МИНОБРНАУКИ РОССИИ
Федеральное государственное автономное образовательное
учреждение высшего образования
«ЮЖНЫЙ ФЕДЕРАЛЬНЫЙ УНИВЕРСИТЕТ»
Научно-исследовательский институт физической и органической химии

СОВРЕМЕННЫЕ ЛИПОСОМАЛЬНЫЕ ПРОТИВООПУХОЛЕВЫЕ ПРЕПАРАТЫ

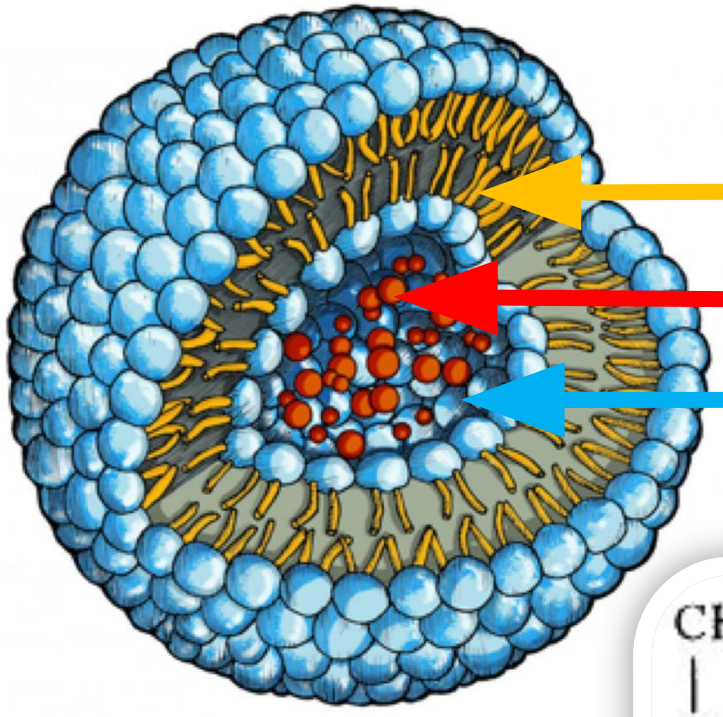
Выполнила: студентка 2 курса магистратуры

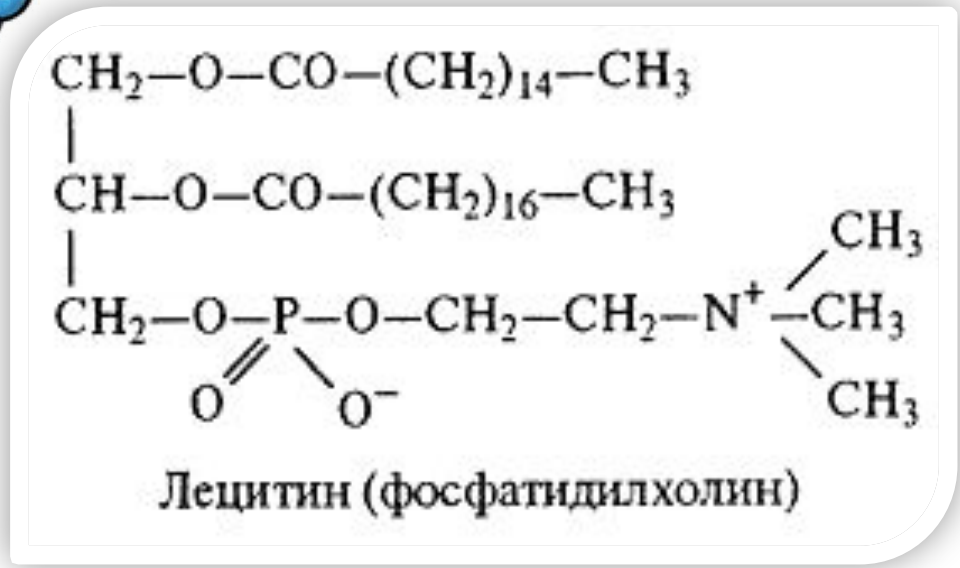
Полфёрова Виктория

Ростов-на-Дону
2019 г.

