EFFECTS OF SULFONATED ZINC PHTHALOCYANIN AND LOW INTENSITY LASER IRRADIATION IN INDUCING PHOTODYNAMIC DAMAGE IN BREAST CANCER CELLS (MCF-7)

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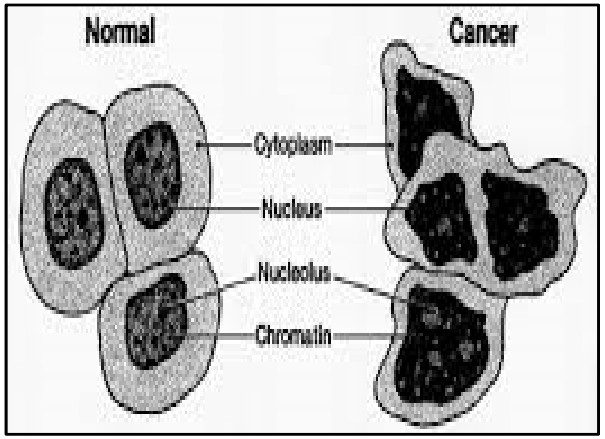
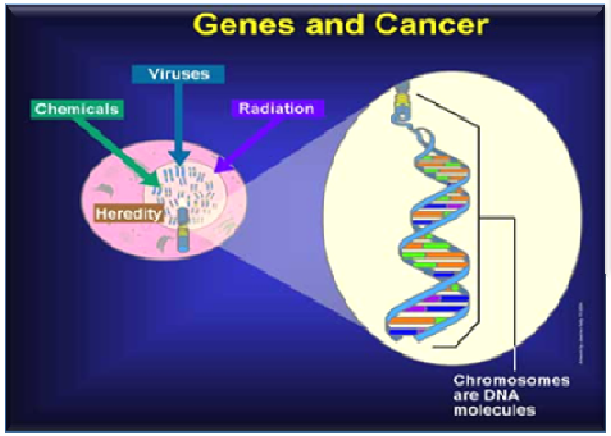
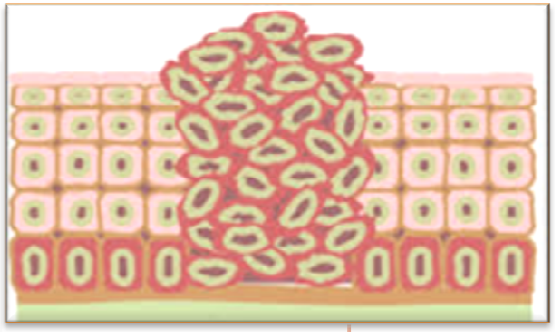
BACKGROUND

- The term "disease" refers to conditions that impair normal tissue function.
- Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues.
- Cancer cells stimulate their own growth, resist inhibitory signals, resist their own apoptosis, stimulate the growth of blood vessels to supply nutrients to tumours, multiply forever and invade local tissue and spread to distant sites.







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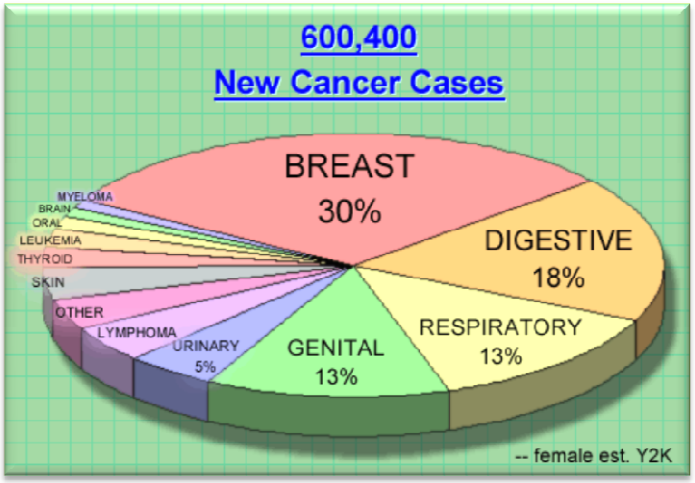
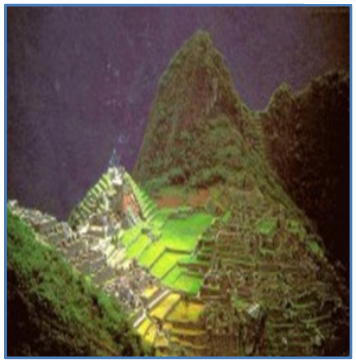
TIMELINE OF CANCER

