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Development and Evaluation of AT11-guided Liposomes for Human Papilloma Virus cancer

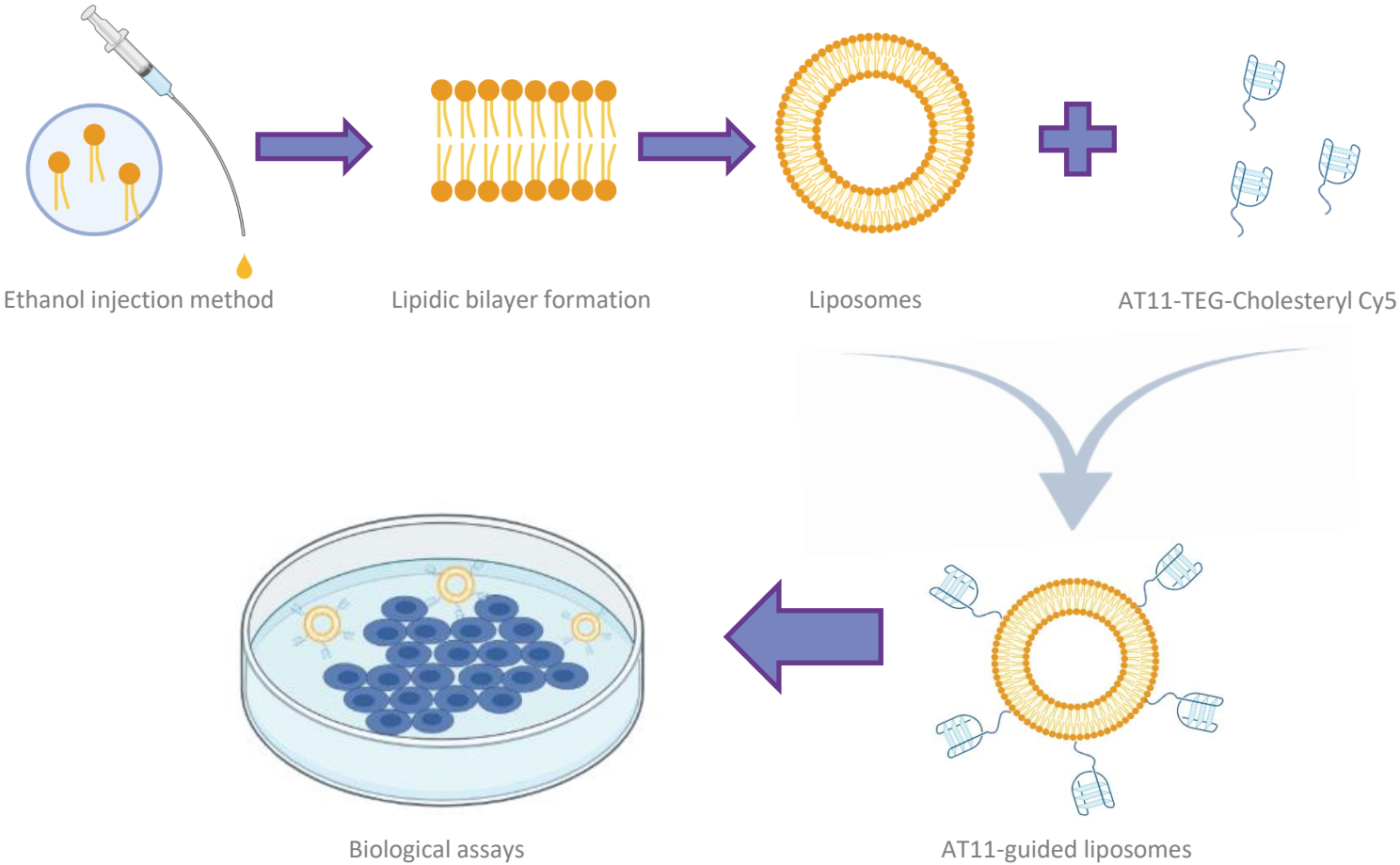
Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals MDPI

Jéssica Lopes-Nunes[1], Paula A. Oliveira[2], and Carla Cruz [1*]