Липосомы: история открытия и химический состав

Липосома (от греч. липос – жир и сома – частица)



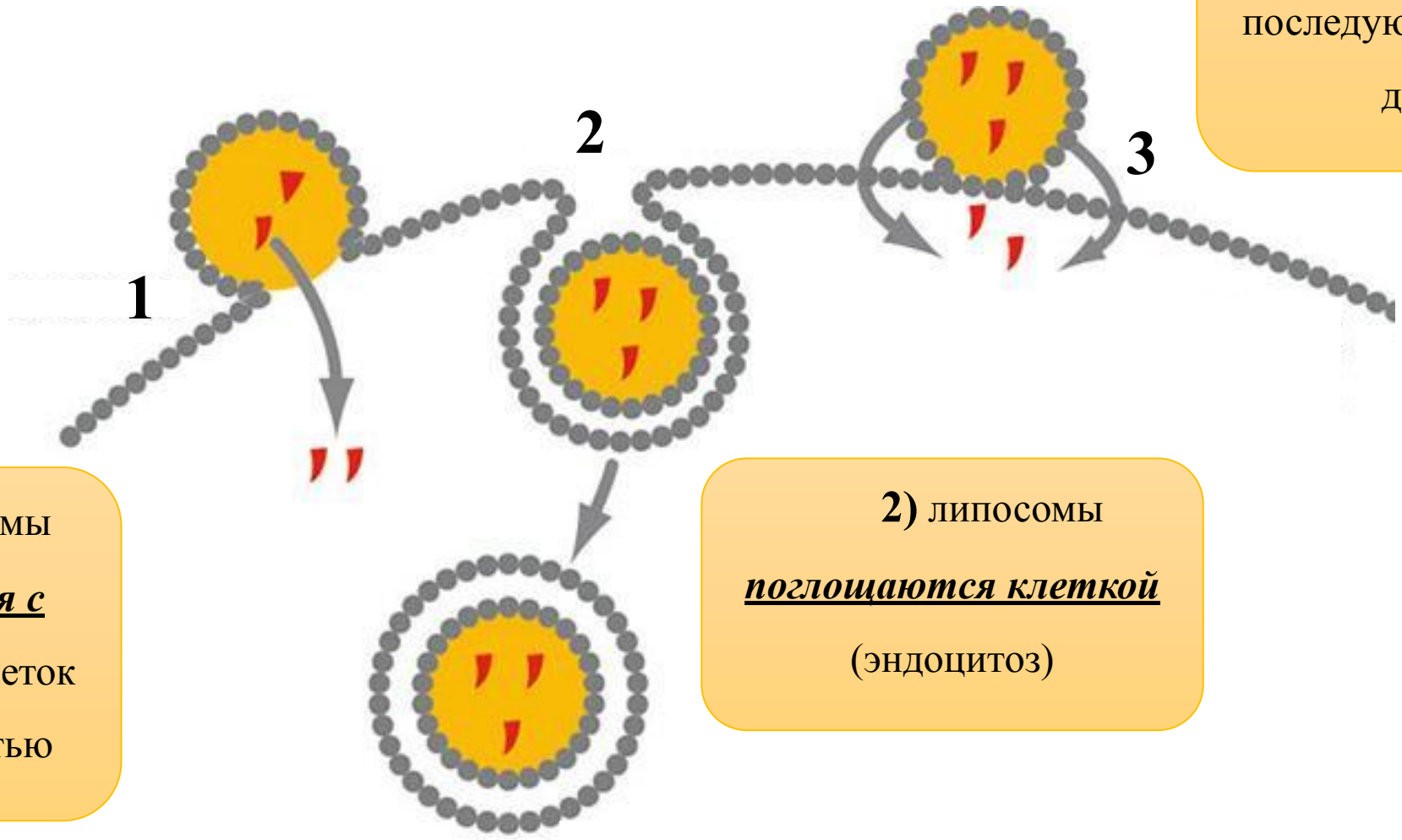


Лецитин (фосфатидилхолин)

Алек Бэнгхем – «отец» липосом

Механизм действия липосом

3) ***адсорбция*** липосом на клеточной поверхности с последующей облегченной диффузией

