About OMICS Group

OMICS Group is an amalgamation of Open Access Publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 500 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 500 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

OMICS International Conferences

OMICS International is a pioneer and leading science event organizer, which publishes around 500 open access journals and conducts over 500 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

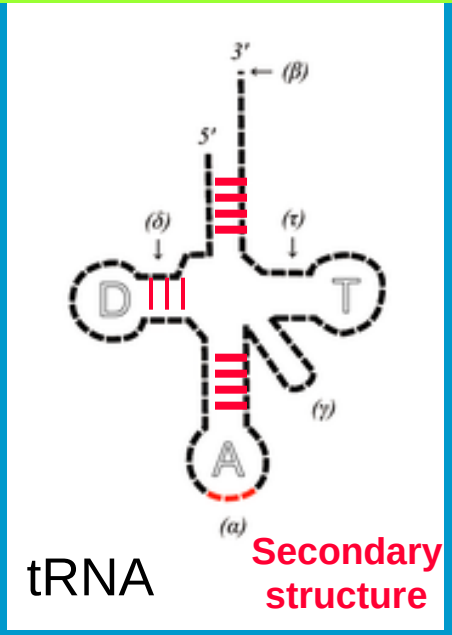
Cancer stem cells targeted delivery of siRNA to overcome induced chemoresistance

Wei DUAN
School of Medicine, Deakin University, Melbourne, Australia

Nucleic Acids Base Pairing leads to *distinct* 3-D fold

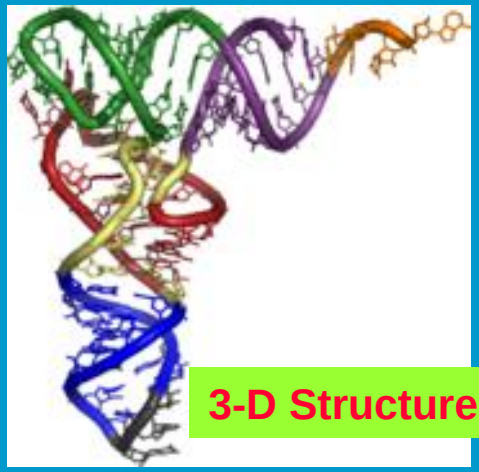
Aptamers (from Latin *aptus*, means "fitting" also known as "chemical antibodies"