[1] CICS-UBI Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal;
[2] Centro de Investigação e Tecnologias Agroambientais e Biológicas (CITAB), Inov4Agro, Universidade de Trás os Montes e Alto Douro (UTAD), Quinta de Prados, 5000-801 Vila Real, Portugal
* Corresponding author: carlacruz@fcsaude.ubi.pt

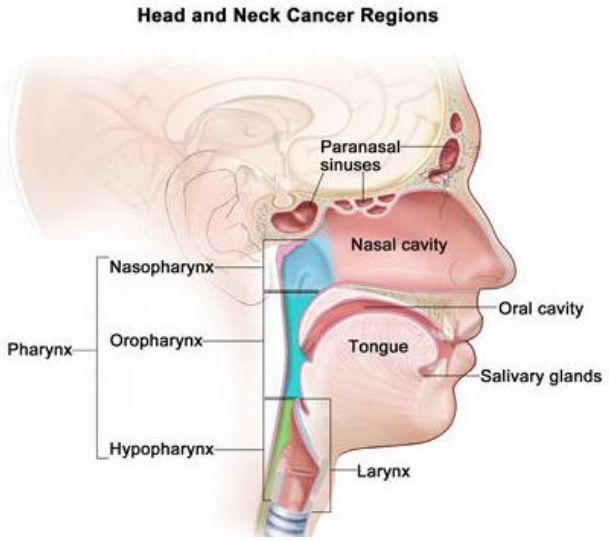
Development of AT11-guided Liposomes for Human Papilloma Virus

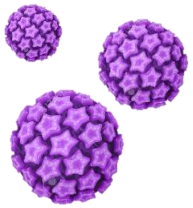


Conventional anticancer therapies present low specificity, leading to several secondary effects. To improve these drawbacks, aptamers able to fold into G-quadruplex (G4) are being used to promote drug accumulation in cancer cells. AS1411 is a G4 aptamer able to recognize nucleolin, a protein overexpressed in cancer cells' surface. This aptamer was tested in phase II clinical trials but showed low response rates and suboptimal pharmacokinetics. Nevertheless, AS1411 is being used as targeting agent. Moreover, AS1411 derivatives were developed, with improved toxicity and high affinity to nucleolin. Thus, we propose to use AT11, an AS1411 derivative, to functionalize liposomes and improve the selectivity of C8 (a potential anticancer drug) into oral cancer. Therefore, we produced liposomes (blank or C8-associated) by ethanol injection method to, then, functionalize with AT11-TEG-Cholesteryl. The resulting liposomes were characterized by DLS. C8 association was determined by UV/vis spectroscopy and the AT11 functionalization was determined by SDS-PAGE. The effect of blank and C8-associated liposomes on oral cancer and healthy cells' viability was determined by MTT and its internalization of was visualized by confocal microscopy. Liposomes with hydrodynamic diameters of 148-168 nm were obtained and C8 was efficiently associated (~100%). When the cells were treated with blank liposomes, cell viability was almost unaffected. After treating with C8-associated liposomes, both cell lines showed a dose-response effect. Additionally, we observed that AT11-liposomes can internalize and reach the cytoplasm of cells. Overall, these findings suggest that the tested liposomes are promising drug carriers for oral cancer therapy.

Keywords: Aptamer; liposomes; oral cancer.

Head and neck squamous cell carcinomas (HNSCC) has been a steady rise.





HPV16 is the predominant HPV type responsible for this disease

Standard Care for HNSCC patients

- Surgery;
- Chemotherapy;
- Radiotherapy.

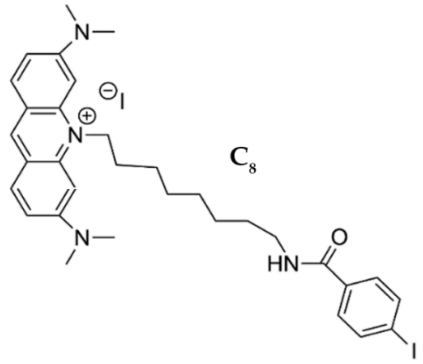
Adverse effects on life quality and mortality are associated with these treatments

Yete, et al. Oncology (doi:10.1159/000485322)