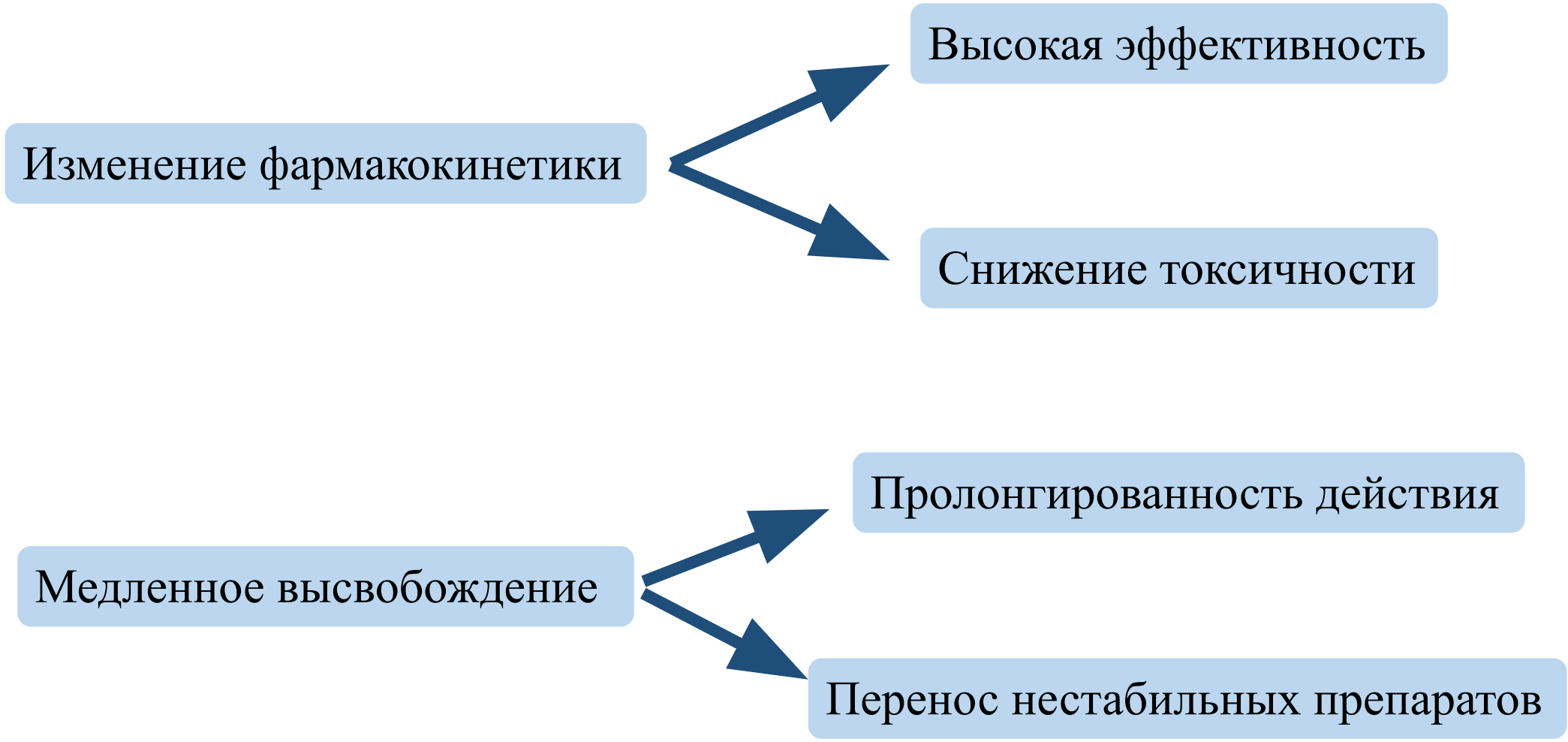


1) липосомы могут ***слиться с мембранами*** клеток и стать их частью

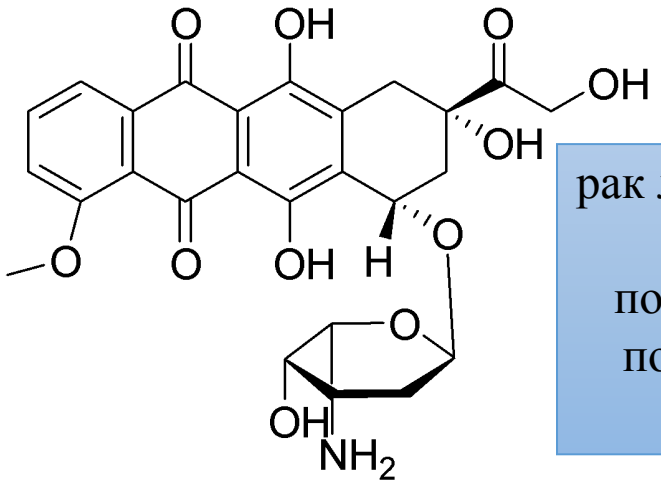
2) липосомы ***поглощаются клеткой*** (эндоцитоз)

Современные липосомальные препараты: применение в онкологии

Преимущества:



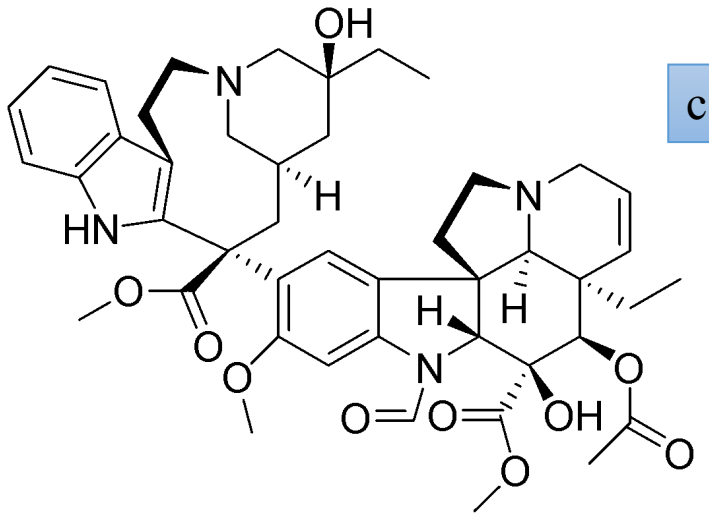
Cuelyx, Myocet - липосомальная форма доксорубицина **1**



