JAGIELLONIAN UNIVERSITY IN KRAKÓW

J-PET

Studies of alive normal and cancer cell lines and tissues in vitro with Positron Annihilation Lifetime Spectroscopy

Ewelina Kubicz

5.09.2019

15th International Workshop on Slow Positron Beam Techniques & Applications (SLOPOS-15)

European Funds Smart Growth
Republic of Poland
FNP Foundation for Polish Science
European Union European Regional Development Fund

Outline

1. Motivation
2. Cancer vs normal cells
3. PALS studies of tumor and normal tissues in vitro
4. PALS studies of cells cultures in vitro
5. Summary and future plans

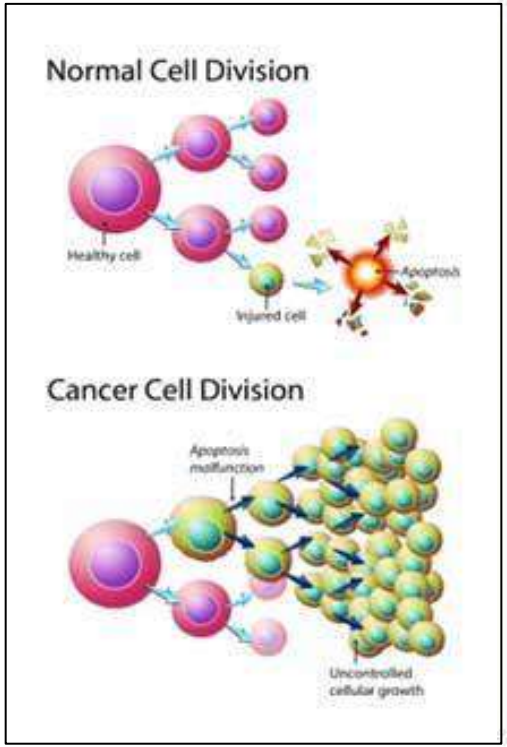
1. Motivation

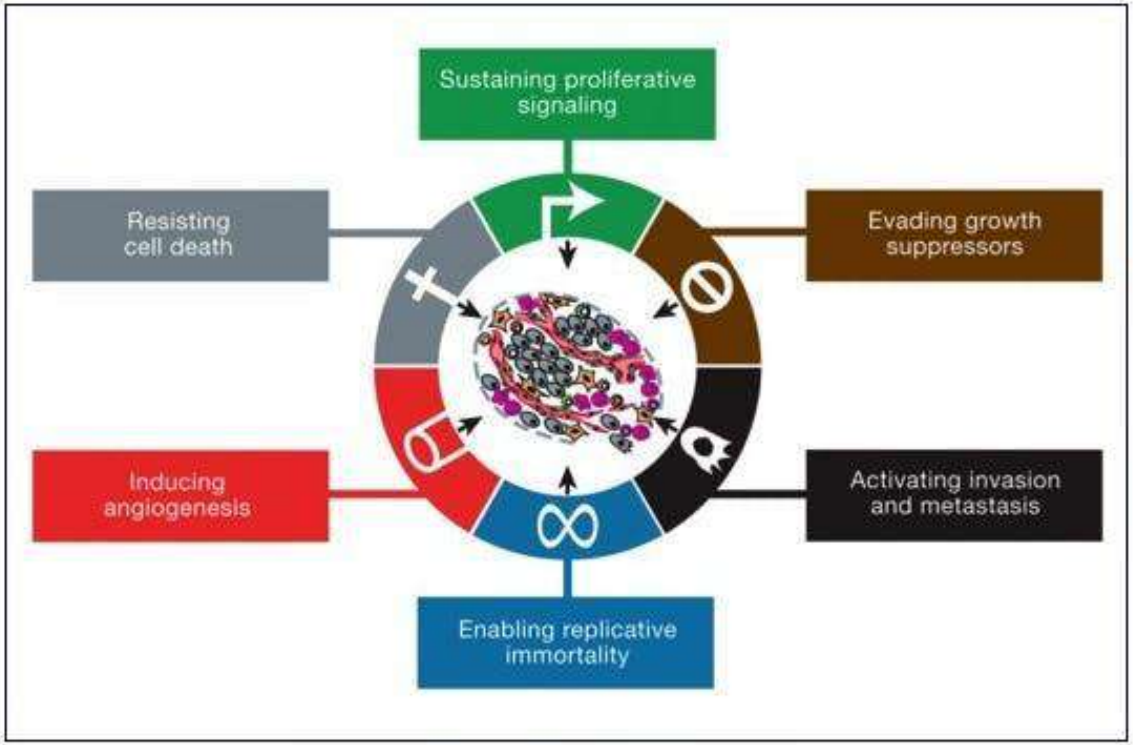
→ Positronium lifetime and intensity are related with temporal dynamics of nanostructures in cells and tissues