Improving treatment options for HNSCC patients infected with HPV16 is a priority

No effective treatment for HPV infection

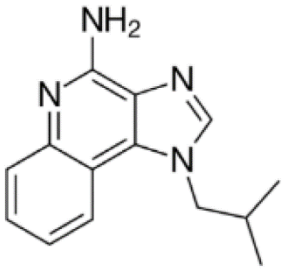
Acridine orange derivative (C_8)



- G4-ligand able to stabilize G-rich regions present in the HPV genome
- Potential anticancer effect
- High non-specific toxicity

Carvalho et al., Biochimie (doi:10.1016/j.biochi.2017.11.004)

Imiquimod (IQ)



- Boost the adaptative immune response, inducing high levels of IFN and TNF-alfa
- Induces apoptosis of tumor cells through caspase activation
- Effective in lesions caused by low-risk HPV, but it has been suggested that it may also have anticancer effects in malignant cells resulting from high-grade HPV infections
- Uncommonly systemic side effects

Yuan et al., Hum Vaccin Immunother. (doi:10.1080/21645515.2018.1445947)

Aptamers

Single-stranded oligonucleotides

Recognize and bind specifically

Targets

- Proteins
- Small molecules
- Ions
- Whole cells

Suitable drug-carriers to achieve an effective drug delivery

G4 Aptamers

Benefits comparatively to monoclonal antibodies:

- ✓ Do not stimulate an immune response;
- ✓ Higher stability;
- ✓ Easier to manufacture and store;
- ✓ Smaller size;
- ✓ Cheaper.

G4 structures have a higher negative charge density than duplex DNA, which favors their interaction with cationic proteins or small molecules

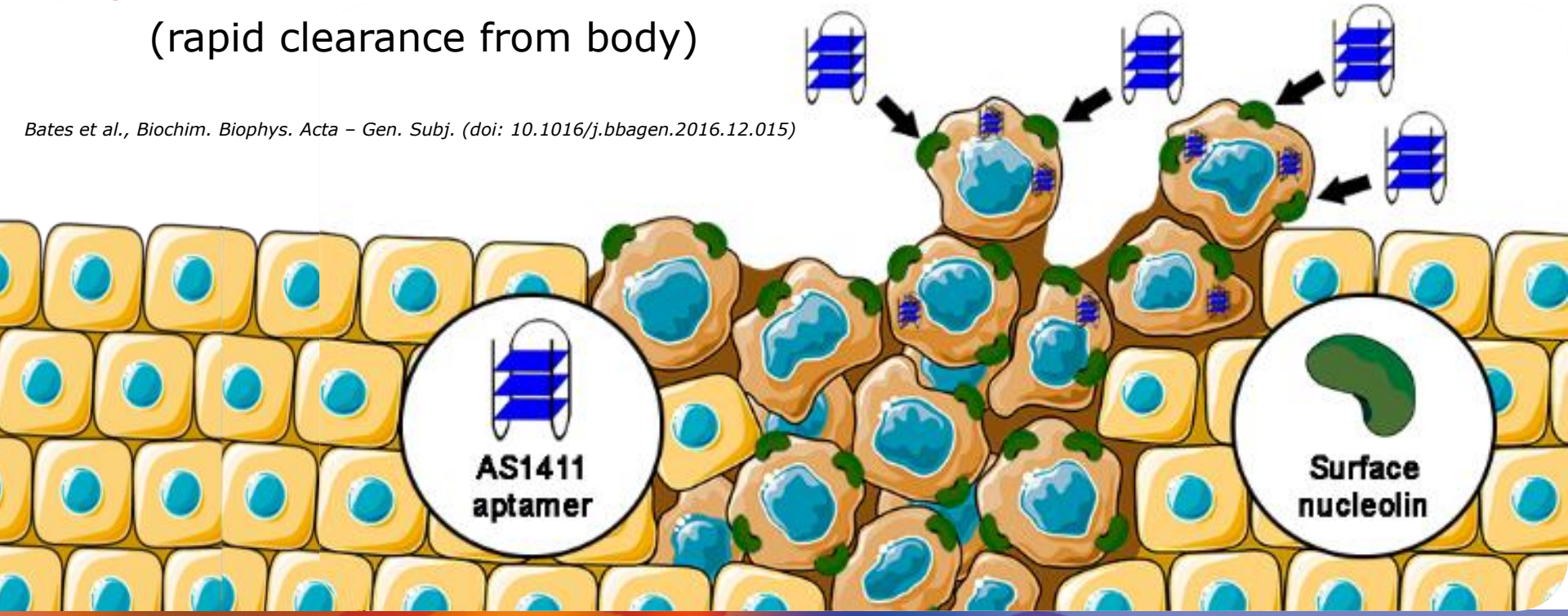
Alshaer et al., Adv. Drug Deliv. Rev. (doi: 10.1016/j.addr.2018.09.011);
Carvalho et al., Trends Mol. Med. (doi:10.1016/j.molmed.2020.05.002).

