# Na,K-ATPase isoform-selective cardiac glycosides- a potential anti-cancer drug ?

**Adriana Katz,**

**Cherniavsky-Lev M., Ainbinder E., Tal D. and Karlish SJD**
**Weizmann Institute of Science, Israel**

# Ion channels and pumps as cancer targets!

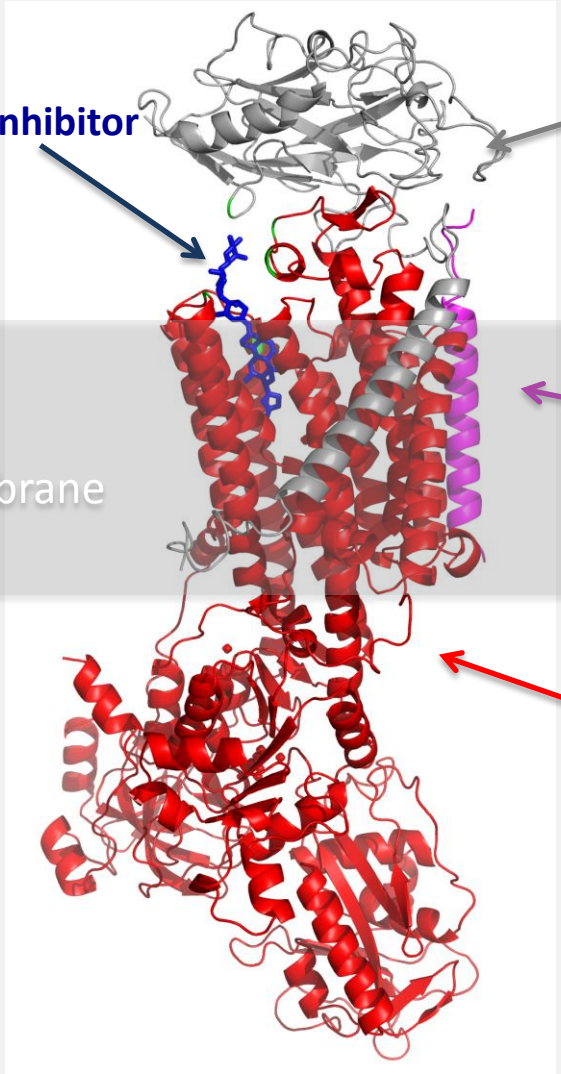
***In vitro* antiproliferative and/or apoptotic effects of cardiac glycosides in cancer cells**

| Cancer type | Compounds tested | Cancer cell lines |
|---|---|---|
| Breast | Digitoxin, digoxin, proscillaridin A, ouabain, digoxigenin, gitoxin, gitoxigenin | MCF-7, MDA-MD-435 |
| Prostate | Oleandrin, ouabain, digoxin, bufalin, cinobufagenin | PC-3, LNCaP, DU145 |
| Melanoma | Digoxin, oleandrin, digitoxin, proscillaridin A, ouabain, digitonin | UACC-62, BRO |
| Lung | Digitoxin, digoxin, ouabain, UNBS1450, oleandrin | A549, NCI-H-358, Calu1, Sklu1, NCI-H6, H69AR |
| Leukaemia | Bufalin, oleandrin, digitoxin, proscillaridin A, ouabain | HL60, U-937, CCRF-CEM, CEM-VM-1 |
| Neuroblastoma | Digoxin, ouabain | SH-SY5Y, Neuro-2a |
| Renal | Digitoxin, digoxin, digitoxigenin, proscillaridin A, ouabain | TK-10, ACHN |
| Myeloma | Digitoxin, digoxin, proscillaridin A, digitoxigenin, ouabain, digitonin, lanatocide C | 8226-S, 8226-LR5, 8226-DOX-40 |
| Pancreatic | Oleandrin | PANC-1 |

**Na+ /K+ -ATPase could be targeted to combat chemoresistant cancers.**

# Na,K ATPase is a vital protein in all mammalian cells.

- Na,K -ATPase is an oligomeric transmembrane protein, localized to the basolateral plasma membrane in most epithelial cells.
- Na,/K-ATPase pumps Na+ and K+ against their physiological gradients.




β Subunit

targets into the membrane, **3 isoforms:**

β1
β2
β3

Extracellular space

**FXYD** accessory protein, regulator

Cytoplasm

**α subunit**

Catalytic moiety, 4 isoforms:
**a1**, ubiqutous , all tissues
a2, skeletal muscle, heart, eyes
**a3**, neurons brain
a4, sperms

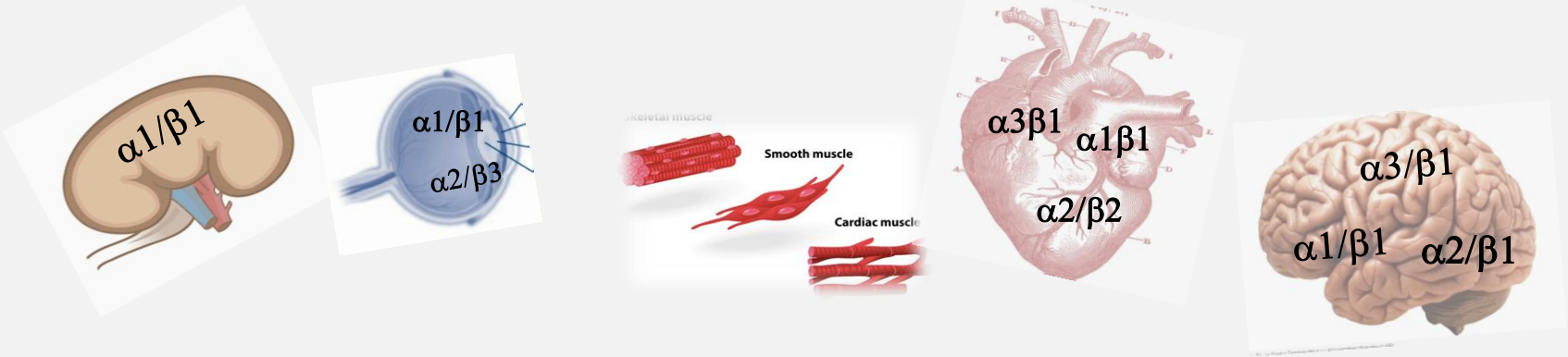
## Na,K pump is essential for many physiological processes

- ✧ Renal function, and regulation of hypertension .
- ✧ Cardiac contraction
- ✧ Regulation of intra ocular pressure, IOP.

And many more….

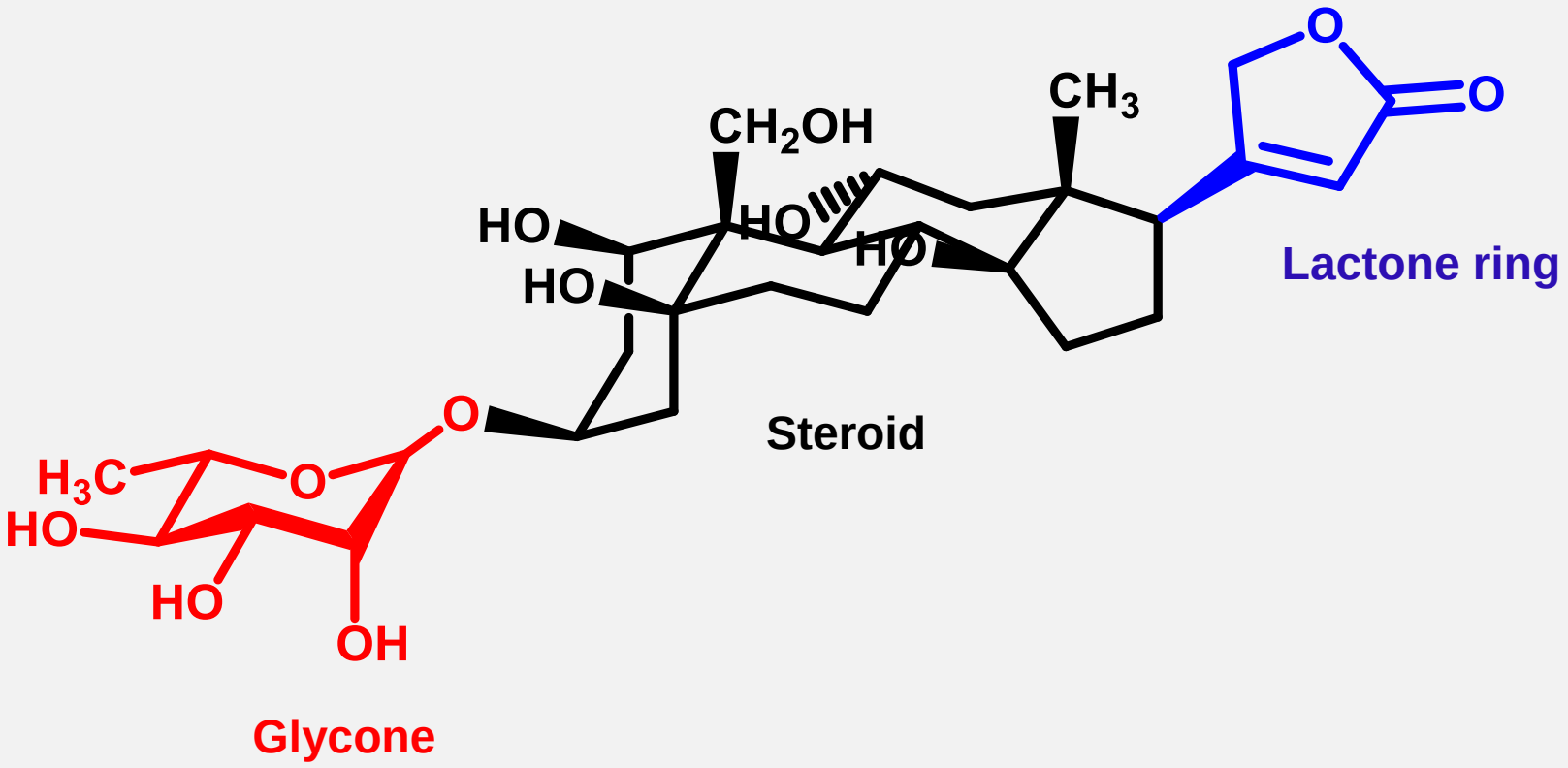
## Expression of isoforms in different tissues.