1

рак легких, рак молочной, щитовидной и поджелудочной желез, почек, яичников, тела матки

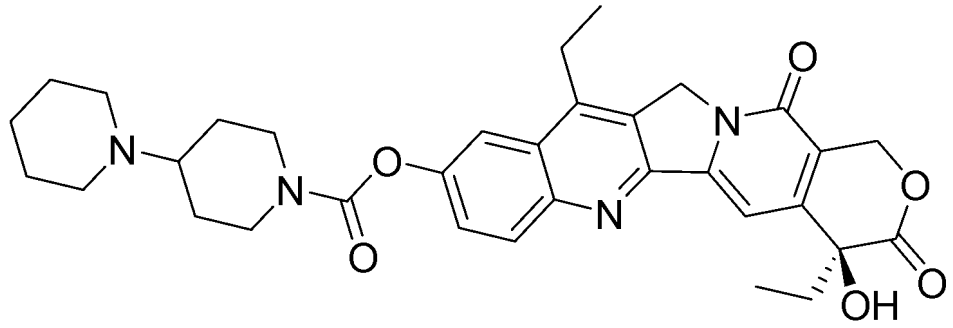
VSLI (onco TCS) — липосомальная форма винкристина **2**



2

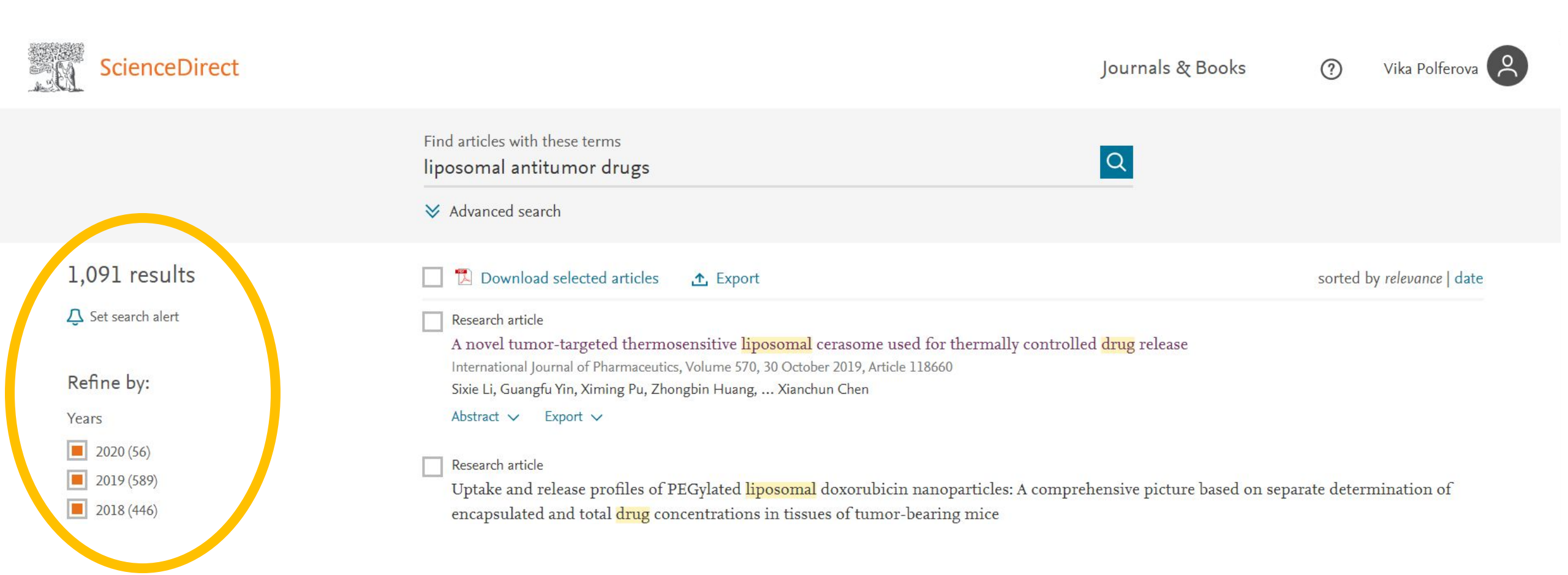
саркома мягких тканей

CPT-11 – липосомальная форма иринотекана **3**



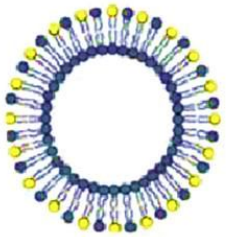
3

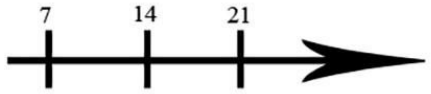
карцинома легких



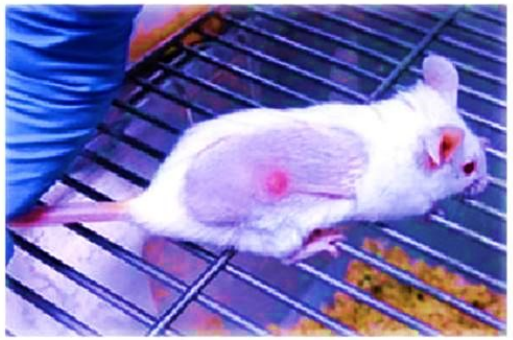
1. Sixie Li / A novel tumor-targeted thermosensitive liposomal cerasome used for thermally controlled drug release / Sixie Li, Guangfu Yin, Ximing Pu, Zhongbin Huang, Xiaoming Liao, Xianchun Chen // International Journal of Pharmaceutics. – 2019. – P. 1-27.
2. Amin Reza Nikpoor / Cell cytotoxicity, immunostimulatory and antitumor effects of lipid content of liposomal delivery platforms in cancer immunotherapies. A comprehensive in-vivo and in-vitro study / Amin Reza Nikpoor, Mahmoud Reza Jaafari, Parvin Zamani, Manouchehr Teymouri, Hamed Gouklani, Ehsan Saburi, Shahrzad Amiri Darban, Ali Badiee, Ali Bahramifar, Mahdi Fasihi-Ramandi, Ramezan Ali Taheri // - International Journal of Pharmaceutics. – 2019. – V. 567. – 118492.