AS1411

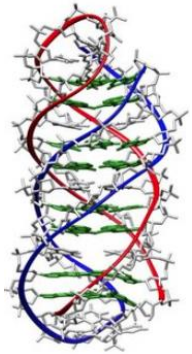
Reached human clinical trials Phase 2

- ✗ Shown low potency (μM)
- ✗ Suboptimal pharmacology (rapid clearance from body)
- ✓ Proved to be safe for humans

Bates et al., Biochim. Biophys. Acta – Gen. Subj. (doi: 10.1016/j.bbagen.2016.12.015)



AS1411



Nucleolin

Overexpressed on the cell membrane of cancer cells

Proliferation → Induces cancer cell death

- ✓ Inhibition of NF-κB activation;
- ✓ Block of DNA replication;
- ✓ Initiation of cycle arrest and apoptosis;
- ✓ Inhibition of cellular viability by overexpression of p53;
- ✓ Downregulation of Akt1 and Bcl-2.

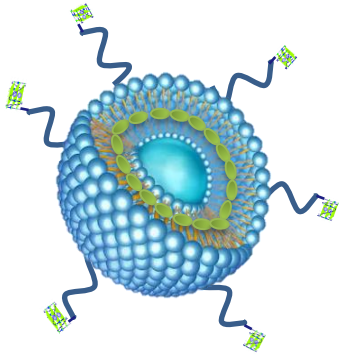
AT11 G4 → **AS1411 derivative with improved *in vitro* efficacy and nucleolin affinity**

- ✓ Cancer-selective properties of AS1411 and its derivatives may be used for conveying therapeutic compounds for HPV-positive cells.
- ✓ Synthesis of aptamer-based nanosystems as potential drug delivery system that can enhance malignant cell selectivity/accumulation, increase toxicity and efficiently deliver chemotherapeutic drugs.

Lopes-Nunes et al., Pharmaceuticals. (doi:10.3390/ph14070671)

Liposomes

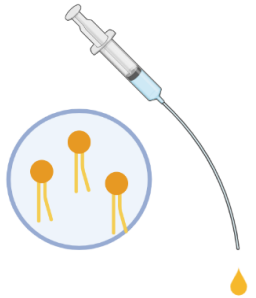
One of the widest used and successful platforms for drug delivery.



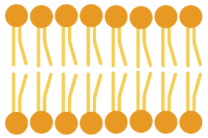
- ✓ Biocompatible;
- ✓ Low immunogenicity;
- ✓ Biodegradable;
- ✓ Lowered systemic toxicity of drugs;
- ✓ Prolonged drug half-life;
- ✓ Liposomes loaded with anticancer drugs (e.g., doxorubicin) are already available for the treatment of several cancers;
- ✓ Shown to increase the plasma residence time of aptamers.

AIM: To produce AS1411 derivatives-functionalized liposomes to improve the selectivity of Imiquimod and C8 into oral cancer cells.