All of the isoforms are expressed in a tissue and functional specific manner.



# Cardiac glycosides (CG)

The cardiac glycosides are an important class of naturally occurring drugs whose actions include both beneficial and toxic effects on the heart.

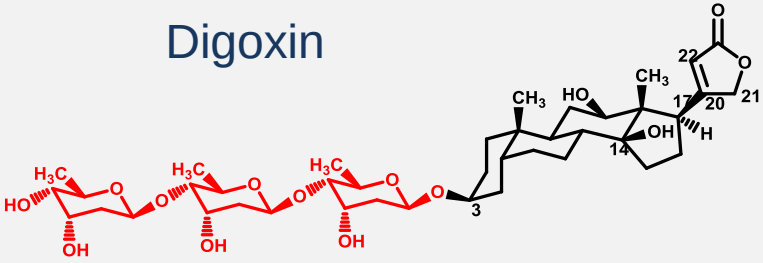


# Cardiac glycosides, naturally occurring in plants and animals.

**Withering W (1785). "An account of the foxglove and some of its medical uses: with practical remarks on dropsy and other diseases."**

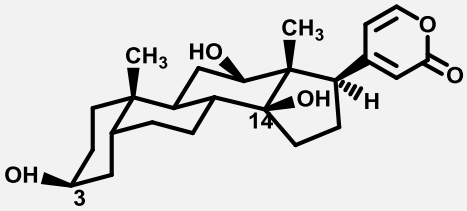
Digitalis purpurea

Digoxin

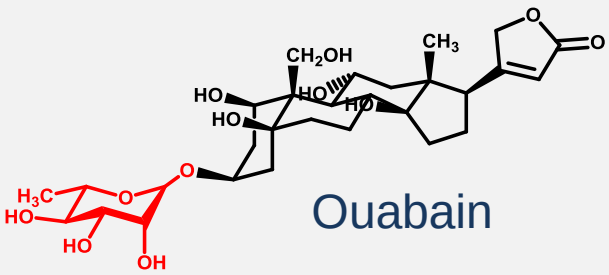


Strophanthus gratus

Bufo bufo



Bufalin

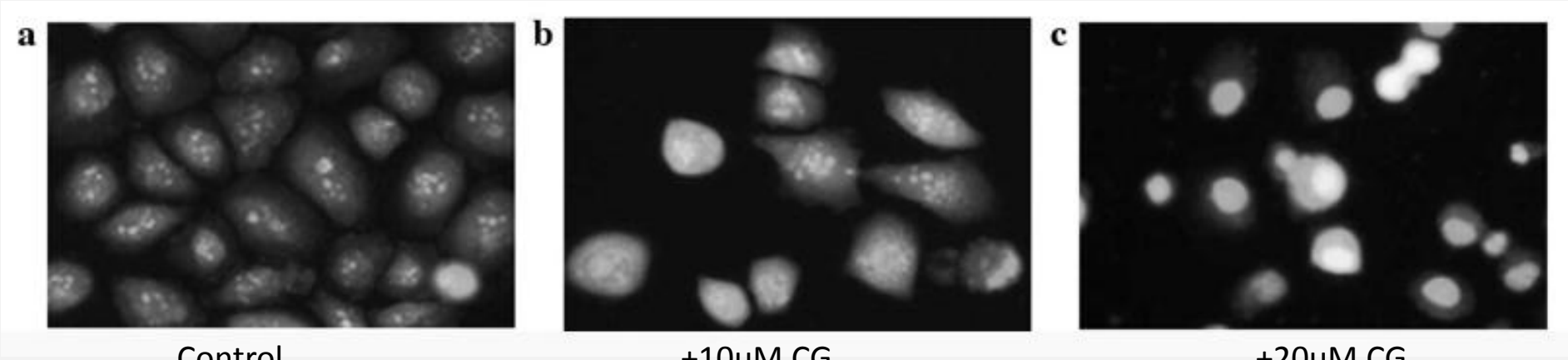


Ouabain

## Cardiac glycosides have a long history of therapeutic application.

- Plants containing cardiac steroids have been used as poisons and heart drugs at least since 1500 B.C.
- The early understanding of their positive inotropic effects facilitated their use as effective drugs for the treatment of heart-related pathologies, yet their toxicity remains a serious problem.

- More recently, considerable in vitro, in vivo and epidemiological data support novel roles for CG's such as inducing apoptosis and inhibit the growth of cancer cell lines.