3. Xuwu Zhang / A chemo-photothermal synergetic antitumor drug delivery system: Gold nanoshell coated wedelolactone liposome / Xuwu Zhang, Yanping Liu, Liyao Luo, Lei Li, Shanshan Xing, Tian Yin, Kexin Bian, Ruiyan Zhu, Dawei Gao // Materials Science & Engineering C. – 2019. – V. 101. – P. 505-512.
4. Sixie Li / A novel tumor-targeted thermosensitive liposomal cerasome used for thermally controlled drug release / Sixie Li, Guangfu Yin, Ximing Pu, Zhongbin Huang, Xiaoming Liao, Xianchun Chen // International Journal of Pharmaceutics. – 2019. – P. 1-27.
5. Halevas E. / Magnetic cationic liposomal nanocarriers for the efficient drug delivery of a curcumin-based vanadium complex with anticancer potential / Eleftherios Halevasab, Barbara Mavroidib, Claudia H. Swansonc, Graham C. Smithc, Alexandra Moschonad, Spyros Hadjispyroue, Athanasios Salifogloue, Anastasia A. Pantazakif, Maria Pelecanoub, George Litsardakis // Journal of Inorganic Biochemistry. – 2019. – V. 199. – 110778

Cell cytotoxicity, immunostimulatory and antitumor effects of lipid content of liposomal delivery platforms in cancer immunotherapies. A comprehensive *in-vivo* and *in-vitro* study

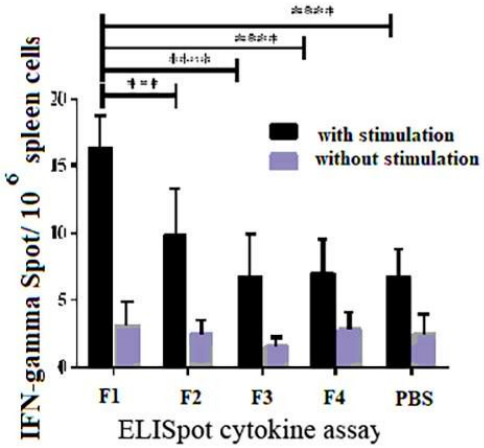


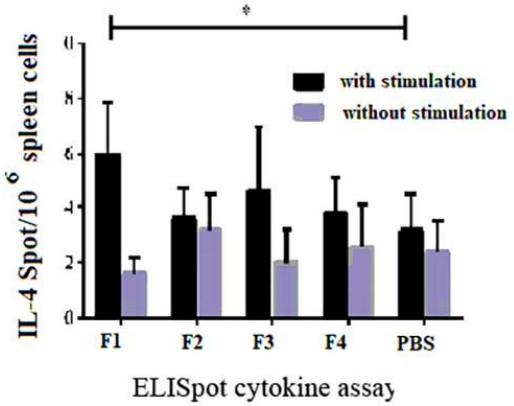


Mice bearing C26 colon carcinoma



F1: DOTAP, DOPE, Chol
F2: DMPC, DMPG, Chol, DOPE
F3: DSPC, DSPG, Chol, DOPE
F4: HSPC, mPEG2000-DSPE,Chol