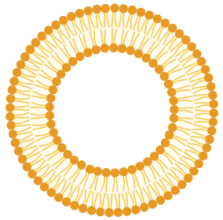
Ethanol Injection Method



Unfavorable interaction of hydrophobic tail with water

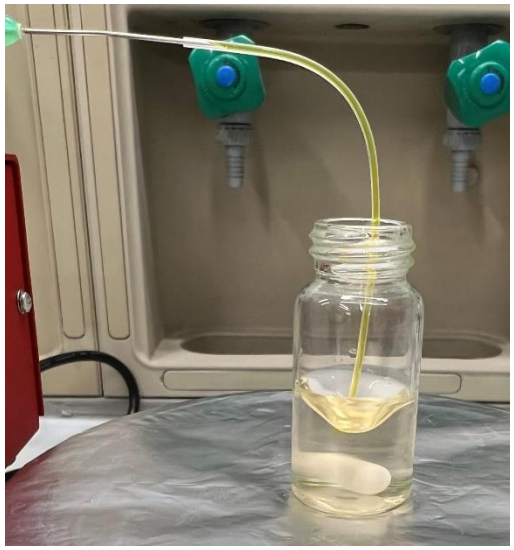


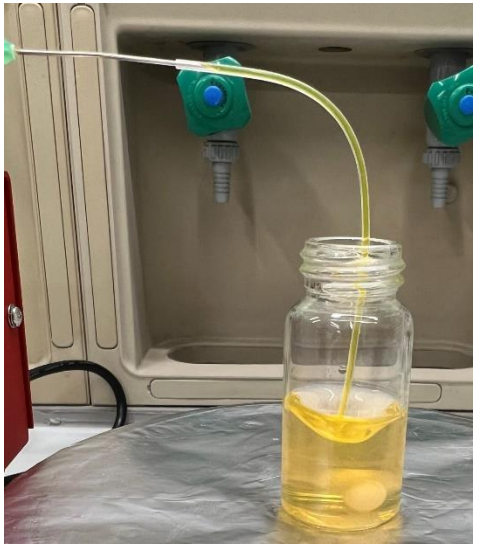
Start aggregation/ Formation of lipidic bilayer



Liposome suspension in water

Ethanol → **Water**

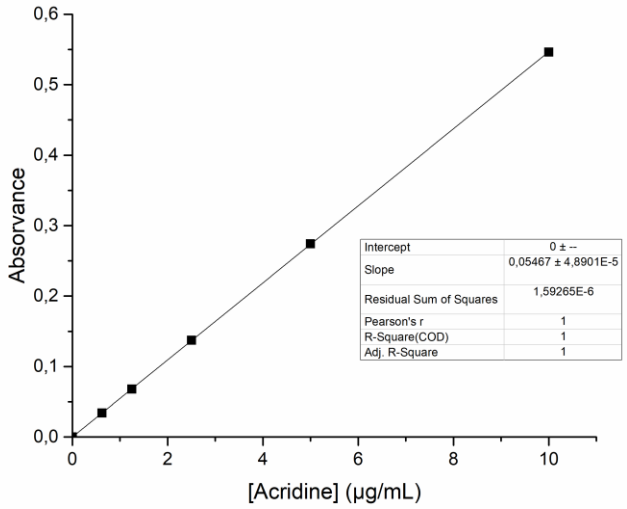




Liposomes were prepared with:
Phosphatidylcholine (PC; 130 mg/mL) + Cholesterol (CHOL; 10/12.5/15 mg/mL)

	Z-average	PDI	Zeta Potential
Blank PC 130 CHOL 10	168.7	0.112	-4.11
PC 130 CHOL 10 + C_8	166.9	0.197	12.4
Blank PC 130 CHOL 12.5	160.6	0.163	-5.61
PC 130 CHOL 12.5 + C_8	164.3	0.202	12.2
Blank PC 130 CHOL 15	147.8	0.147	-8.59
PC 130 CHOL 15 + C_8	150.2	0.178	10.0

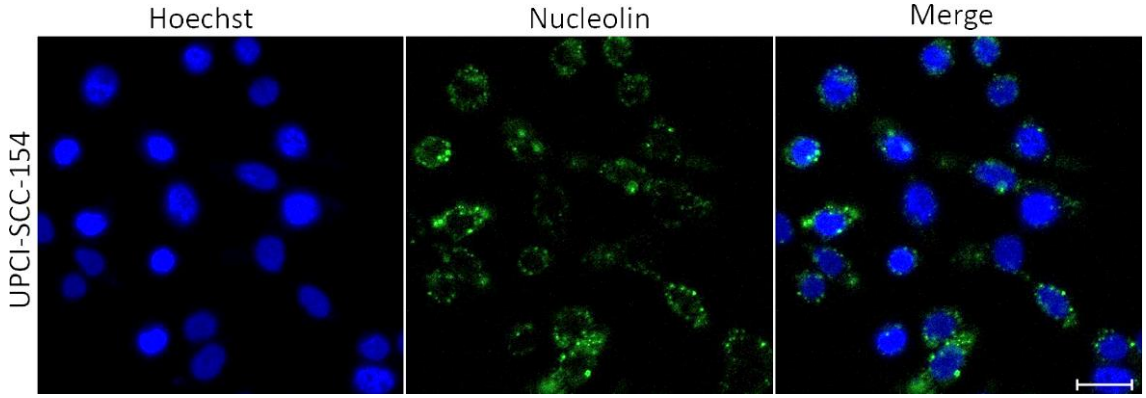
Cholesterol influences liposomes' fluidity and drug retention