→ Possibility to determine early and advanced stages of carcinogenesis by observing changes in biomechanical parameters between normal and cancer cells

→ Combining J-PET scanner with PALS technique – new biomarker in cancer diagnosis

2. Cancer vs normal cells





Cell 144, March 4, 2011 646-674

https://www.dietdoctor.com/6-common-features-cancer

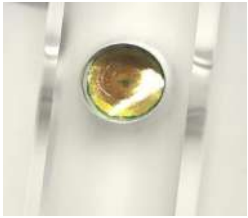
3. PALS setup

→ Two BaF_2 detectors with resolution ~250 ps (FWHM)

→ ^{22}Na source in Kapton foil with activity ~ 1 MBq sandwich between sample

→ PALS spectra analysis with PALS Avalanche program developed by K. Dulski – J-PET collaboration

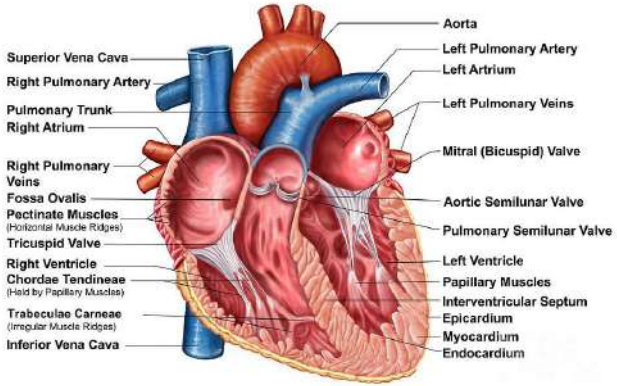
K. Dulski et al., Analysis procedure of the positronium lifetime spectra for the J-PET detector, Acta Phys. Polon. B48 no. 10, 1611 (2017)



3. PALS studies of tumor and normal tissues in vitro

Cardiac Myxoma

→ Primitive connective tissue tumor (benign), very rare in comparison to metastatic tumors
→ 75 % of them are located in the left atrium
→ Occur mainly in people over the age of 50



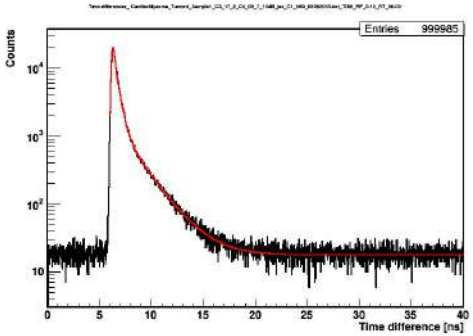
https://healthjade.com/human-heart-health/

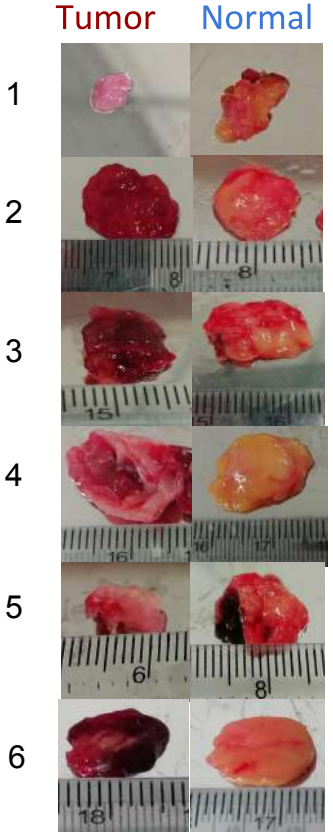
Non fixed - tumor sample measured with mediastinal adipose tissue for comparison