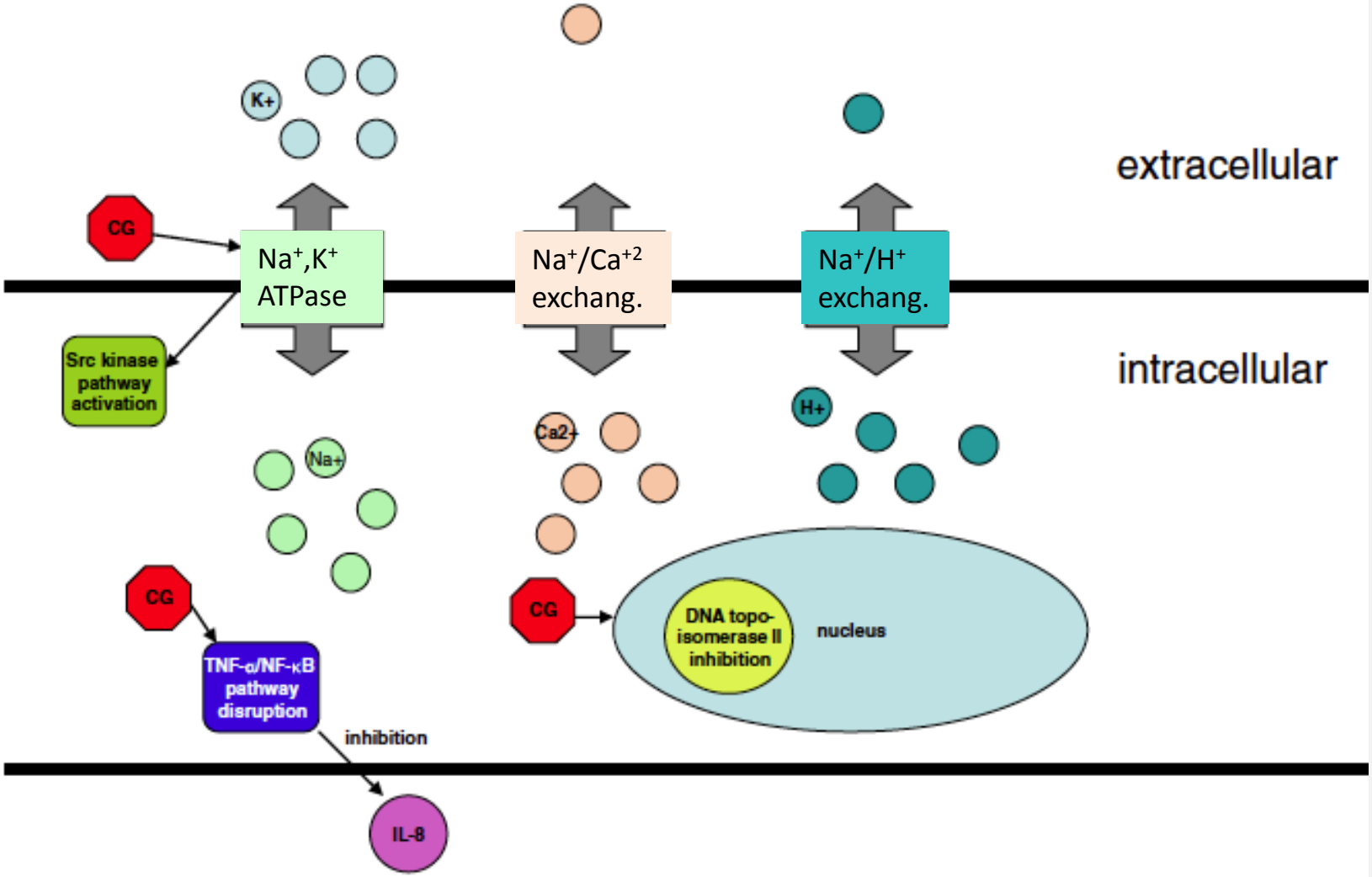



Control +10uM CG +20uM CG

Liang-Fei Ye,et al.2013 Oncology letters

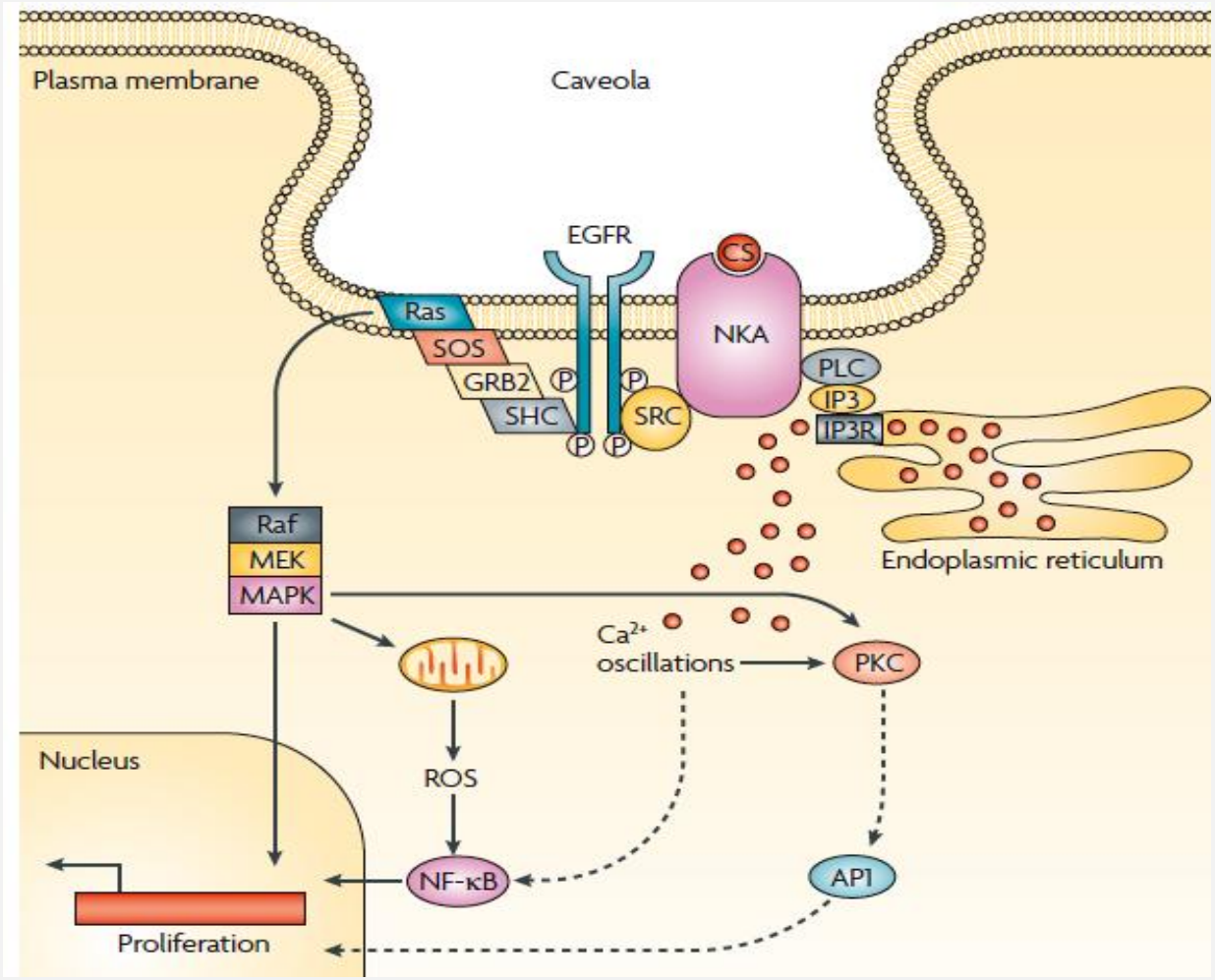
# Proposed mode of action of cardiac glycosides, CG

Invest New Drugs (2013) 31:1087–1094 Slingerland M. et al



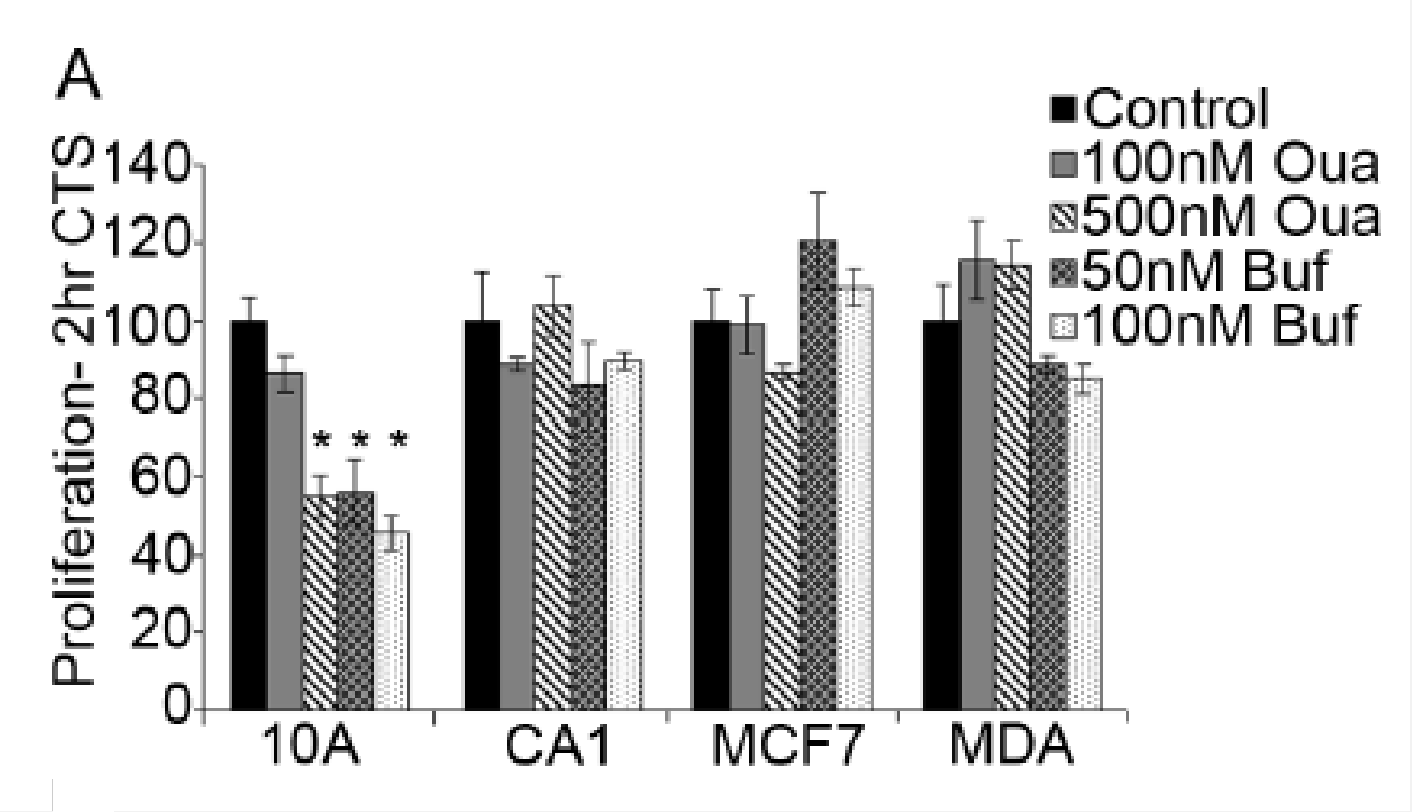

**The decrease in intracellular K+ and increase in intracellular Na+ and Ca2+ following inhibition of the Na+/K+-ATPase may induce apoptosis**

# Na,K-ATPase as a versatile signal transducer ?




Na+/K+-ATPase may also act as a signal transducer. When intact cells are exposed to digitalis drugs (e.g., ouabain and digoxin) specific inhibitors of this enzyme various cell signaling pathways are activated leading to highly cell-specific down-stream consequences.

