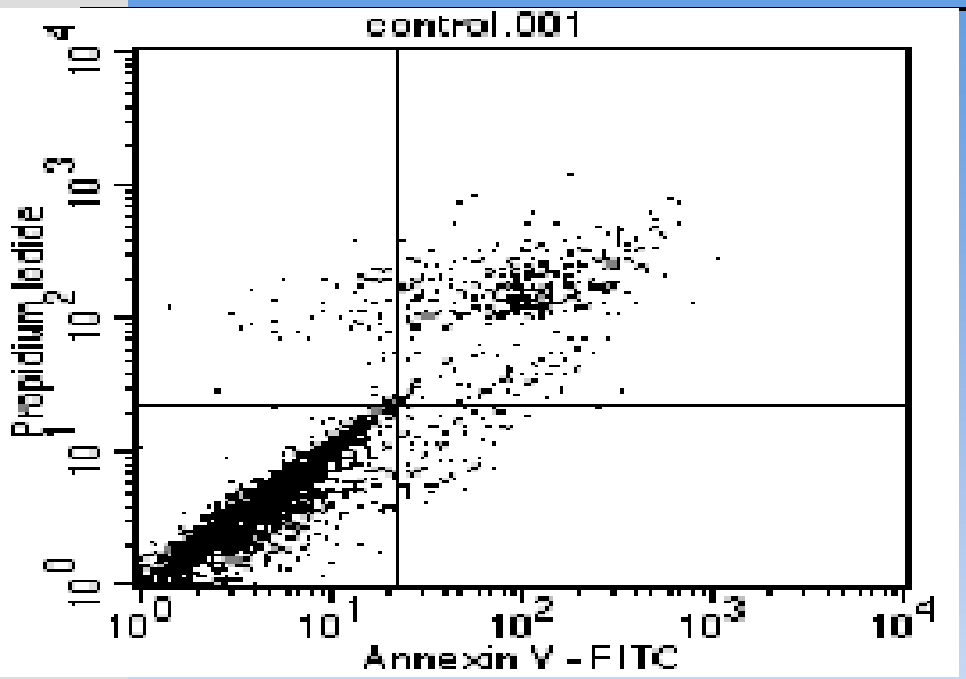
Apoptosis analysis

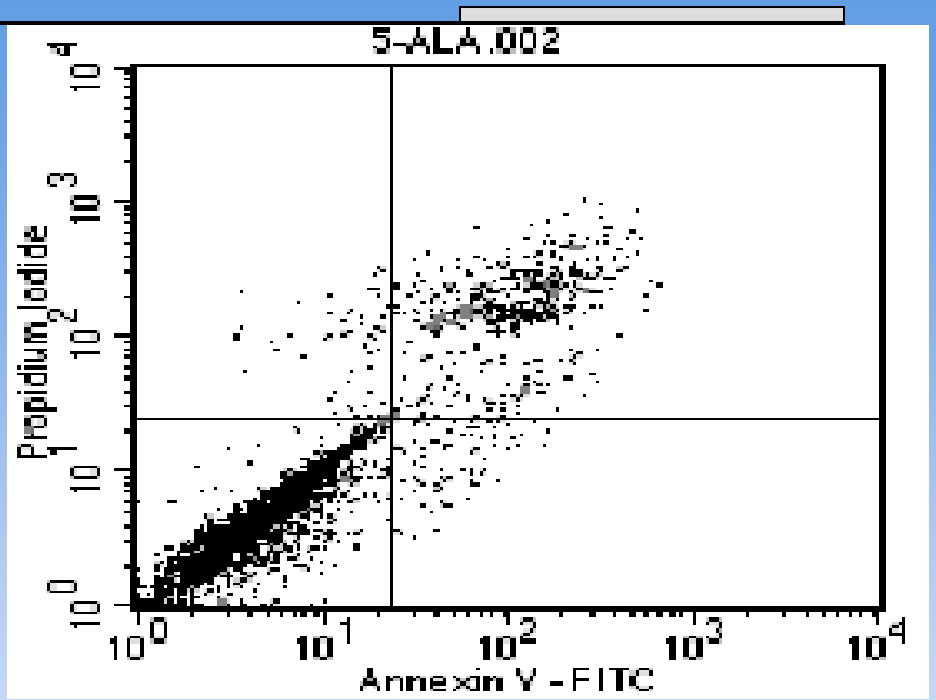
- The assessment of early apoptosis was made using Annexin V—FITC.
- Propidium Iodide was used for detection of late apoptosis.
- It was found out that the most efficient light parameters are 5 mW/cm^2 and a total light dose of 30 J/cm^2.

These values of light parameters gives the highest achieved difference in apoptosis levels (including early and late apoptosis) between control (A) and glioblastoma (B) cells.



Apoptosis - results





Quad	Events	% Gated
UL	183	1.83
UR	1054	10.54
LL	8272	82.72
LR	191	1.91

Quad	Events	% Gated
UL	215	2.15
UR	1597	15.97
LL	7962	79.62
LR	226	2.26

Contour plots of Annexin V –FITC vs Propidium Iodide of control (A) and glioblastoma cells with adherent morphology using 5 mW/cm^2 fluence rate

Conclusions

- Treatment of glioblastoma tumours continues to be a very serious issue and there is growing need in development of new concepts, methods and cancer-fighting strategies. PDT may contribute in accomplishing better results in cancer treatment and can be applied as well in combination with other techniques.

- Further experiments with increasing of the database are foreseen. The main idea of the experiment is to be established personalized PDT treatment of human glioblastoma tumours in hospital environment in our country.

Thank you very much for your attention!

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