***In-vitro* and *In-vivo* studies**

- Apoptosis assay
- Serum cytokine assay
- ELISpot cytokine assay
- Flowcytometry
- Survival assays

<u>Keywords</u>
Liposome
Phospholipid
Immunotherapy
Cellular immune response
Tumor

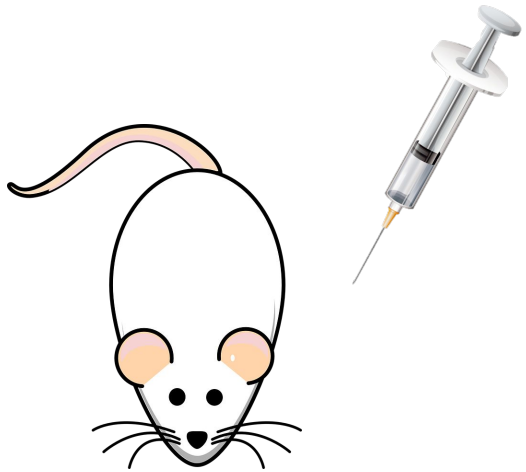
Nikpoor A. R. / Cell cytotoxicity, immunostimulatory and antitumor effects of lipid content of liposomal delivery platforms in cancer immunotherapies. A comprehensive in-vivo and in-vitro study / A. R. Nikpoor, M. R. Jaafari, P. Zamani, M. Teymouri, H. Gouklani, E. Saburi, S. A. Darban, A. Badiee, A. Bahramifar, M. Fasihi-Ramandi, R. A. Taheri // - International Journal of Pharmaceutics. – 2019. – V. 567.

Formulations	Molar ratio
F1: DOTAP, DOPE, Cholesterol	4, 4, 4
F2 : DMPC, DMPG, Cholesterol, DOPE	15, 2, 3, 5
F3: DSPC, DSPG, Cholesterol, DOPE	15, 2, 3, 5
F4:2 HSPC, mPEG2000-DSPE, Cholesterol	13, 1, 10

2.2. Liposome preparation

The nomenclature and lipid compositions of the liposomes used in the study are given in Table 1. The liposomes were prepared via thin film hydration and extrusion method (Nikpoor et al., 2015). Briefly, appropriate amounts of phospholipids were added to a round-bottomed flask, previously prepared as chloroform stock solution and the solvent was removed with a rotary evaporator and one-night freeze drying. The resulting film was hydrated and mixed with phosphate-buffered saline (PBS, 300 mM, pH: 7.4) at 60 °C for 30 min under argon atmosphere followed by one-night incubation at 4 °C. The resulting multi-lamellar liposomes were then passed through respective 400, 200 and 100 nm pore size polycarbonate membranes. Finally, liposomes were sterilized for further experiments using 0.45 μm sterile syringe filters.

	Formulations
F1	**DOTAP** (1,2-dioleoyl-3-trimethylammonium propane), **DOPE** (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine), **Chol** (cholesterol): molar ratio 4/4/4
F2	**DMPC** (1,2-dimyristoylsn- glycero-3-phosphocholine), **DMPG** (1,2-Dimyristoyl-sn-glycero-3-phosphorylglycerol), **Chol**, **DOPE**: molar ratio 15/2/3/5
F3	**DSPC** (1,2-distearoyl-sn-glycero-3-phosphocholine), **DSPG** (1,2-distearoyl-sn-glycero-3-(phospho-rac-(L-glycerol)) (sodium salt)), **Chol**, **DOPE**: molar ratio 15/2/3/5
F4	**HSPC** (hydrogenated soya phosphatidylcholine), **mPEG2000-DSPE** (distearylphosphatidylethanolamine), **Chol**: molar ratio 13/1/10



7 дней

Размер опухоли >3мм^3

F1
(0,6мкмоль/50мкл)

F2
(5мкмоль/200мкл)

F3
(5мкмоль/200мкл)

F4
(2,4мкмоль/100мкл)