Cancer is one of the leading causes of death in the world, particularly in developing countries



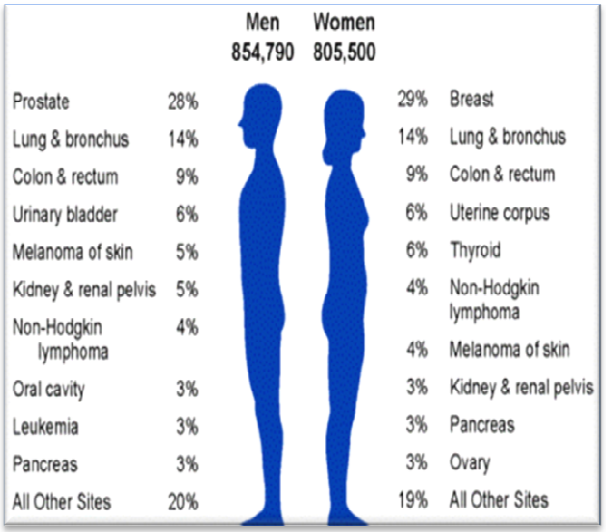
Egyptian Mummies
Evidences of cancer found in mummies from -3000

Egyptian Papyruses (-1600)
Treatment of certain cancers
Breast cancer may be one of the oldest in Human cancer



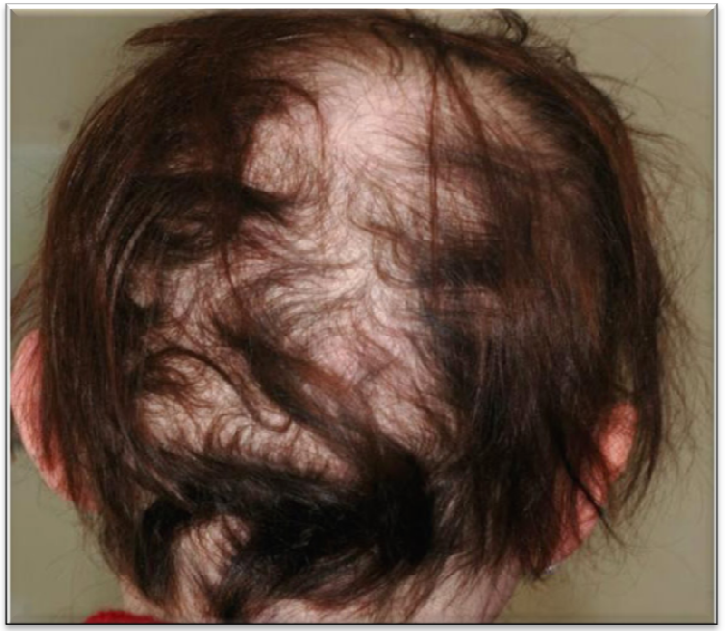
Incas of Peru (-400)
Evidences of cancer in pre-Columbian mummies