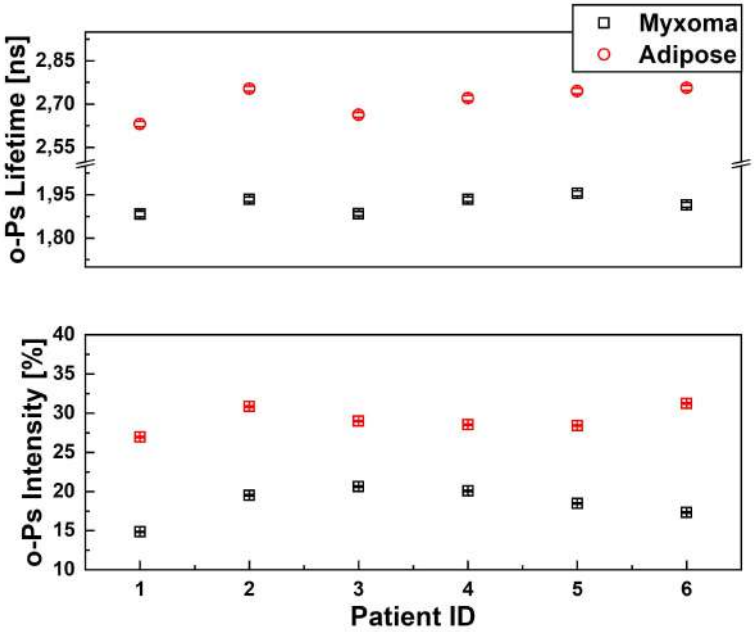
Patient ID	Sex	Age
1	man	70
2	man	58
3	woman	59
4	woman	85
5	woman	60
6	man	64

3. PALS - tissues in vitro - not fixed

Cardiac Myxoma - not fixed



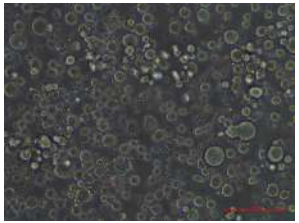




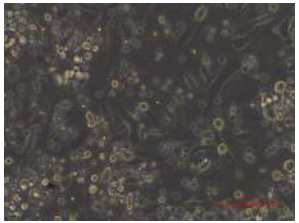
3. PALS - cell vs tissues in vitro - not fixed

Cardiac Myxoma

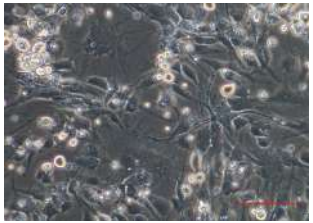
→ Tumor sample placed in Collagenase II 200 U/ml soluble in DMEM+10% FBS + P/S for 48 h
→ Squeezed through 70 um nylon mesh to isolate cells from extracellular matrix
→ Cells were seeded on flasks and culture in DMEM + 10% FBS + P/S