# Proliferation of CG-treated cells.



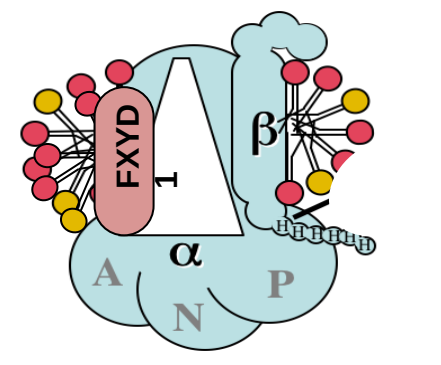

**Normal** **Cancer cells**

**Breast cancer cells are not more sensitive to CG's cytotoxicity than are normal cells.**

Clifford RJ, and Kaplan JH 2013 PLOS ONE 8,

# Purification and stabilization of isoforms of human Na,K-ATPase expressed in *Pichia pastoris*

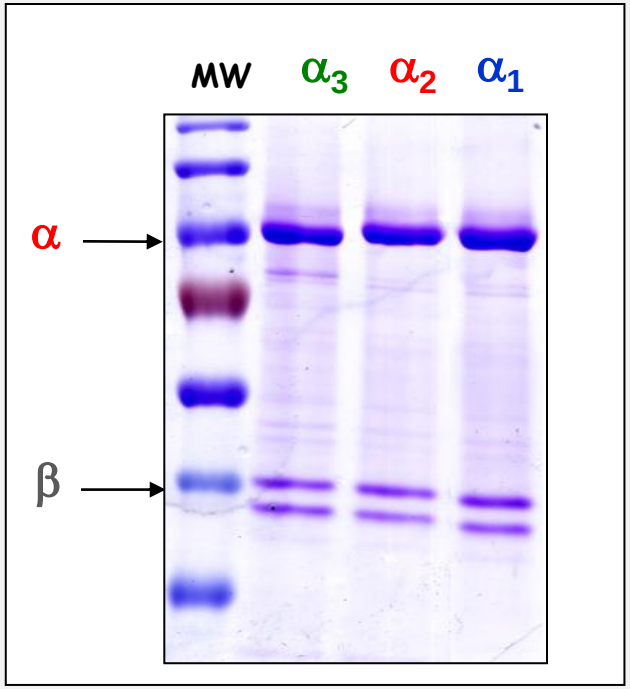
# Isoforms of human Na,K-ATPase expressed in *Pichia pastoris*




**Functional, stable, detergent-soluble αβFXYD complex**

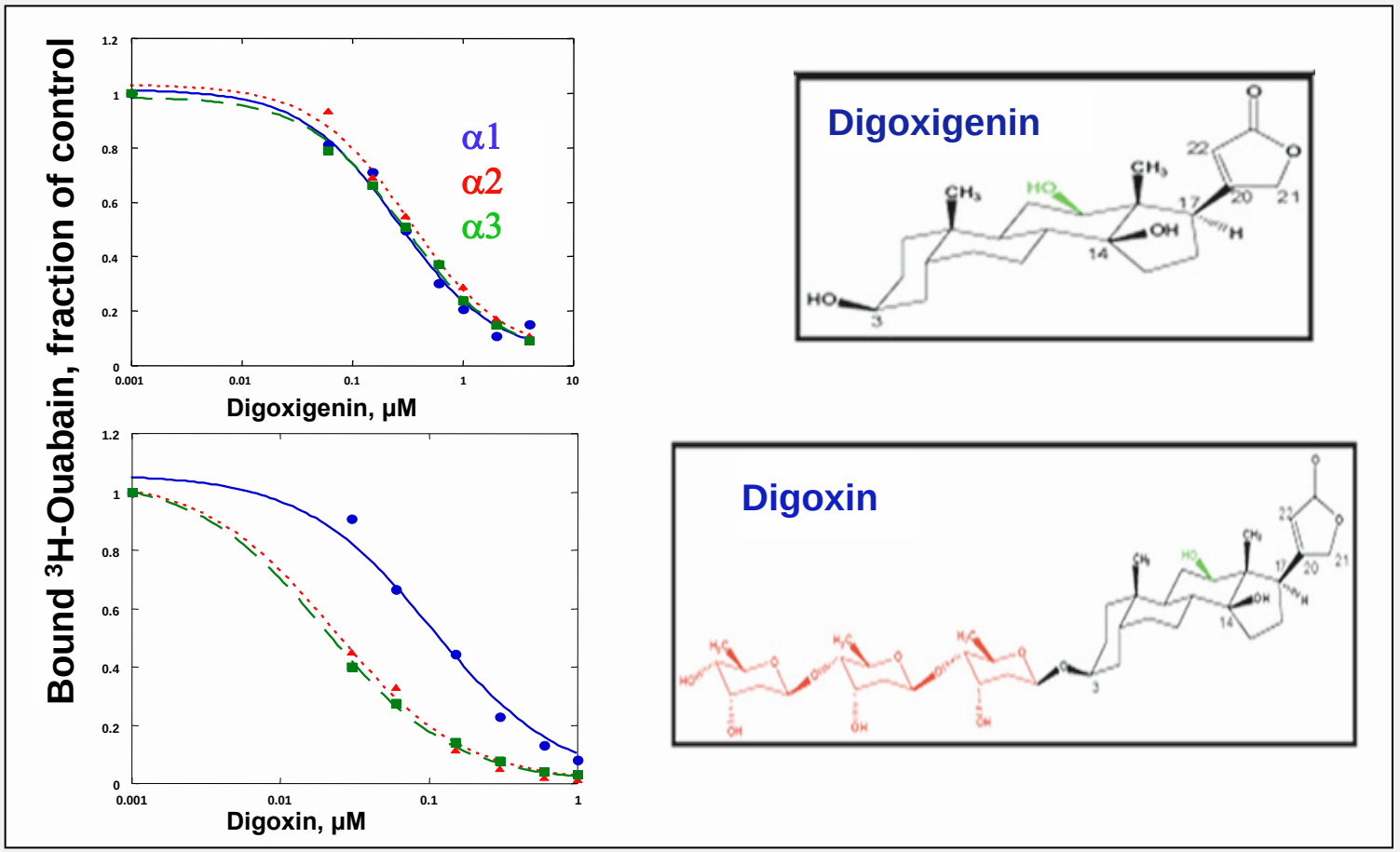
Detergent , C12E8
Phosphatidyl serine,SOPS
Phosphatidyl choline, PC
Cholesterol

**Human α isomers/β1 purified enzymes**




**Enables different combinations of isoforms**

# Cardiac glycoside affinity and selectivity for the α isoforms.

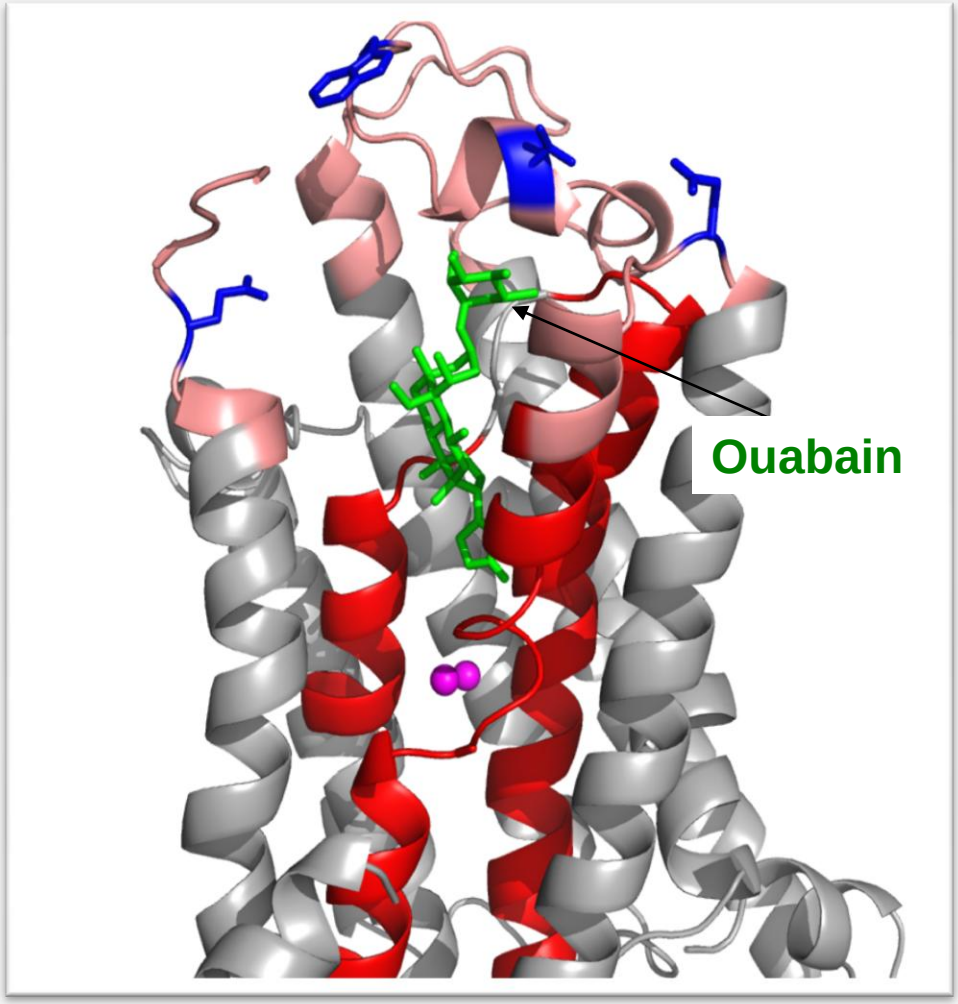



- Isoform selectivity is determined by the sugar component!
- Digoxin, is partially α2-selective CG, while Ouabain shows very low selectivity