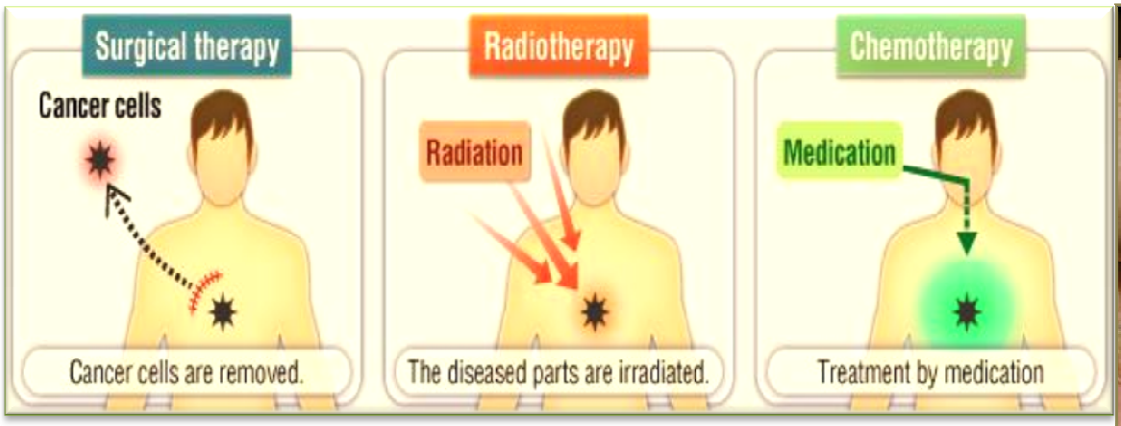
Hippocrates (-300)
Father of Medicine named ranges of tumors as carcinos

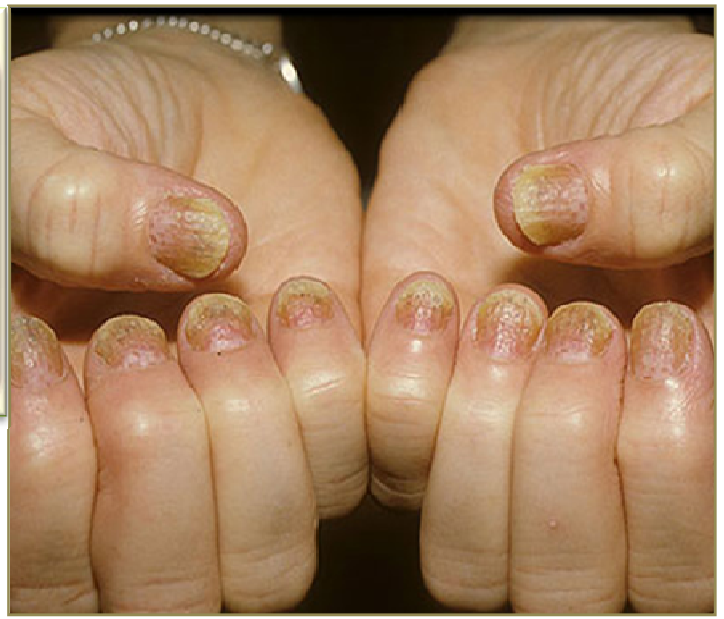


CONVENTIONAL CANCER THERAPY

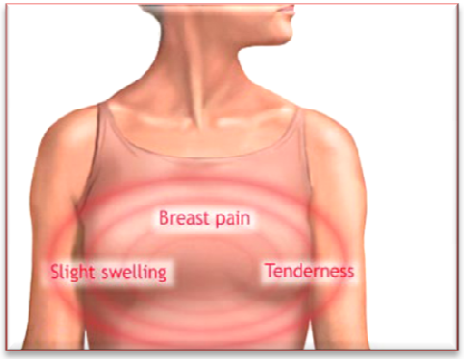
- Surgery
- Radiation therapy
- Chemotherapy
- Hormone therapy
- Immunotherapy