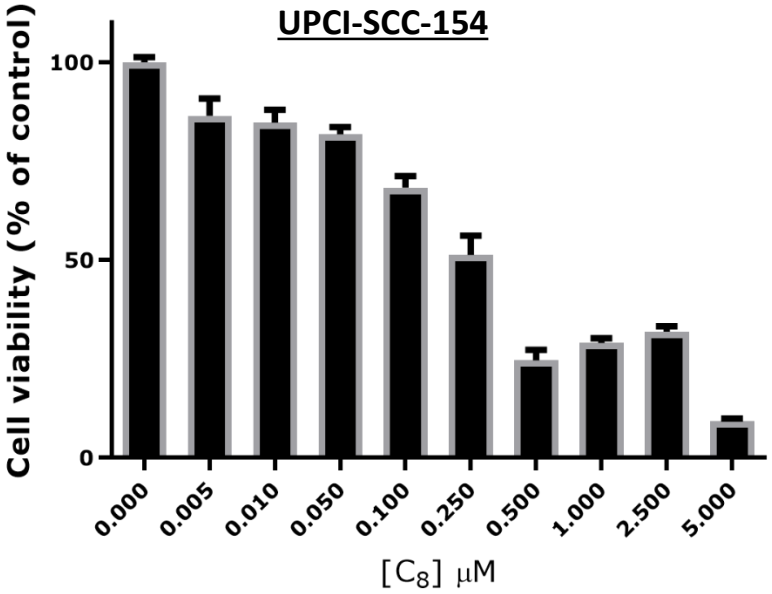
Filtrate absorbance at 495 nm
CHOL 10: 0.00058739
CHOL 12.5: 0.0024036
CHOL 15: 0.0017503

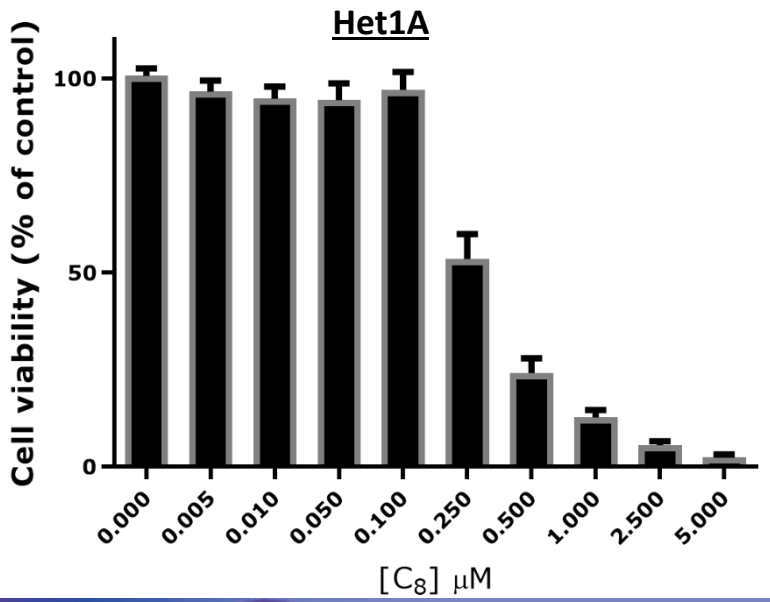
C_8 Loading ~ 100%

Nucleolin is overexpressed in the UPCI-SCC-154 cells surface



Free C_8 is highly toxic in oral cancer (UPCI-SCC-154) and healthy (Het1A) cell lines