Вводили внутривенно в течение 3 недель → Спустя неделю собирали спленоциты

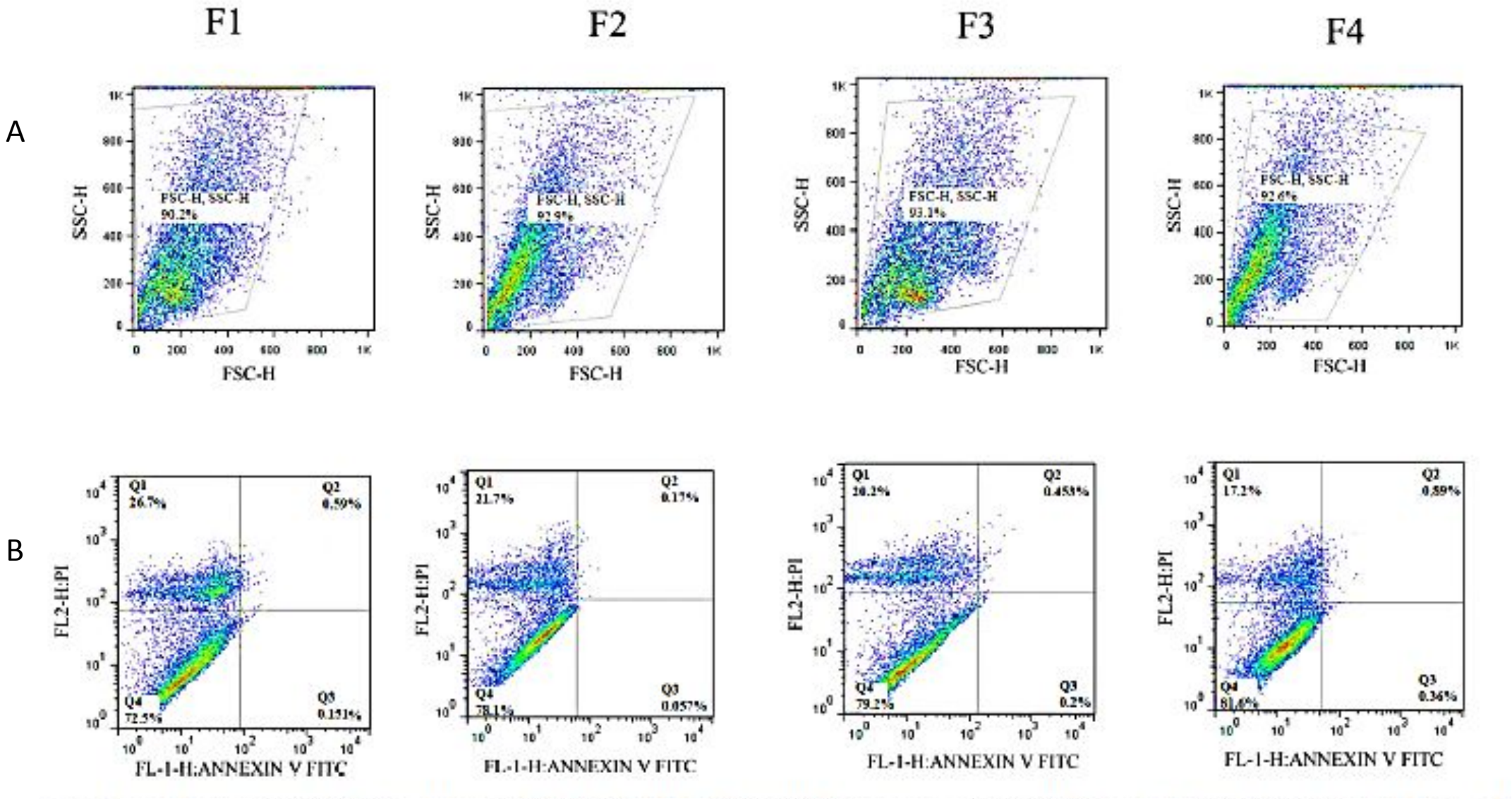


Fig. 1. The apoptotic effects of liposomal formulations on BALB/c splenocytes during 24-h culture with liposomes was studied using Annexin V and Propidium iodide (PI). To this aim, the splenocytes were extracted from BALB/c mice spleens and were cultured. The splenocytes were treated for 24 h with liposomes. Then, 10^5 cells/100 µl with 5 µl Annexin V-FITC incubated for 35 min at 37 °C. The PI dye was then added to the samples five minutes before running; apoptosis was evaluated using a BD FACSCalibur flow cytometry. A: The Live cells were gated through FSC-H and SSC-H parameters. B: The frequencies of live cells (lower left side of quadrant), early apoptotic cells (lower right side of quadrant), middle apoptotic cells (upper right side of quadrant) and fully apoptotic cells (upper left side of quadrant) of stained cells were calculated using FL-1 H and FL-2 H channels in a logarithmic mode.