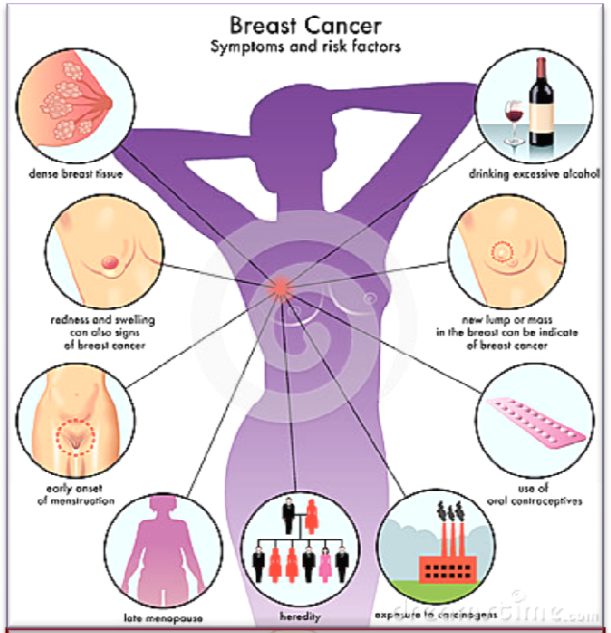


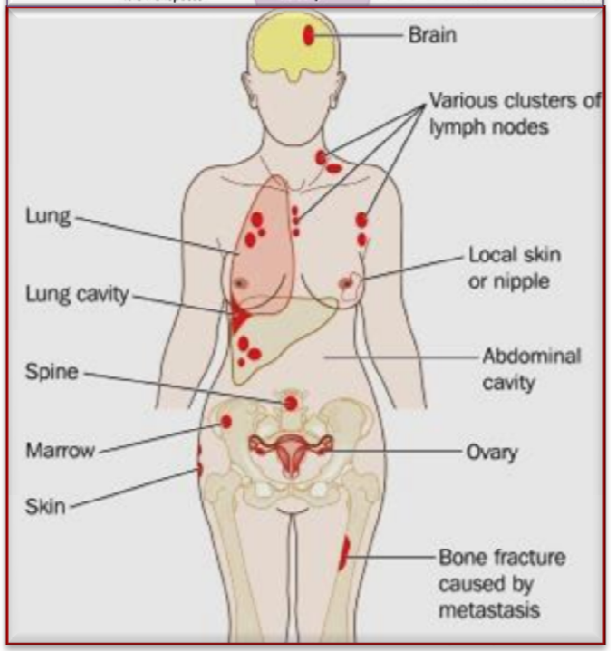




BREAST CANCER







- More than 100,000 South Africans are diagnosed with cancer every year
- Most commonly diagnosed cancer of women worldwide.
- Cancer in SA is a rising health concern with breast cancer being the leading cancer faced by SA women
- Risk factors: Gender, age, genetics, hormonal change, life style, diet, obesity, alcohol, smoking etc.