# Isoform selectivity of Cardiac glycosides

| CG | Calculated Kd±SEM, nM | | | Ratio of Kd's, ± SE | |
|---|---|---|---|---|---|
| | α1 | α2 | α3 | α1/α2 | α1/α3 |
| Ouabain | 9.8±0.33 | 21.9±0.56 P=0.0001 | 11.1±1.3 | **0.44±0.01** | **0.88±0.1** |
| Digoxin | 87±6.0 | 25.6±2.8 P=0.001 | 25±2.4 P=0.001 | **3.39±0.43** | **3.48±0.41** |
| Digoxigenin | 270±21 | 332±11 | 307±27 | **0.81±0.06** | **0.88±0.1** |
| β-Methyl digoxin | 129±20 | 43±7 P=0.018 | 31±4 P=0.009 | **3.0±0.67** | **4.16±0.84** |
| Digitoxin | 38±3 | 18.3±4 P=0.02 | 14±3.9 P=0.008 | **2.07±0.48** | **2.70±0.78** |
| Digitoxigenin | 101±13.4 | 125±13.6 | 134±15.6 | **0.8±0.13** | **0.75±0.13** |
| Bufalin | 42.5±6.5 | 45±8 | 40±11 | **0.94±0.22** | **1.06±0.33** |
| Marinobufagenin | 2240±13 | 2470±45 | 2430±205 | **0.91±0.0** | **0.92 ±0.1** |

# Na,K-ATPase structure with bound ouabain



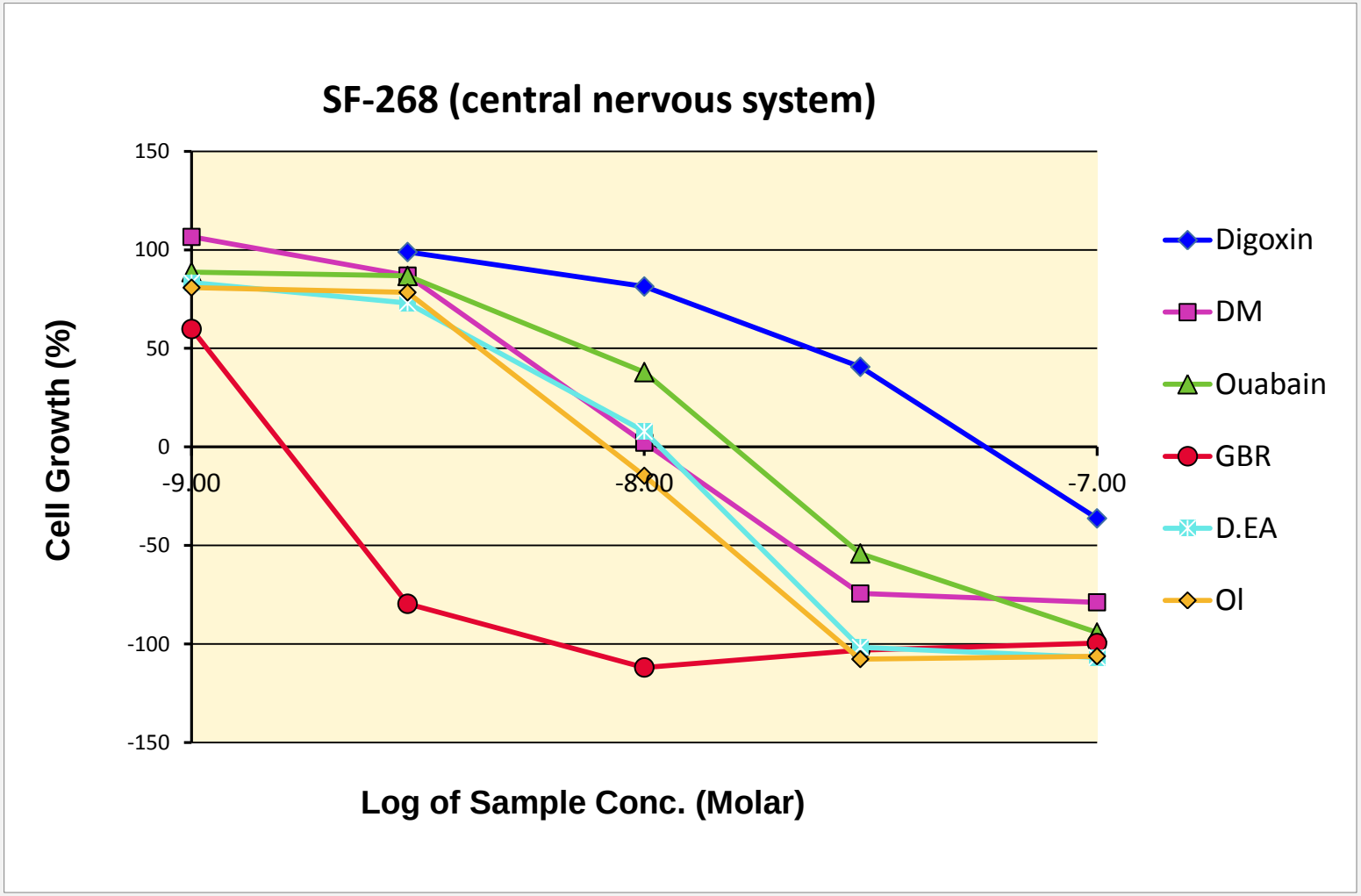

The residues common to α2 and α3 and different in **α1** are all located on the extracellular loops at the entrance to the Ouabain binding site, close to the sugar moiety.

Inhibition of cancer cell proliferation at low concentrations is used to assess the therapeutic potential of drug candidates in preclinical studies.

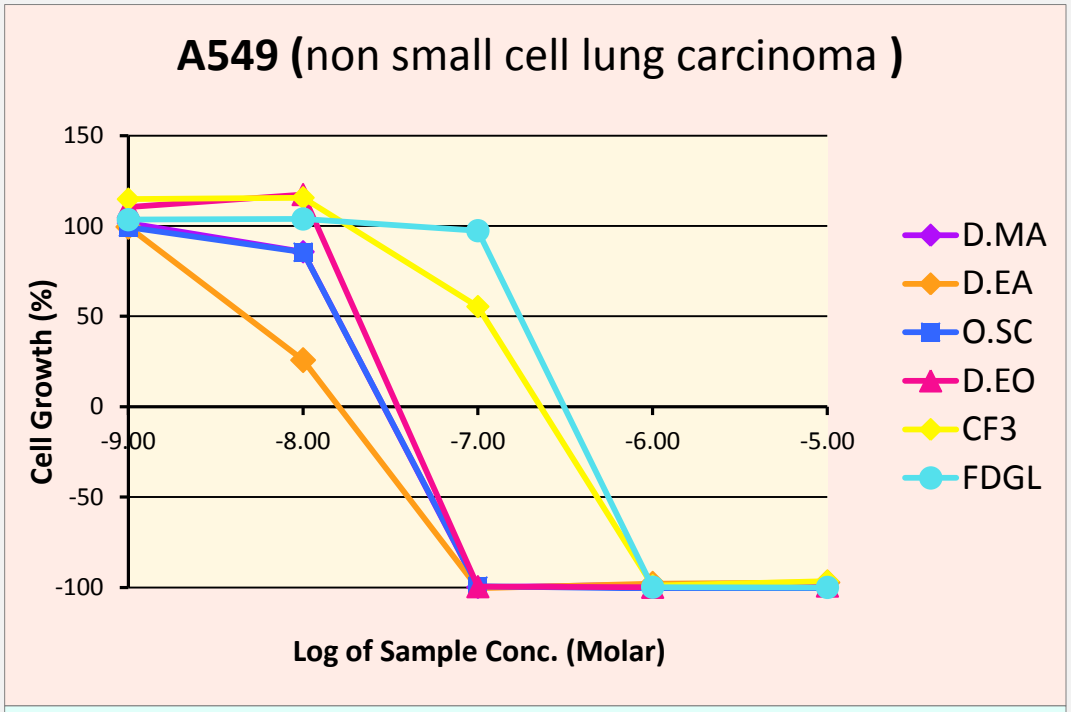
| Compounds | $IC_{50}$ (nM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A549 | U373 | Hs683 | T98G | MCF-7 | SKMEL-28 | PC-3 | HT-29 |
| **Cardenolides** | | | | | | | | |
| Ouabain* | 37 ± 2 | 78 ± 1 | 34 ± 3 | 80 ± 3 | 100 ± 17 | 83 ± 13 | 63 ± 10 | 84 ± 8 |
| Ouabagenin* | 866 ± 25 | 5,281 ± 250 | 1,902 ± 167 | 3,600 ± 36 | 4,011 ± 202 | 2,563 ± 183 | 2,708 ± 62 | 3,482 ± 19 |
| Digoxin* | 60 ± 7 | 227 ± 42 | 51 ± 6 | 274 ± 17 | 363 ± 35 | 220 ± 26 | 215 ± 71 | 208 ± 20 |
| Digoxigenin* | 395 ± 34 | 3,188 ± 129 | 794 ± 22 | 1,171 ± 99 | 5,321 ± 170 | 4,005 ± 116 | 3,880 ± 87 | 4,458 ± 83 |
| Digitoxin** | 11 | 61 | 15 | 32 | 187 | 59 | 37 | 198 |
| Digitoxigenin** | 92 | 369 | 79 | 166 | 2,134 | 2,495 | 298 | 230 |
| Gitoxin** | 68 | 351 | 79 | 174 | 828 | 679 | 361 | 352 |
| Gitoxigenin** | 1,715 | 4,477 | 653 | 3,589 | 5,193 | > 10,000 | 3,964 | 4,403 |
| Uzarigenin-rhamnoside** | 45 | 247 | 38 | 38 | 469 | 222 | 77 | 41 |
| Uzarigenin** | 3,169 | 7,918 | 2,252 | 5,138 | > 10,000 | > 10,000 | 6,281 | 6,439 |