http://www.archemix.com/website/index.php



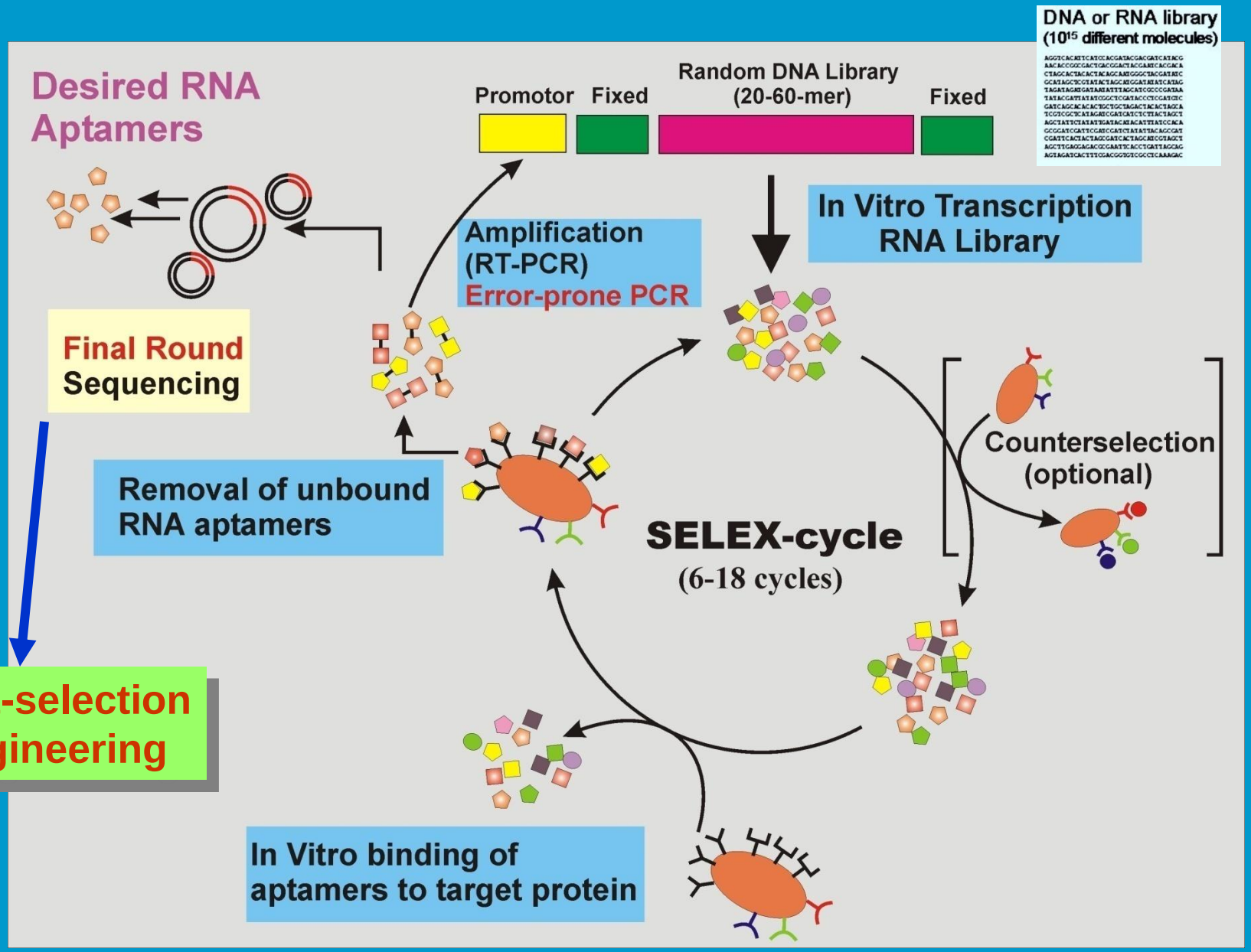
tRNA

Secondary structure



3-D Structure

SELEX: Systemic Evolution of Ligands by Exponential enrichment.



Features/Advantages of APTAMERS

1. Highly specific. High affinity (K_D: 1 pM vs 100 pM for Ab)

2. Highly stable: may tolerate a wide range of temperature, pH (~4-9) and organic solvents.

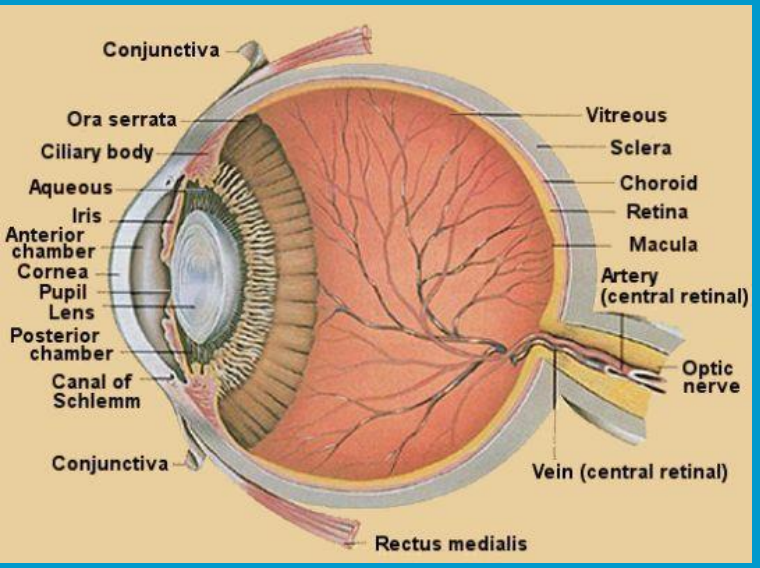
3. Exhibit superior tissue penetration (due to their small size (6-15 kDa vs 150 kDa, 20-25 times smaller).

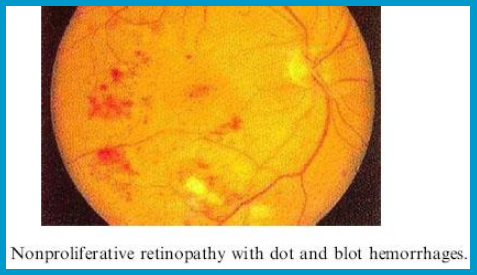
4. Entirely chemical synthesis, low batch variability, faster turnover, relatively low cost.