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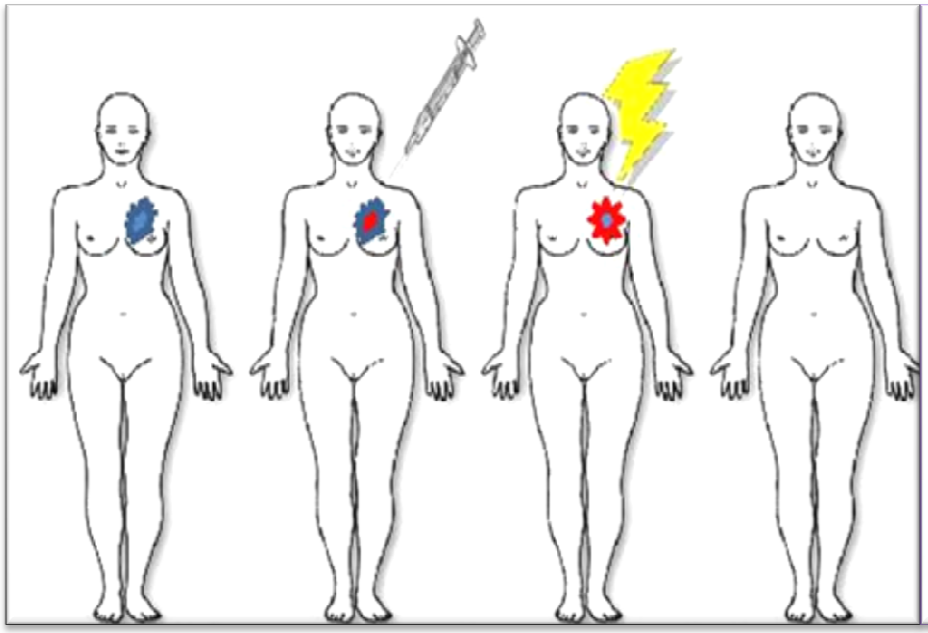
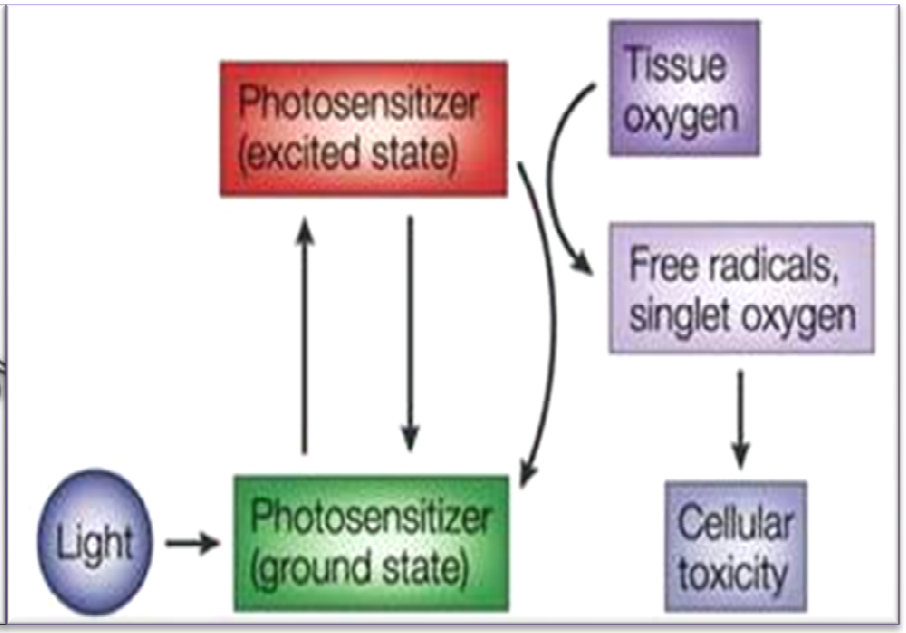
PHOTODYNAMIC CANCER THERAPY (PDT)

- PDT is an alternative therapy for the management of cancer
- Compared to conventional therapies, PDT is promising and efficient treatment because this is **neoplastic selective**
- PDT has been approved and recommended in the USA, Canada, Japan and most of the European Union countries
- A frequent problem encountered with PDT is the accessibility of tumour cells to laser light and the prolonged light sensitivity





OBJECTIVES OF THIS PROJECT

After determining the cellular localization of $ZnPcS_{mix}$ in MCF-7 breast cancer cells, optimal laser fluency and PS concentration:

- ✓ Identify the induced cell death pathway subsequent to PDT
- ✓ Evaluate the expression of genes involved in cell death following PDT

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MATERIALS AND METHODS

MCF-7 cell line

$ZnPcS_{mix}$
0.05, 0.1, 0.5 and 1 µM

Laser Irradiation
5, 10 and 15 J/cm²

Group 1
No Irradiation
No PS

Group 2
No Irradiation
PS

Group 3
Irradiation
No PS

Group 4
Irradiation
PS

Cell death
Flow cytometry
ELISA
RT-PCR Array

Tynga et al., J Photochem Photobio B 120 (2012)