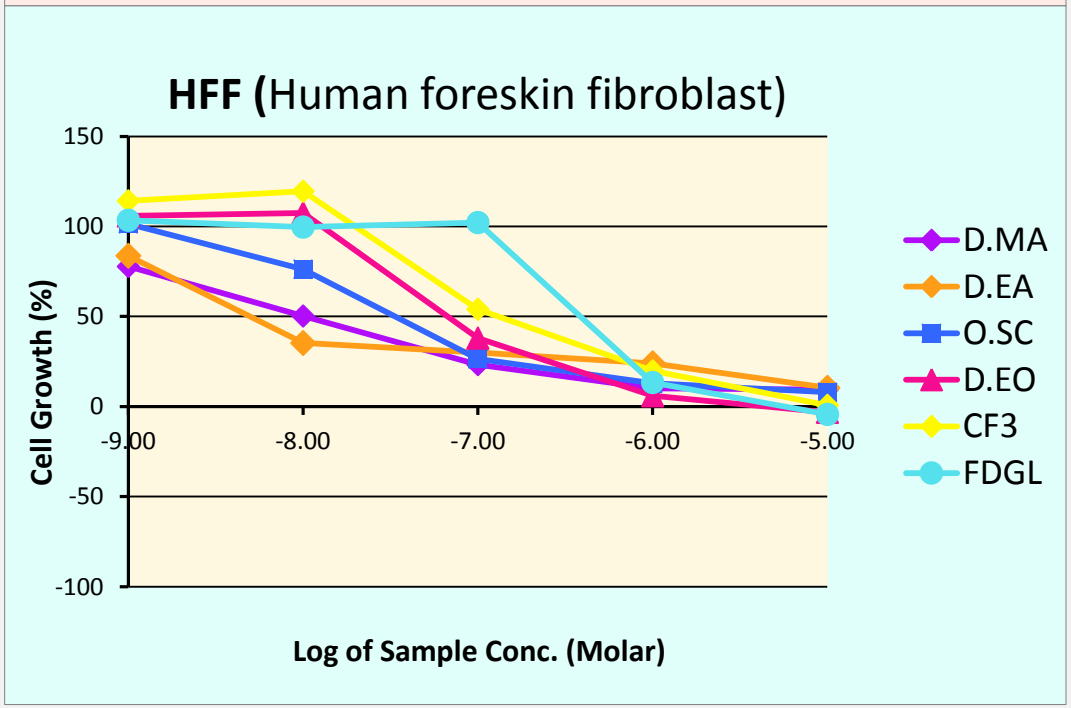
**In vitro growth inhibitory concentrations at 50% (IC50) in human cancer cells after three days of culture in the presence of the drug of interest**

**2.**


SF-268 (central nervous system)
Cell Growth (%)
150
100
50
0
-50
-100
-150
-9.00
-8.00
-7.00
Log of Sample Conc. (Molar)
Digoxin
DM
Ouabain
GBR
D.EA
Ol

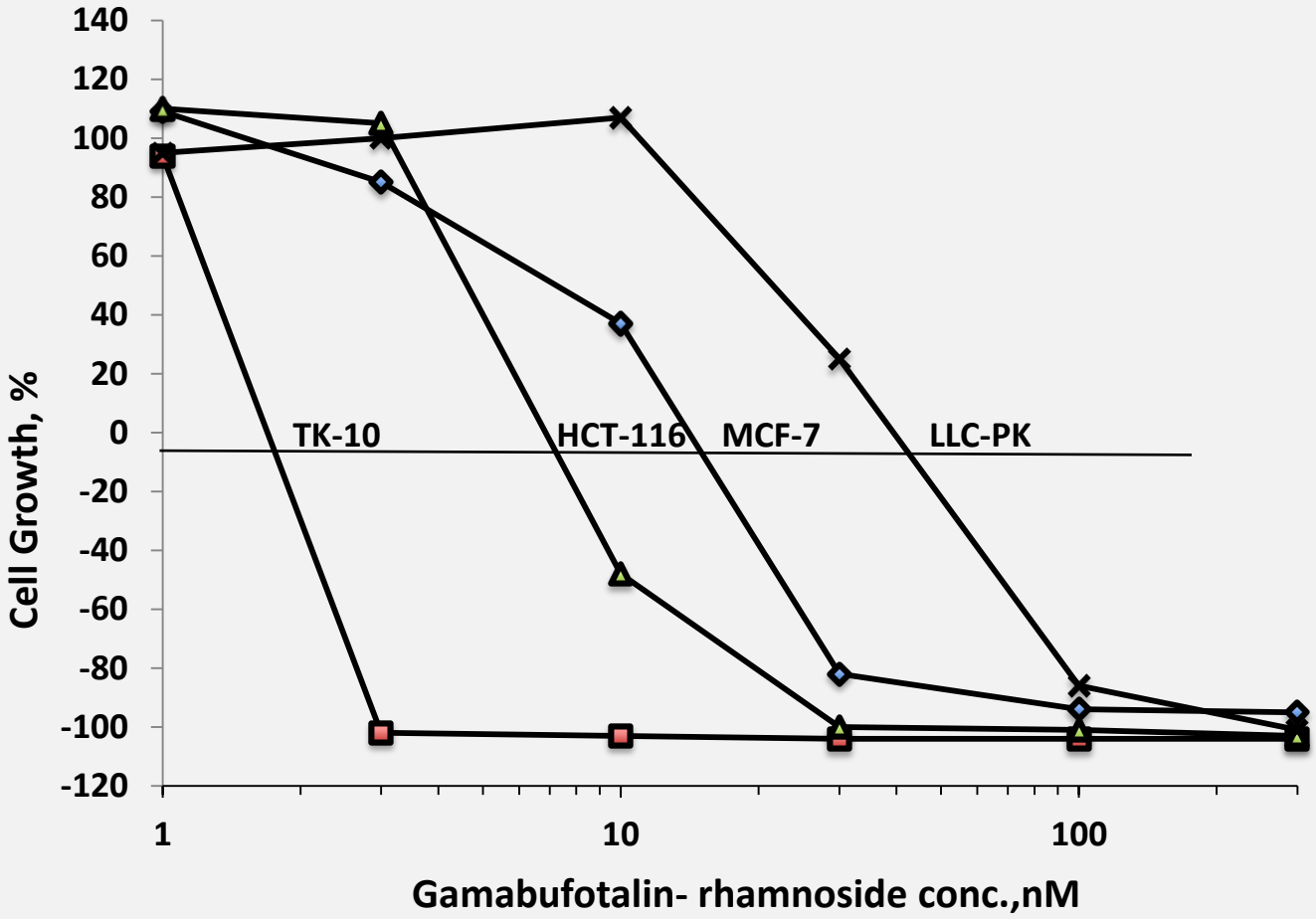
3.