5. No or very low immunogenicity
6. Non-toxic, human-degradable

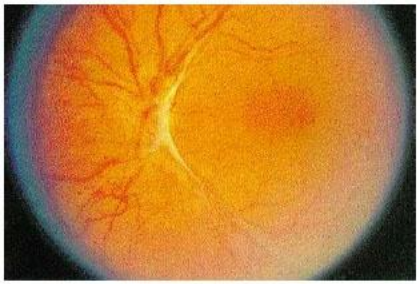
First RNA aptamer drug was approved by FDA in 2004

Macugen, (Pegaptanib sodium) from Eyetech Pharmaceuticals, an anti-vascular endothelial growth facti (VEGF) RNA aptamer. For the treatment of all types of neovascular age-related macular degeneration (AMD)

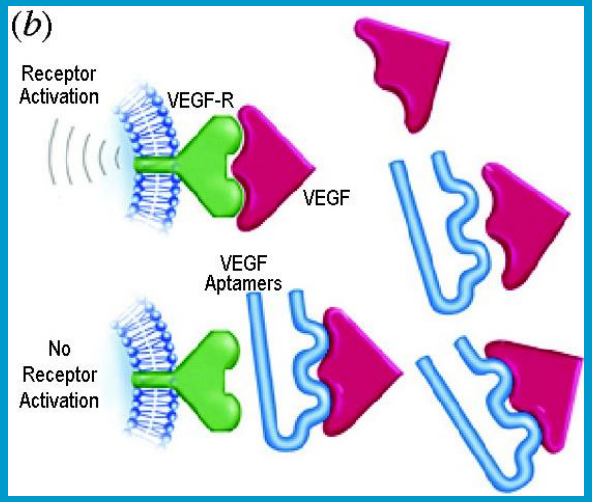




Nonproliferative retinopathy with dot and blot hemorrhages.



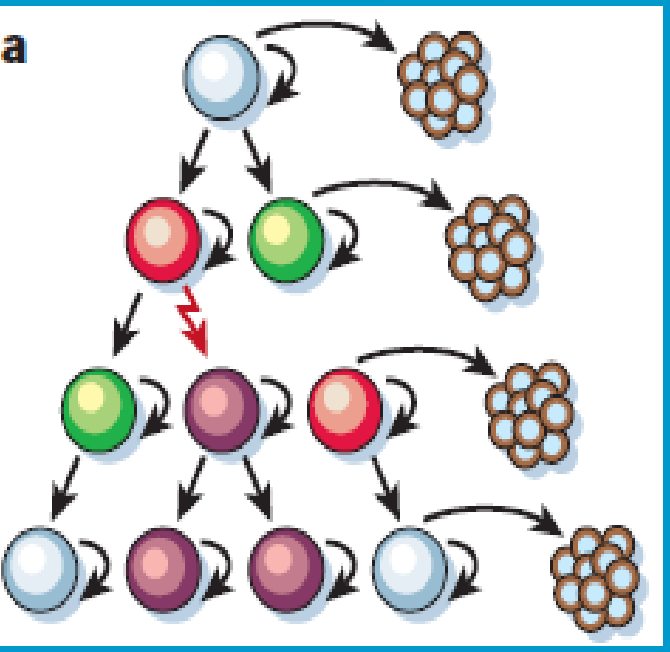
7. Neovascularization (proliferative retinopathy)



The advantages of targeting a cell surface marker(s) expressed in both non-CSC and CSC

Stochastic cancer model

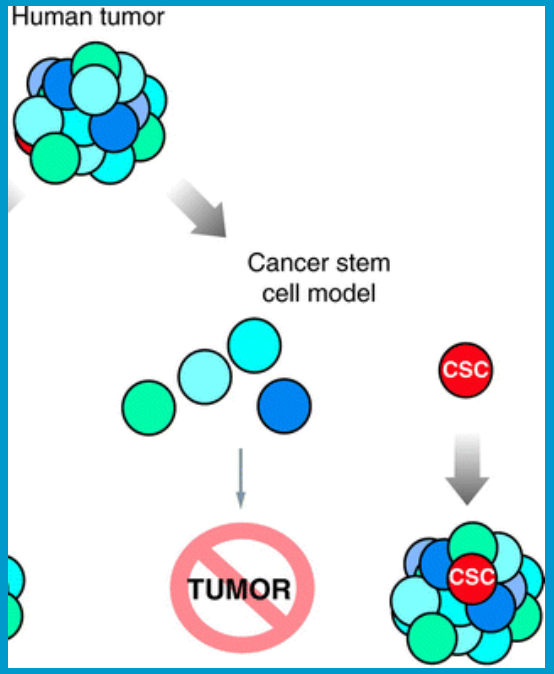
1976



Nowell, P. C. *Science* 194, 23–28 (1976).