CELL DEATH MECHANISM
FLOW CYTOMETRY: Different populations

	Untreated Control	$ZnPcS_{mix}$ control	Actinomycin D	PDT
Normal	89±1.07	91±0.32	24±0.67***	21±0.89***
Early apoptotic	4±0.89	5±1.04	34±0.38**	37±1.06**
Necrotic	3±0.43	2±0.81	11±0.74	13±0.94
Late apoptotic	4±0.52	2±1.12	31±1.23**	29±0.76**

Compared to untreated MCF-7 cells, stat sig shown as **p>0.1 and *** p>0.01

Tynga et al., , Photomed Laser Surg 3650(2014)

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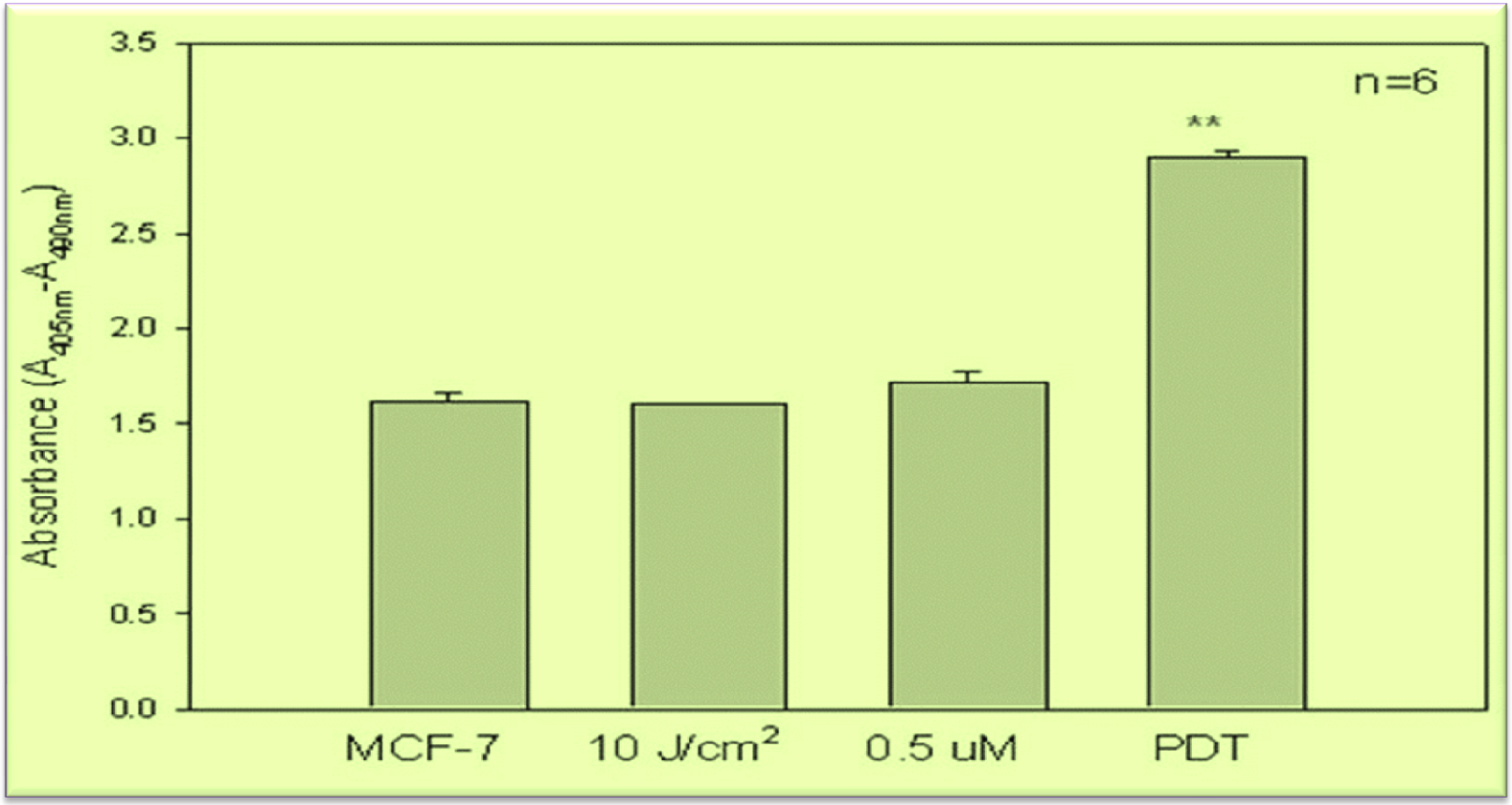
CELL DEATH MECHANISM
ELISA: Nuclear fragmentation