0 h

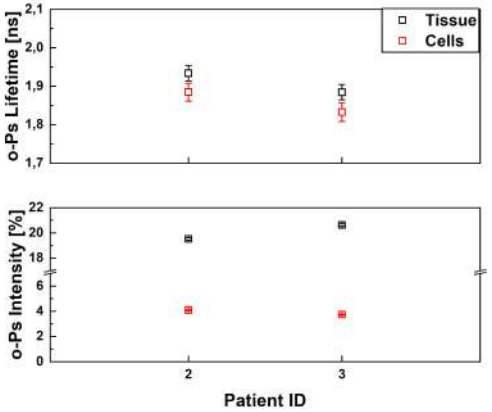


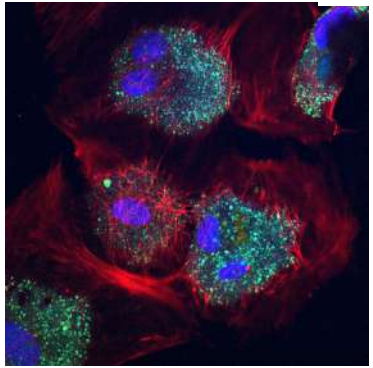
24 h

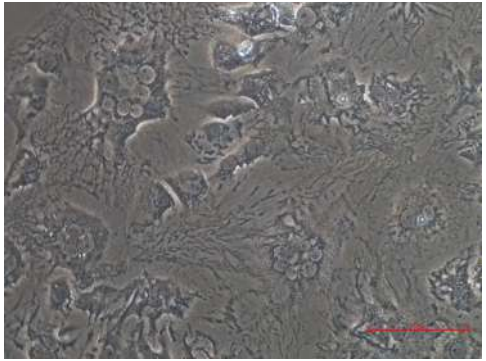


72 h

Patient ID	Sex	Age
2	man	58
3	woman	59







4. PALS studies of cells culture in vitro

Human cell lines:
1) Melanocytes HEMa-LP from ThermoFisher
2) Melanoma WM115 from ATCC
3) Melanoma WM266 from ATCC

→ Cultured in M254/RPMI 1640 medium supplemented with HGMS-2/10% Fetal Bovine Serum, Penicillin 100U/ml and Streptomycin 100 ug/ml
→ Culture was incubated at 37°C in 5% CO_2 humidified atmosphere rinse

HEMa-LP

WM115

WM266

Each samples contains cells from 8 x 75cm^2 flasks, harvest upon 100% confluation (>10^8 cells).

Alive Cells in 37 C deg.

With EGCG or Vit C

HEMa-LP

WM115

WM266

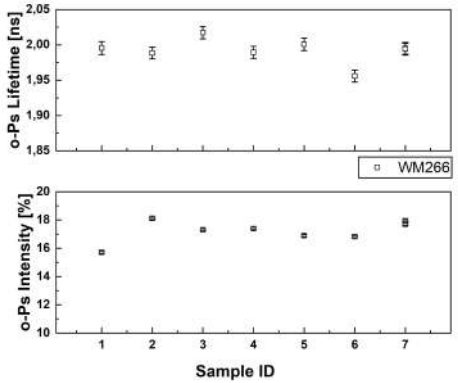
4. PALS - cells culture in vitro

Human cell lines:

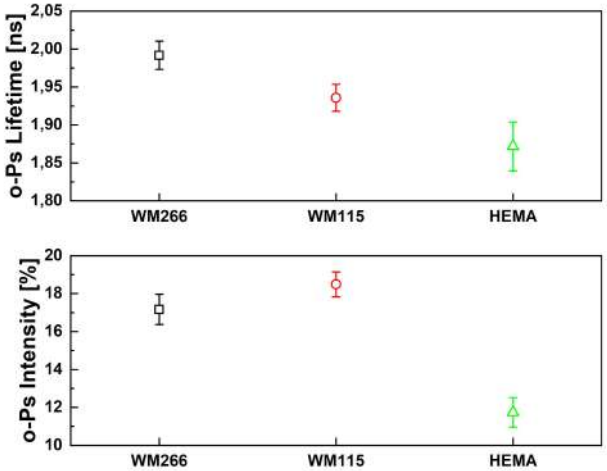
1) Melanocytes HEMa-LP from ThermoFisher
2) Melanoma WM115 from ATCC
3) Melanoma WM266 from ATCC

→ Cells were measured in 37 C deg. for 1 h

Rate of change =
$100\% *(V_{before} - V_{after})/ V_{after}$



Cell Line	Viability Rate of change [%]
HEMa-LP	2.06
WM115	5.22
WM266	2.03



→ For WM266 cell line 7 repetitive measurements were done

→ Results from all are the in the line with each other within 2 sigma uncertainty

→ 3 repetition from each cell line were measured

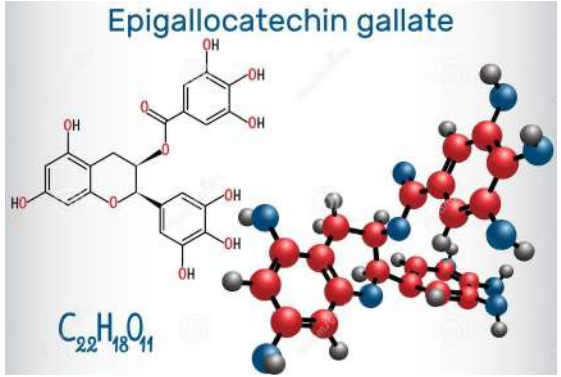
→ Given results are calculated as an average value from all repetitive measurements

4. PALS - cell culture with Vitamin C and EGCG

FR scavengers → eg. antioxidants, prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition.