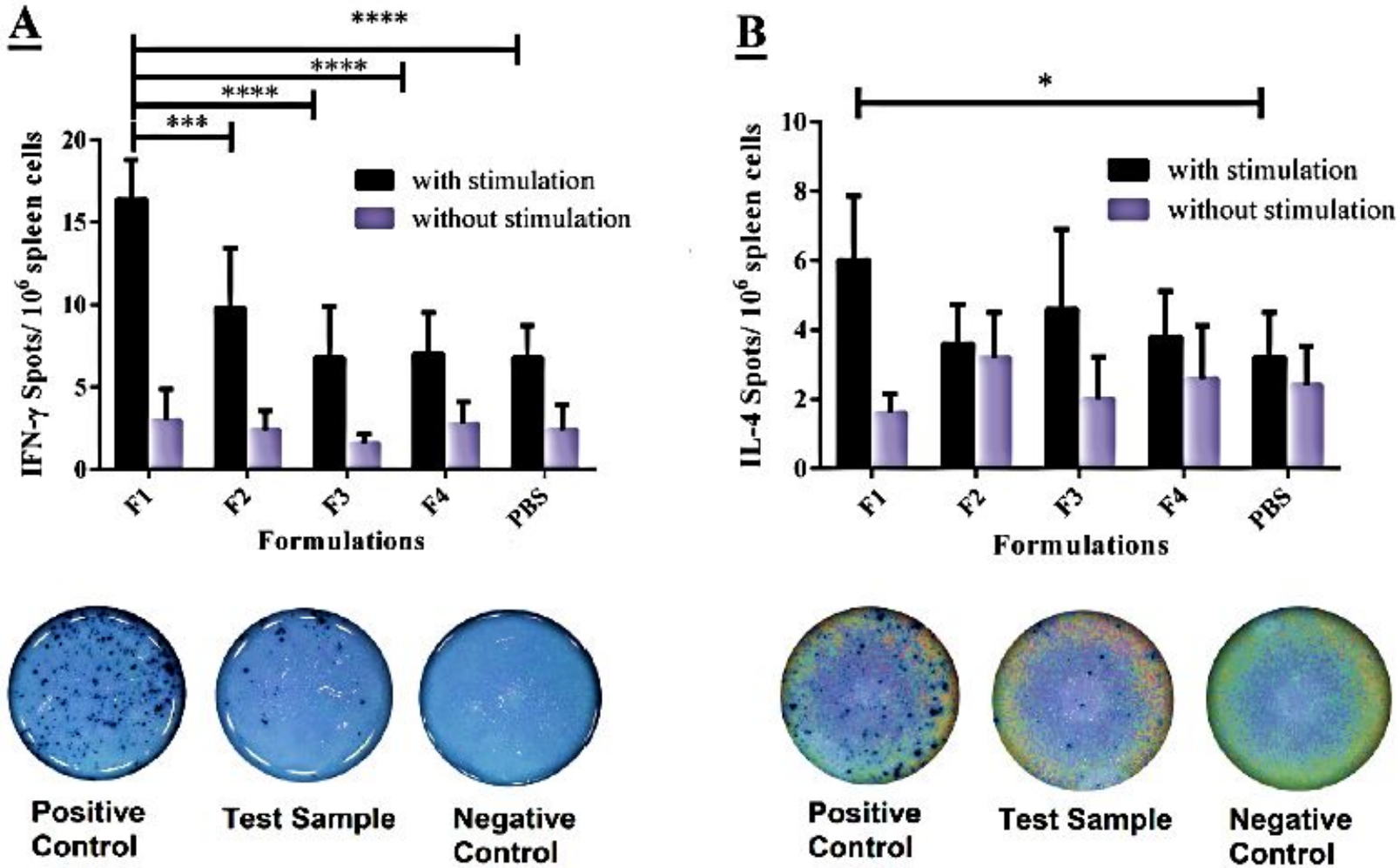


Fig. 3. *In-vitro* secretion assay of IFN-γ (A) and IL-4 (B) from mice splenocytes following immunization with liposomal formulations using ELISpot. Phytohaemagglutinin (PHA) with the concentration of 10 μg/mL was also used as a polyclonal activator of splenocytes and as a positive control. The number of spots per well, denoting cytokine-secreting colony-forming units (CFU)/10^6 splenocytes, were increased significantly in F1-L-treated mice. At the bottom of the figures, as an example of the ELISpot assay wells are shown. 3×10^5 splenocytes was stimulated by PHA as Positive control, liposomal formulation as sample and culture media as negative control. The number of spots were then count after staining. Data are presented as mean ± SD (n = 5). The spots statistically significant differences are shown as follows: *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$.

● F1 (DOTAP и DOPE)-липосомы наиболее эффективно индуцируют иммунную систему

● DOTAP и DOPE повышают эффективность трансфекции для иммунных клеток

● Все препараты имели размер в диапазоне 120-135нм, что указывает на гомогенную популяцию липосом, способную индуцировать иммуногенные ответы, связанные с Th2 *+F1 – индуцирует ответы Th1*

● F1 проявляет высокую цитотоксичность в отношении спленоцитов

Самое важное: препараты, содержащие DOTAP DOPE (F1) способны ***стимулировать клеточный иммунитет и активировать иммунные ответы***

Фосфолипидный состав липосом заслуживает рассмотрения при разработке липосом для иммунотерапии рака

СПАСИБО ЗА ВНИМАНИЕ!