A549 (non small cell lung carcinoma )
150
100
50
0
-50
-100
-9.00
-8.00
-7.00
-6.00
-5.00
Cell Growth (%)
Log of Sample Conc. (Molar)
D.MA
D.EA
O.SC
D.EO
CF3
FDGL


HFF (Human foreskin fibroblast)
150
100
50
0
-50
-100
-9.00
-8.00
-7.00
-6.00
-5.00
Cell Growth (%)
Log of Sample Conc. (Molar)
D.MA
D.EA
O.SC
D.EO
CF3
FDGL

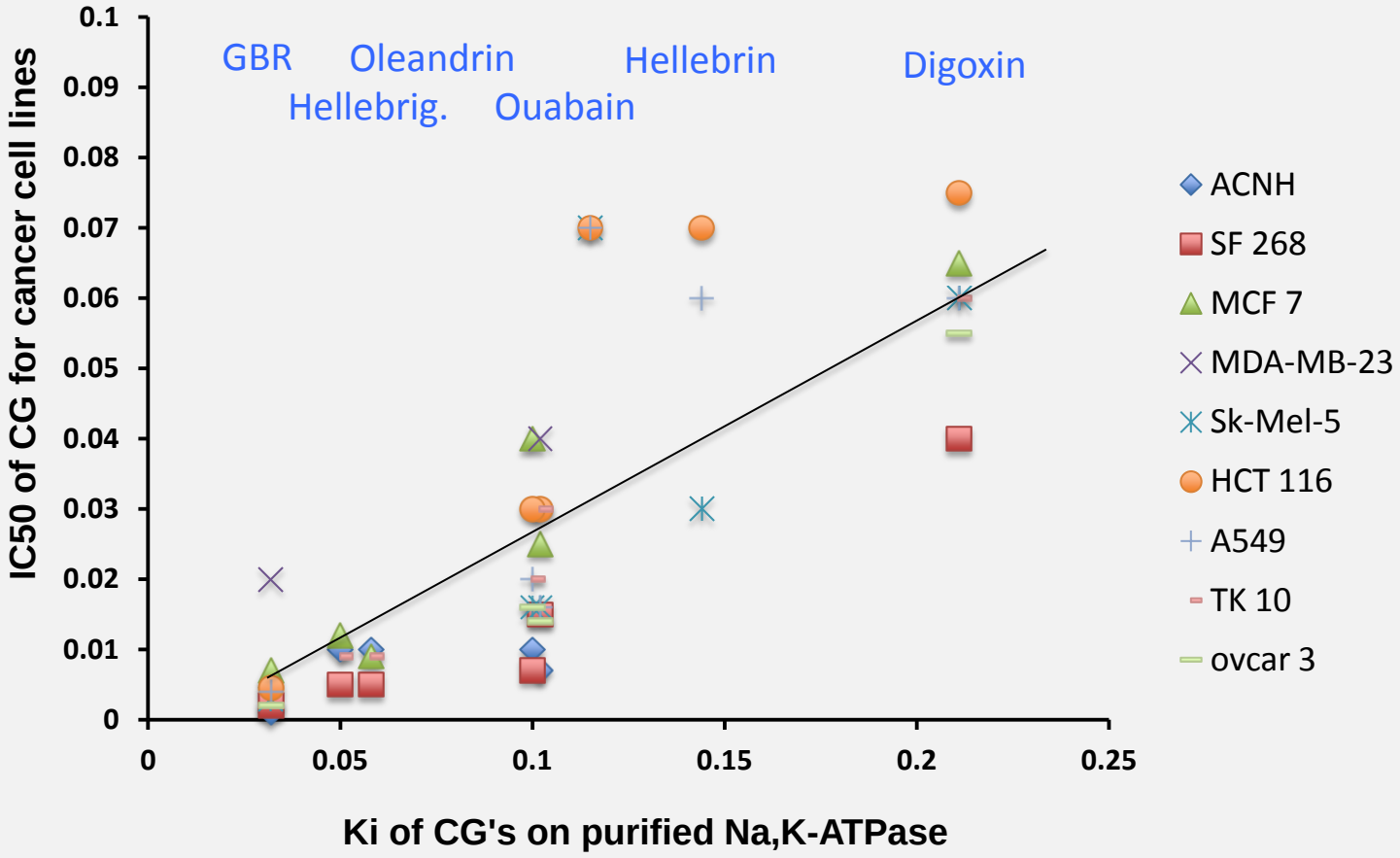
## *In vitro* growth inhibitory concentrations at 50% ($IC_{50}$) in human cancer cells after two days of culture in presence of GBR, the most effective CG.

1.