Vitamin C (L-ascorbic acid) → found in various foods, functions as an antioxidant

EGCG (Epigallocatechin gallate) → found mostly in green tea, 100x more powerful antioxidant than Vit. C



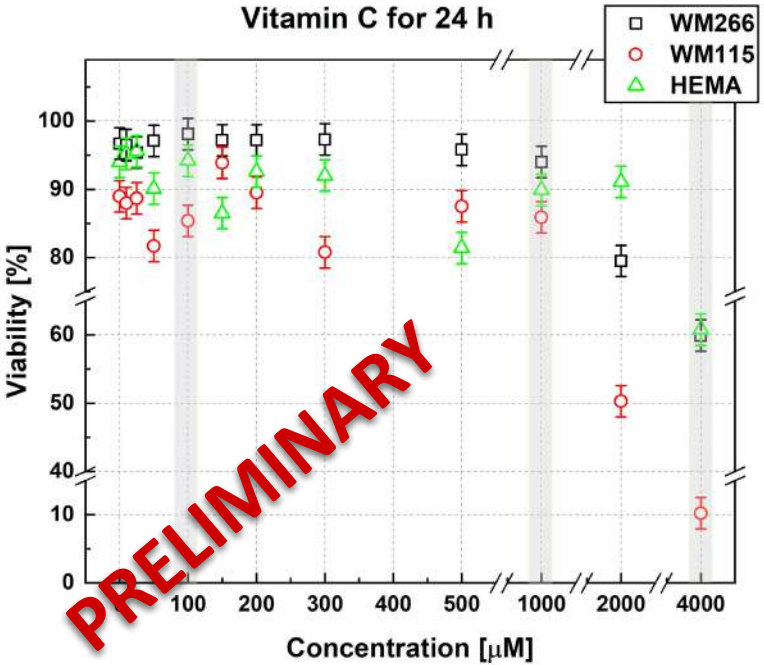
→ Before PALS measurement each flask was incubated for 2 h with media and antioxidant substance in given concentration

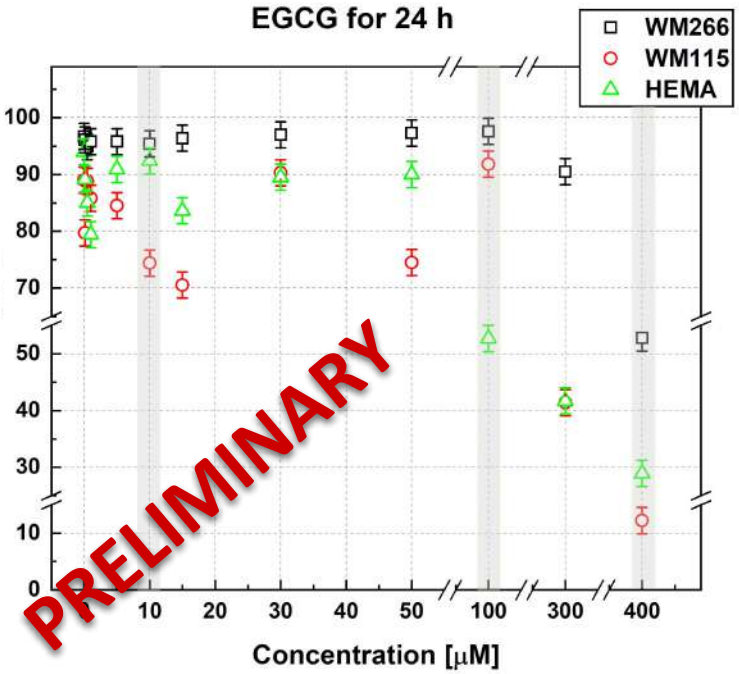
→ Again media with antioxidant in the same concentration was added during passage before centrifuging cells for PALS measurement

https://www.123rf.com/photo_106233294_stock-vector-ascorbic-acid-vitamin-c--structural-chemical-formula-and-molecule-model-vector-illustration.html
https://www.dreamstime.com/epigallocatechin-gallate-egcg-most-abundant-catechin-i-tea-structural-chemical-formula-molecule-model-vector-image110021327