Hierarchical CSC model

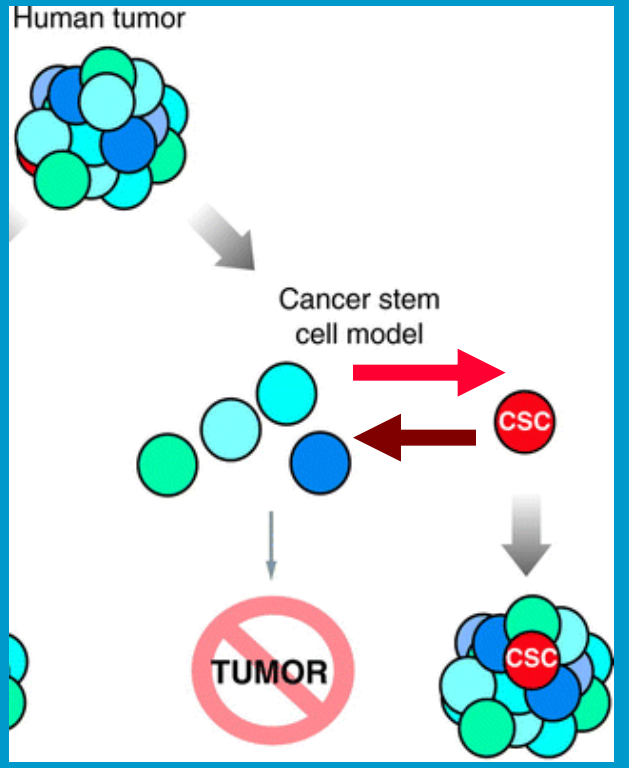
1997, 2003



Bonnet, D. & Dick, J. E. *Nature Med.* 3, 730–737 (1997)
Al-Hajj, M., *et al. Proc. Natl Acad. Sci. USA* 100, 3983–3988 (2003)