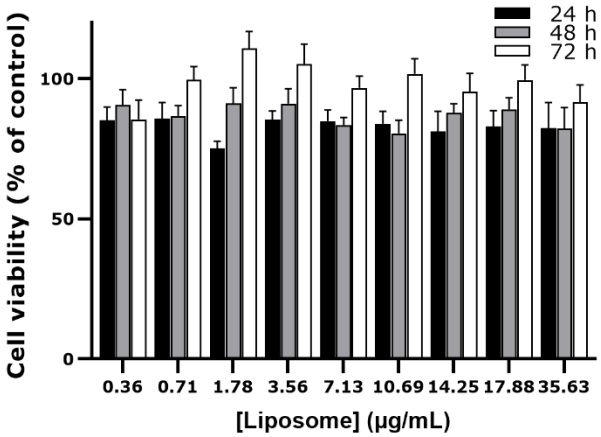


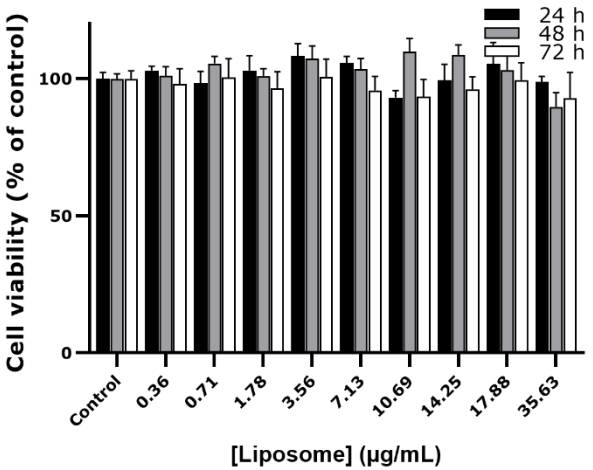
Blank liposomes are non-toxic and C8-associated liposomes present a dose-response effect