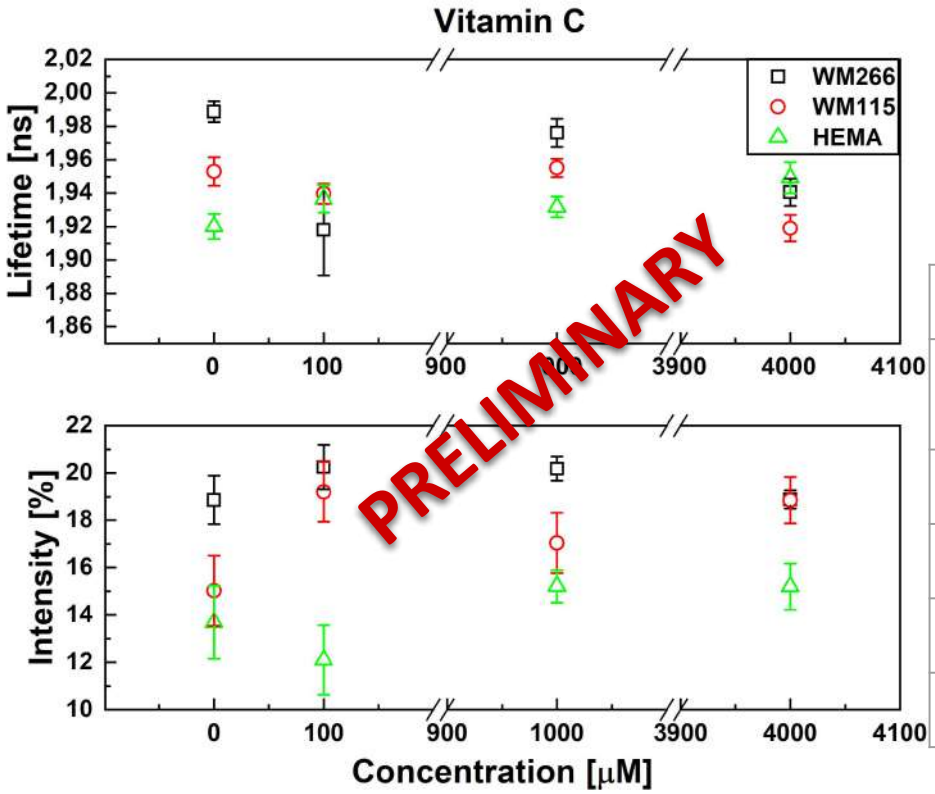
4. PALS - cell culture with EGCG and Vit. C

Cytotoxicity of EGCG and Vit C. → cell viability was checked after 24 h incubation in given concentration