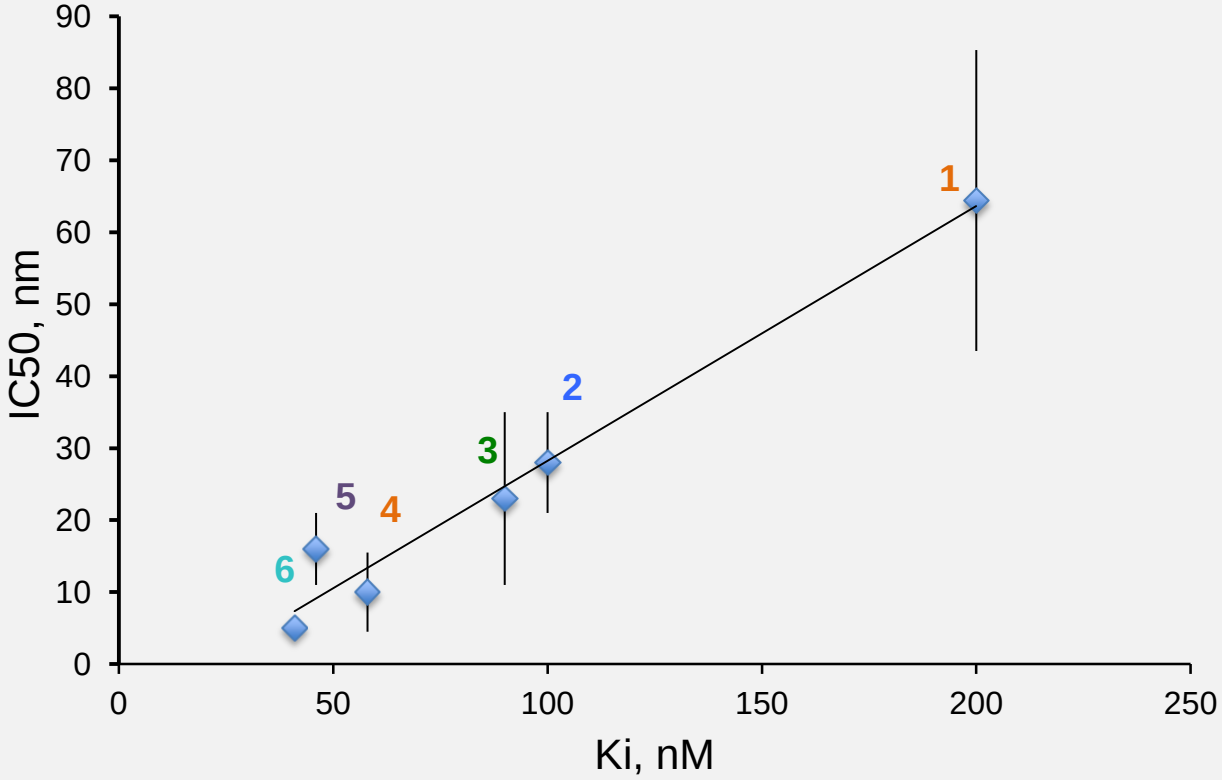



**Cell lines: LLC-PK pig kidney, MCF-7 breast cancer , HCT-116 colon cancer, TK-10 renal cancer.**

# Correlation of Ki for inhibition of human α1β1 by cardiac glycosides and the growth inhibition effects



# Correlation of Ki for inhibition of human α1β1 by cardiac glycosides and the growth inhibition effects

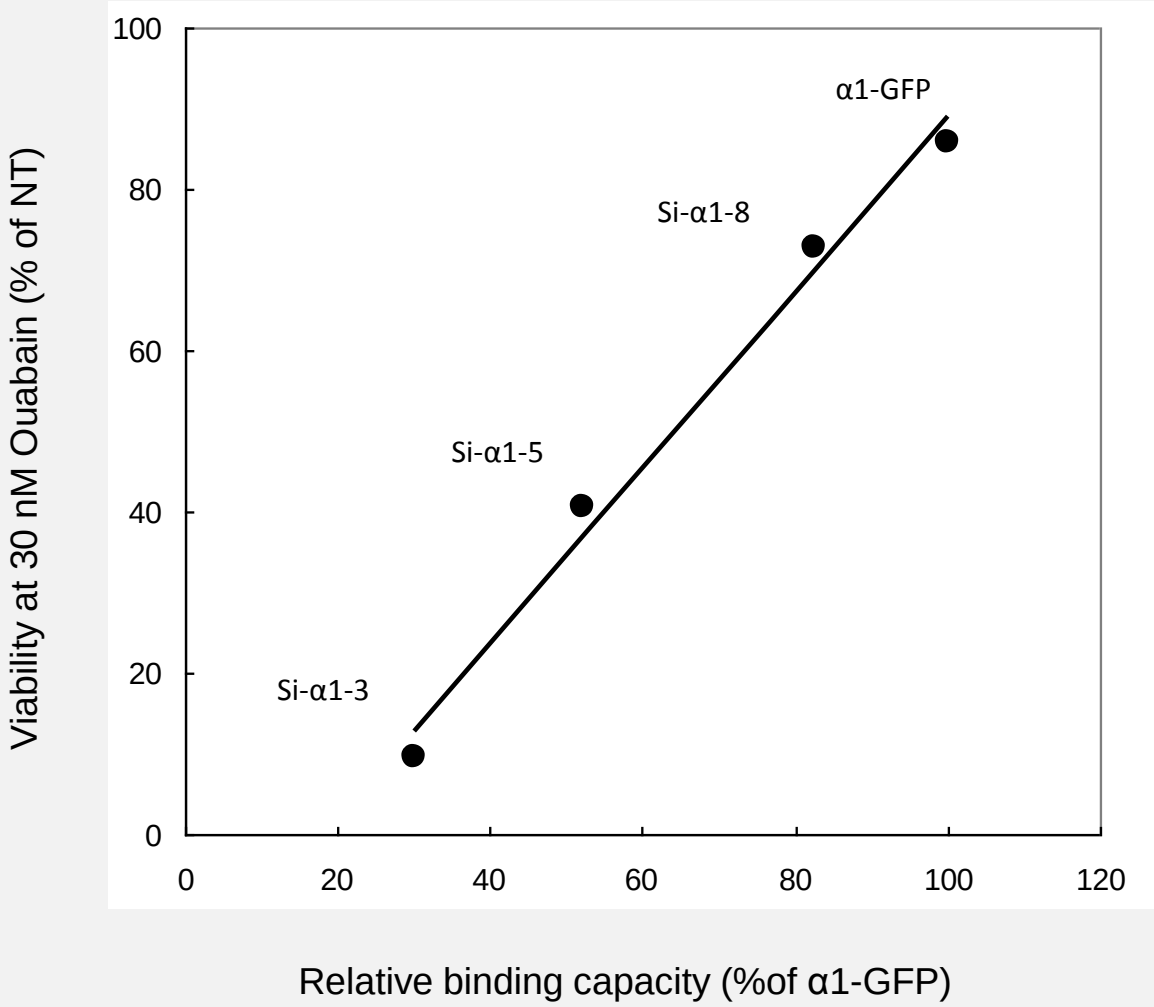



1. Digoxin
2. Hellebrin
3. Ouabain
4. Oleandrin
5. Hellebrigenin
6. Gamabuf. Rhamnoside

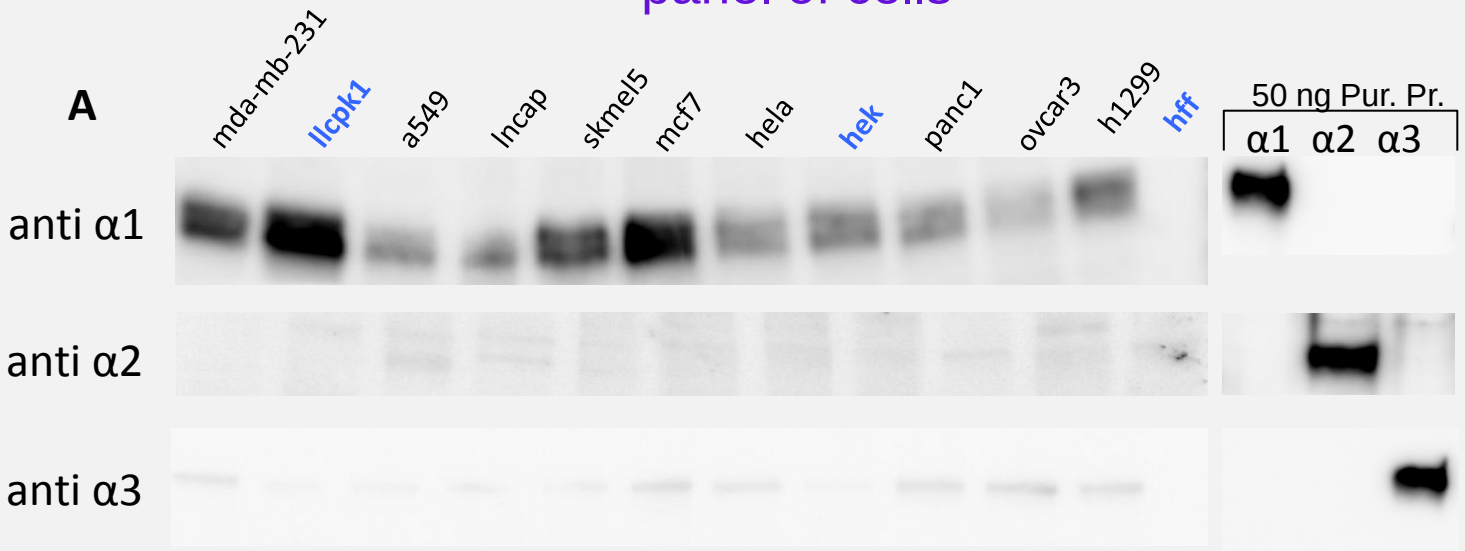
4 repetitions with 14 different cancer cells.

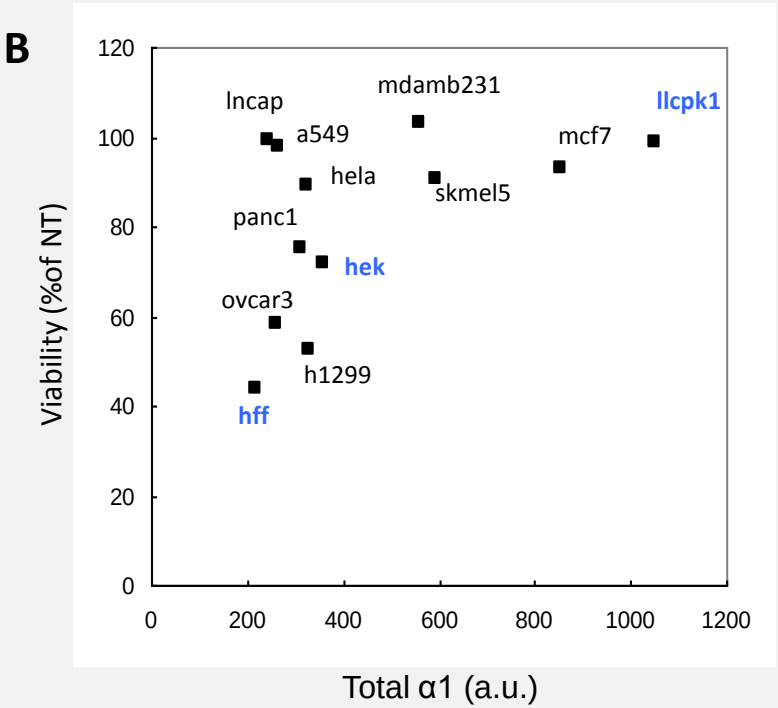
**Cell growth is linearly correleted to the affinity of individual CG to the Na,K-ATPaes**

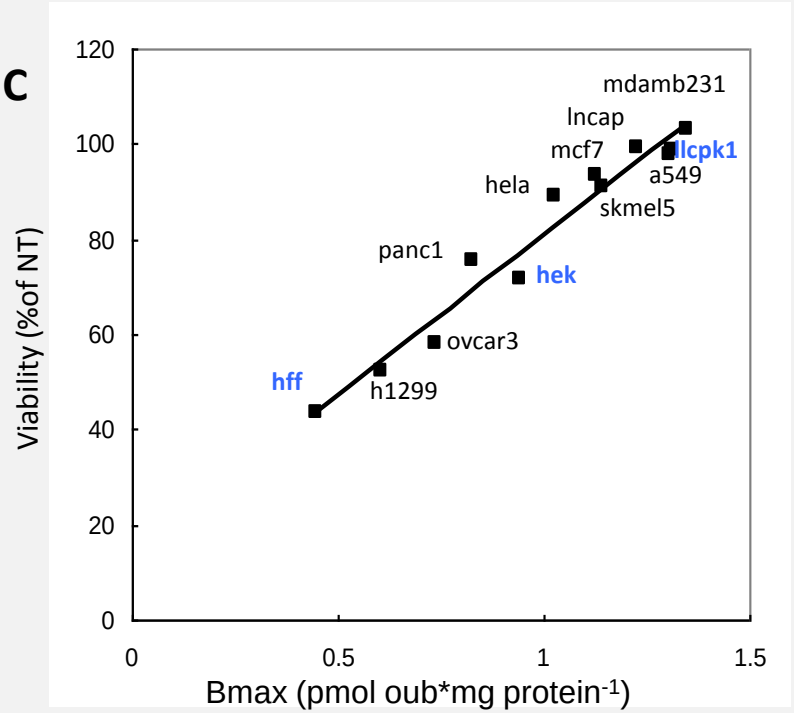
# Viability is linearly correlated with number of active pumps in partially silenced H1299 cells



# Viability is linearly correlated with number of active pumps in a panel of cells



✧Viability is linearly correlated with number of active pumps– CG affect cancer cell viability only through binding and inhibition of NaK ATPase transport

✧ Although cardiac glycosides can inhibit the proliferation of cancer cells at very low concentrations (nM), they inhibit the proliferation of human nonmalignant cells at similar concentrations; this strongly suggests that their potential for cancer therapy is low.

✧ More experimental data are needed to further decipher the structure-activity relationship between CG's and cancer cell cytotosicity.

# Acknowledgments

**Steven Karlish**

**Marina Cherniavsky-Lev**

**Elena Ainbinder**

**Daniel Tal**

**Yotam Nadav**

**Michael Habeck**

**Thank you for your attention.**