Compared to untreated MCF-7 cells, stat sig shown as **p>0.1

Tynga et al., , Photomed Laser Surg 3650(2014)

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CELL DEATH MECHANISM
RT-PCR Array: Gene expression

Cell death	Gene subunits
Pro-Apoptotic	ABL1, APAF1, BCL2L11, BIRC2 (c-IAP2), CASP1 (ICE), **CASP2** , CASP6, CASP7, CASP9, CD40 (TNFRSF5), CD40LG (TNFSF5), CFLAR (CASPER), **DFFA** , FASLG (TNFSF6), GADD45A, NOL3, TNFRSF10A (TRAIL-R).
Anti-Apoptotic	BCL2A1 (Bfl-1/A1), BIRC3 (c-IAP1), IGF1R, MCL1, TNFRSF11B, TRAF2, XIAP.
Apoptosis and Autophagy	AKT1, BAX, **BCL2** , BCL2L1 (BCL-X), CASP3, FAS (TNFRSF6), TNF, TP53.
Apoptosis and Necrosis	ATP6V1G2, CYLD, SPATA2, SYCP2, TNFRSF1A
Autophagy	APP, ATG12, ATG16L1, ATG3, ATG5, ATG7, BECN1, CTSB, CTSS, ESR1 (ERa), GAA, HTT, IFNG, IGF1, INS, IRGM, MAP1LC3A, MAPK8 (JNK1), NFKB1, PIK3C3 (VPS34), RPS6KB1, SNCA, SQSTM1, ULK1.
Necrosis	BMF, C1orf159, CCDC103, COMMD4, DEFB1, DENND4A, DPYSL4, EIF5B, FOXI1, GALNT5, GRB2, HSPBAP1, JPH3, KCNIP1, MAG, OR10J3, PARP1 (ADPRT1), PARP2, PVR, RAB25, S100A7A, TMEM57, TXNL4B.

BCL-2	**Apoptotic gene and expressed in response to high level of apoptogenic proteins**
CASP-2	**Protease and involved in Cytochrome C and apoptogenic proteins release from mitochondria**
DFFA	**DNA fragmentation factor and activated by Cas-3**

Tynga et al., Photomed Laser Surg 3650(2014)