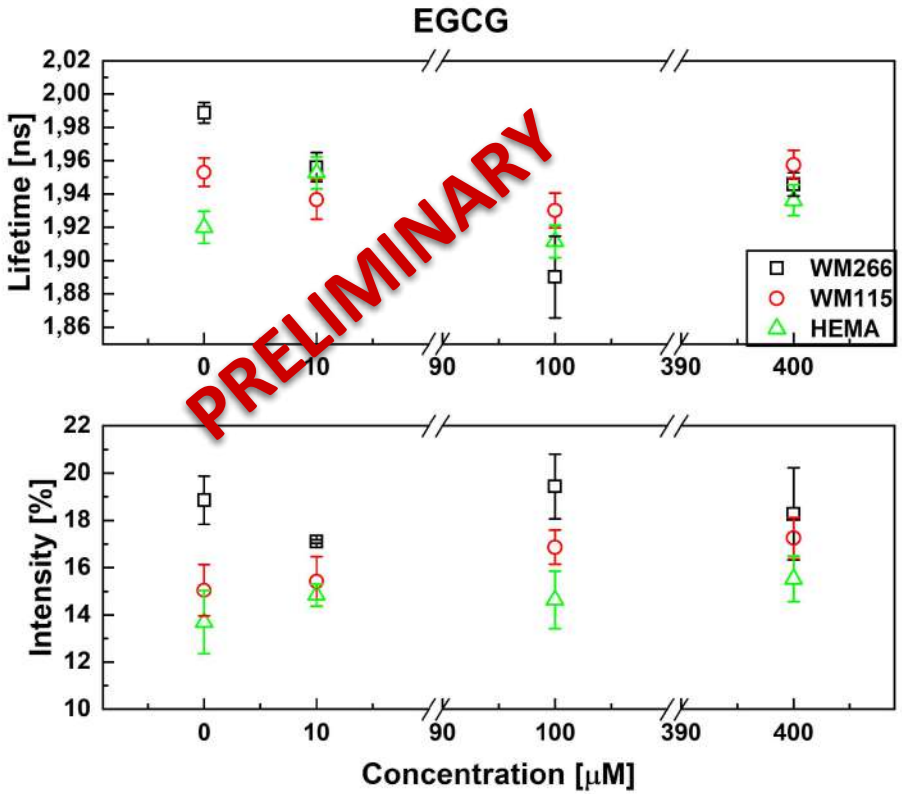
4. PALS - cell culture with Vit. C



Rate of change =
$100\% *(V_{before} - V_{after})/ V_{after}$

Vit. C	HEMA	WM115	WM266
Concentration [uM]	Viability RoC [%]	Viability RoC [%]	Viability RoC [%]
0	2,13	5,99	0,21
100	10,31	4,75	0,52
1000	4,76	1,68	0,31
4000	7,14	4,00	0,32

4. PALS - cell culture with EGCG



Rate of change =
$100\% *(V_{before} - V_{after})/ V_{after}$

EGCG	HEMA	WM115	WM266
Concentration [uM]	Viability RoC [%]	Viability RoC [%]	Viability RoC [%]
0	2,13	5,99	0,21
10	6,00	0,44	1,36
100	9,46	4,18	0,52
400	4,10	0,11	0,72

5. Summary and future plans

→ PALS is applicable to study biological structures

→ Preliminary results shown that PALS parameters differ for normal and cancer cells and tissue

→ Studies with alive cell cultures and tissues – comparing normal vs cancer → increasing statistic

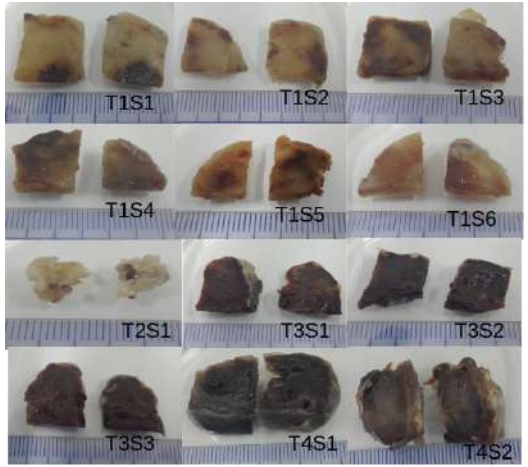
→ Checking for possible o-Ps formation model in living cells