UPCI-SCC-154

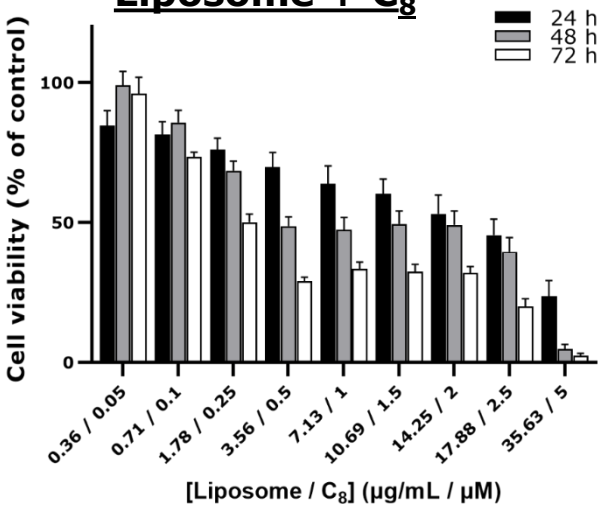
Het1A

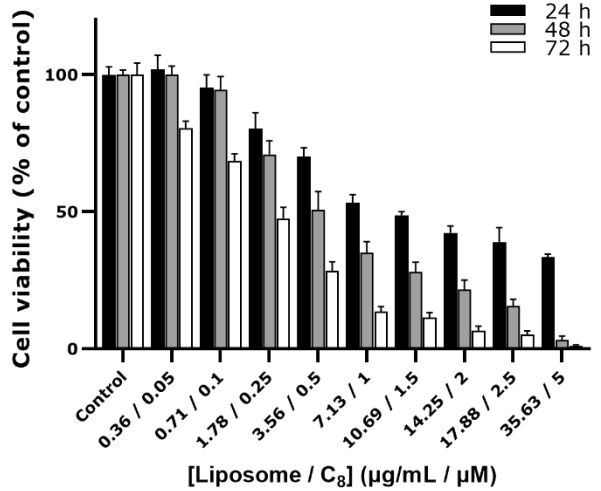
Blank Liposome



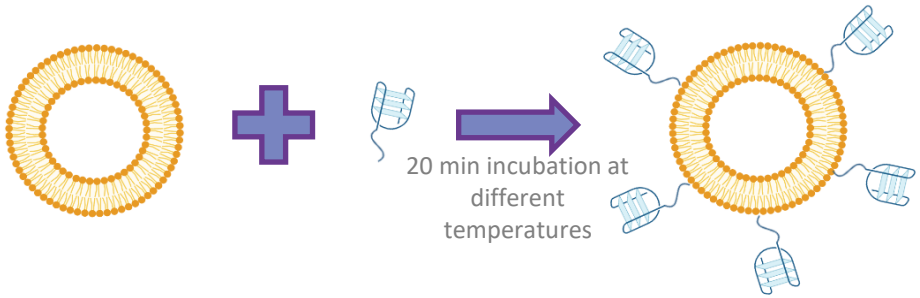


Liposome + C_8





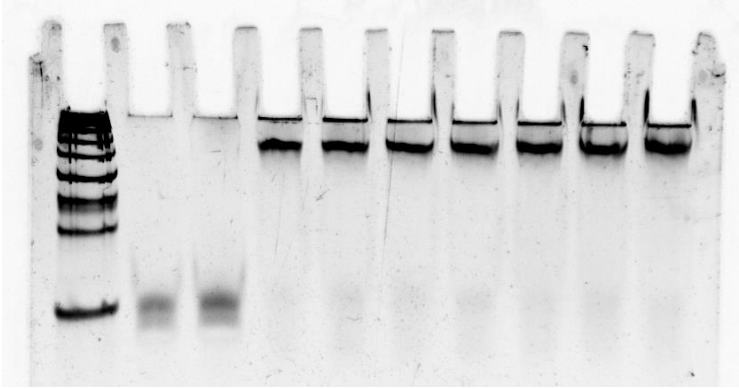
Liposomes were successfully functionalized with AT11-TEG-Cholesteryl Cy5



Liposome

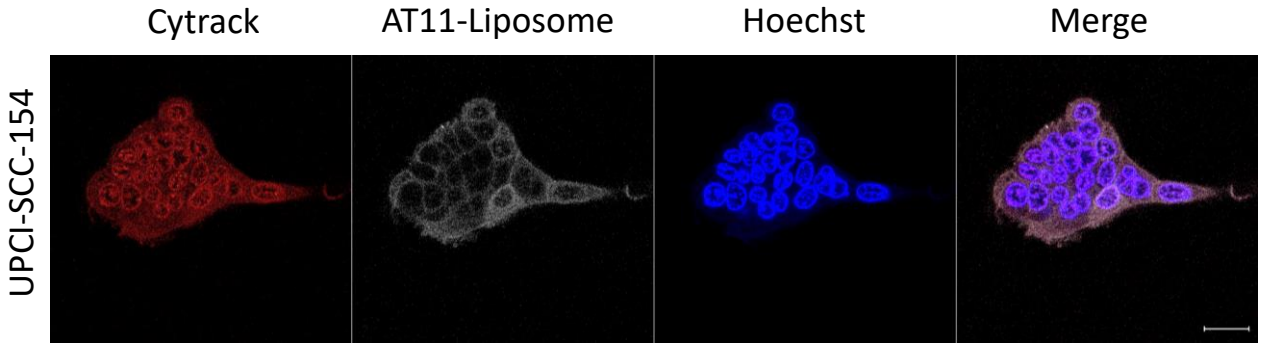
AT11-TEG-Cholesteryl Cy5

AT11-TEG-Cholesteryl Cy5 tagged liposome



1 –25 bp Marker; 2- AT11-TEG-Cholesteryl Cy5; 3- AT11-TEG-Cholesteryl Cy5 55 ˚C 20 min; Liposomes + AT11-TEG-Cholesteryl Cy5 incubated for 20 min at different temperatures: 4- RT; 5- 30 ˚C; 6- 35˚C; 7- 40 ˚C; 8- 45 ˚C; 9- 50 ˚C; 10- 55 ˚C.

Confocal Microscopy



AT11-Liposomes are efficiently internalized by UPCI-SCC-154 cells

Conclusions

Liposomes with hydrodynamic diameters of 148-168 nm were obtained and C8 was efficiently associated

After treating with C_8-associated liposomes, both cell lines showed a dose-response effect.

AT11-liposomes can internalize cells and we expect that they are able to improve C_8-associated liposomes selectivity.

Overall, these findings suggest that the tested liposomes are promising drug carriers for oral cancer therapy.

Acknowledgments

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