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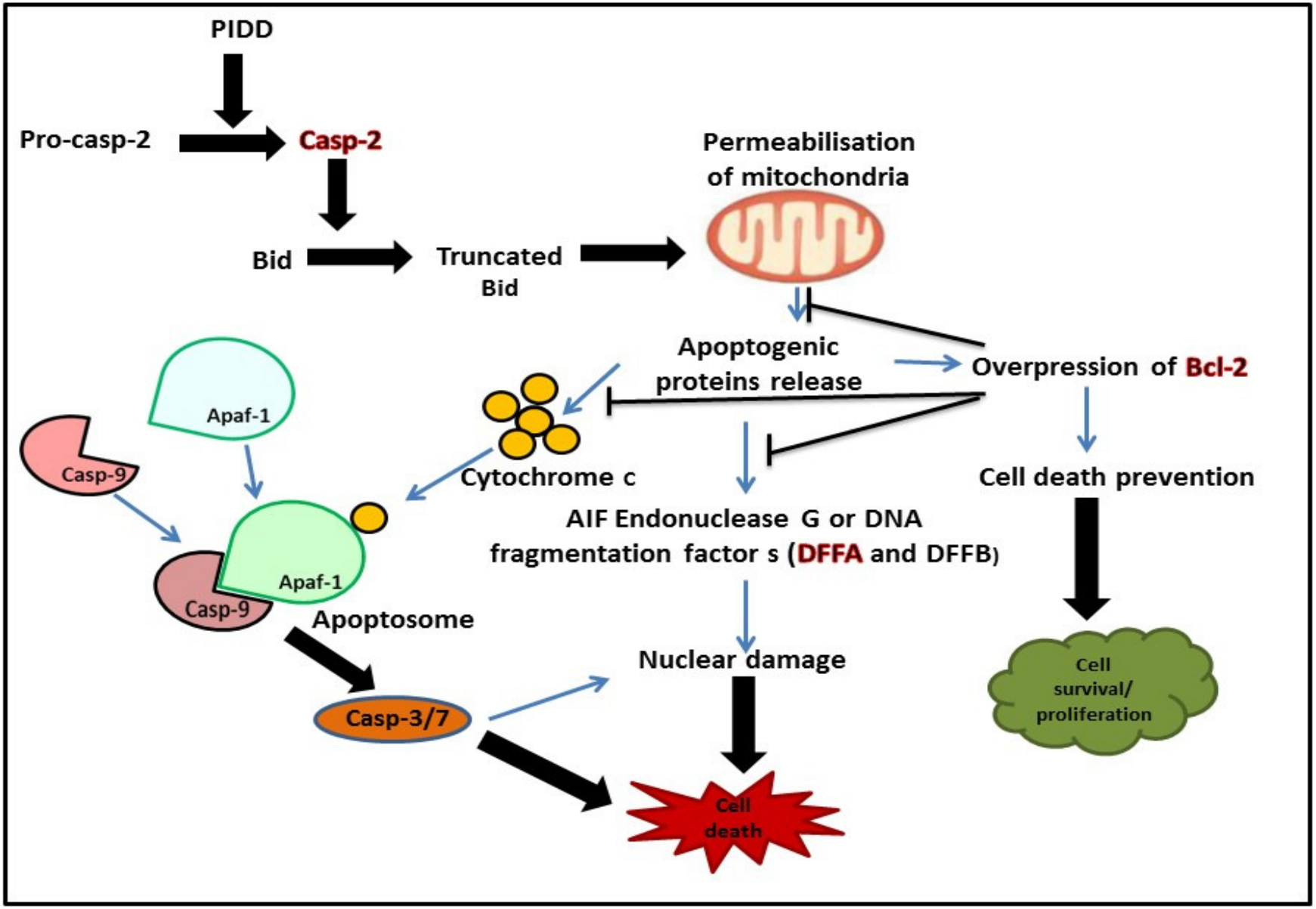
PROPOSED CELL DEATH MECHANISMS



CONCLUSION

- $ZnPcS_{mix}$ mediated PDT was able to induce cell death; 0,5 µM $ZnPcS_{mix}$ and 10 J/cm^2 was found to be combination with a decrease around 50% viability and the photosensitiser localised in lysosomes, mitochondria and around nuclei

- Most cells were apoptotic, nuclear condensation and fragmentation as well as three genes (BCL-2, CASP-2 and DFFA) involved in the apoptotic pathway were found to be up-regulated

- **Future Work**: Antibodies, Gold nanoparticles, $ZnPcS_{mix}$

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obrigado
Dank U
Merci
mahalo
Köszi
спасибо
Grazie
Thank you
mauruuru
Takk
Gracias
Dziękuję
Děkuju
danke
Kiitos