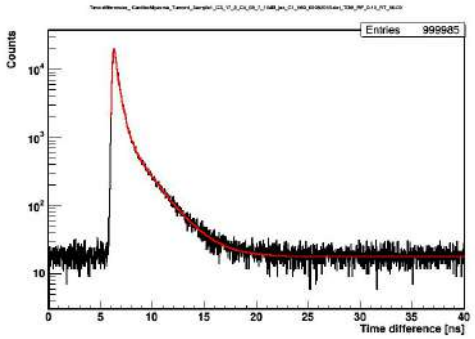
Thank you for attention

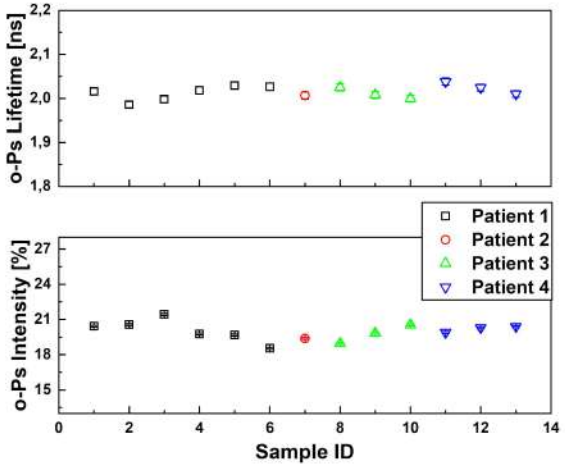
3. PALS - tissues in vitro - fixed

Cardiac Myxoma - fixed

Fixed in formalin, samples ~2 mm thick





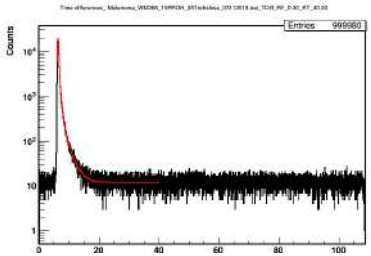


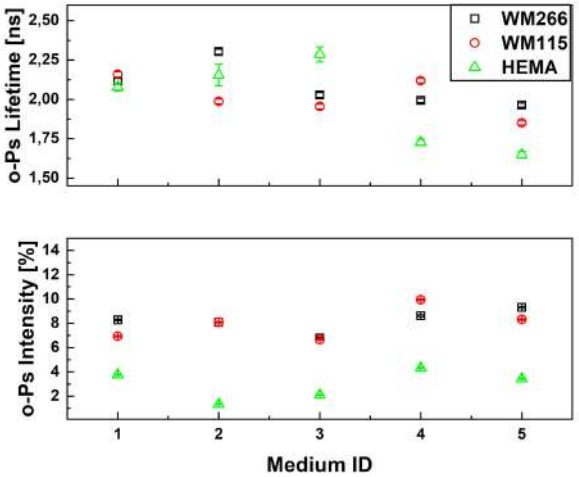
Patient ID	Sex	Age	Sample ID
1	woman	72	1-6
2	man	61	7
3	man	59	8-10
4	woman	54	11-13

4. PALS - cells culture - freeze dried

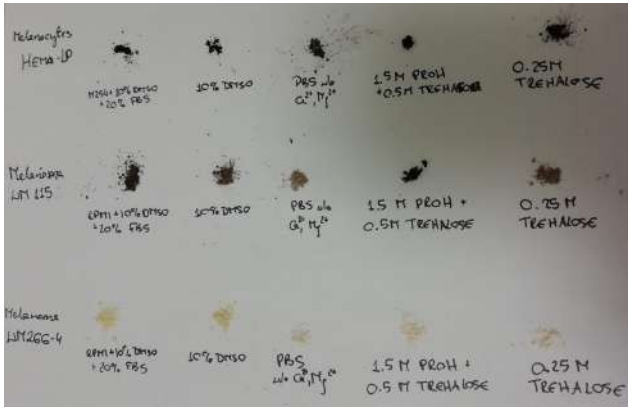
Human cell lines:

1) Melanocytes HEMa-LP from ThermoFisher
2) Melanoma WM115 from ATCC
3) Melanoma WM266 from ATCC





Cells were freeze - dried in -80 C deg., 0.0375 mbar for 24 h



Freeze Mediums:

1) M254/RPMI 1640+ P/S+ 20% FBS + 10% DMSO
2) 10% DMSO + PBS w/o $^{2+}$Ca, $^{2+}$Mg
3) PBS w/o $^{2+}$Ca, $^{2+}$Mg
4) 1.5 M PROH(propylene glycol) + 0.5 M D-trehalose in PBS w/o $^{2+}$Ca, $^{2+}$Mg
5) 0.25 M D-trehalose in PBS w/o $^{2+}$Ca, $^{2+}$Mg