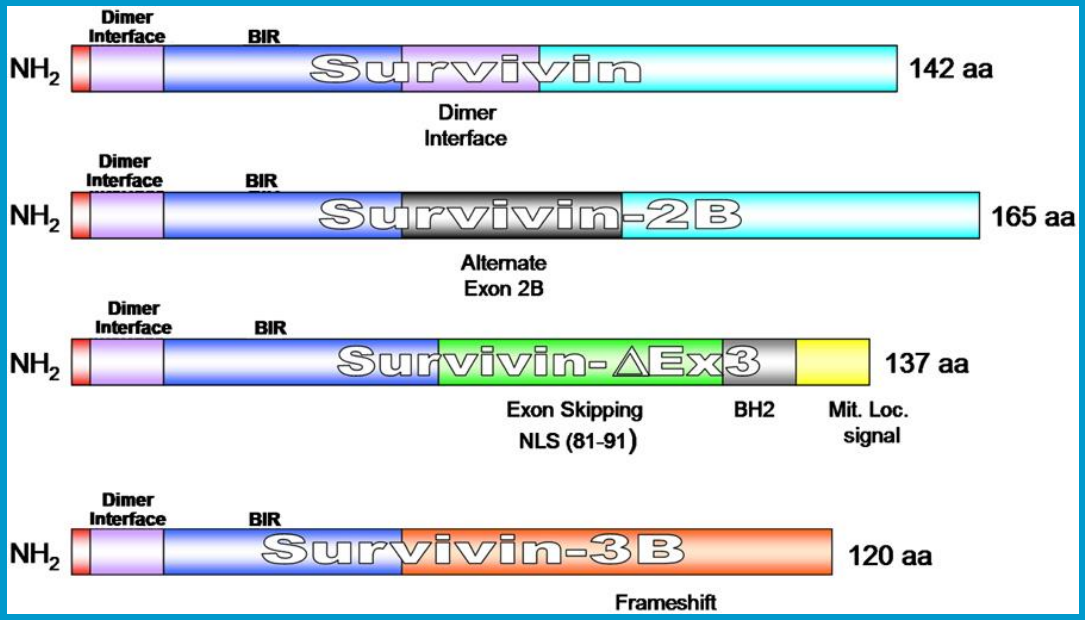
Dynamic CSC model

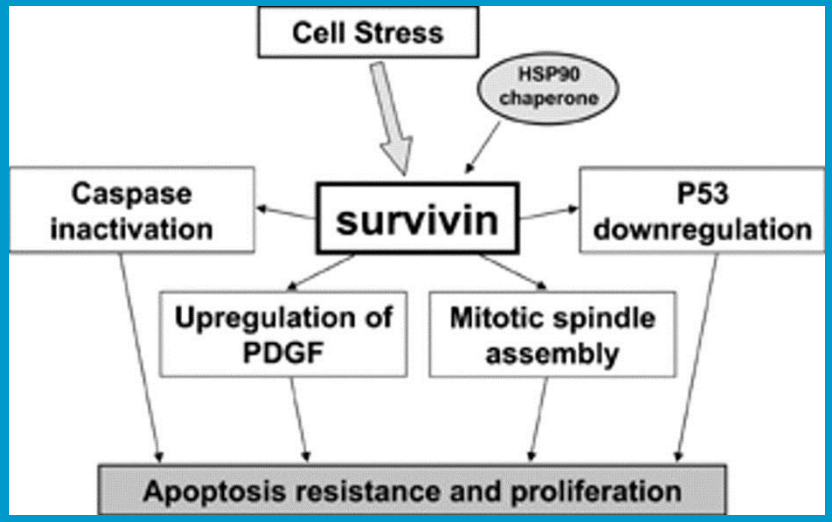
2011



CL Chaffer, I Brueckmann, C Scheel *et al.* Proc Natl Acad Sci USA, 108, 7950–7955 (2011)
C Scheel, EN Eaton, SH Li et al. Cell, 145, 926–940 (2011)

Survivin, a key regulator of apoptosis and mitosis





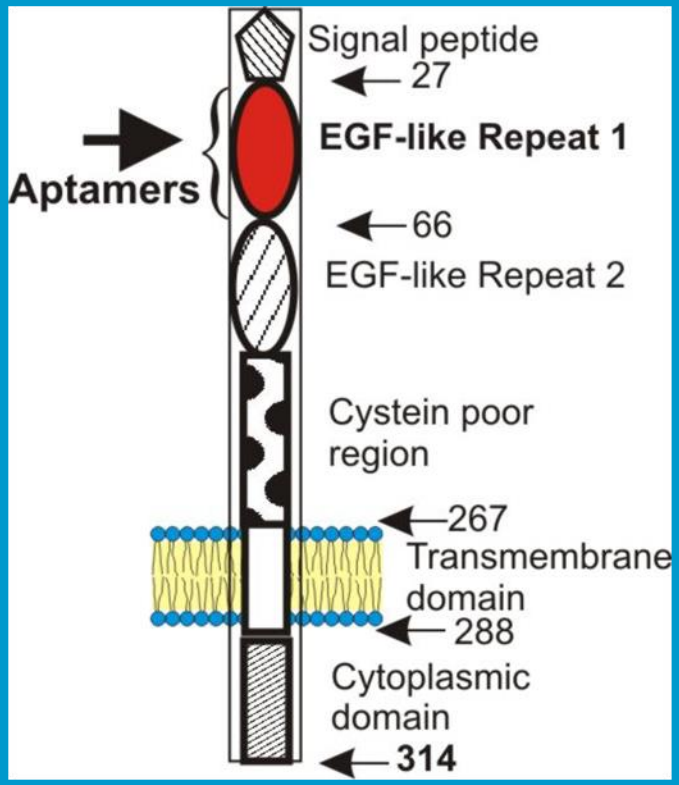
Low expression in normal tissues

Vast overexpression in cancer, especially after chemotherapy

One of the most cancer-specific genes

EpCAM (Epithelial Cell Adhesion Molecule)

Overexpressed in most (~70%) solid cancers



Breast caner CSC marker:

$EpCAM^{+}/CD44^{+}/CD24^{-}/Lin^{-}$

Muhammad Al-Hajj et al, *Proc Natl Acad Sci U S A.*100(7): 3983–3988, 2003.

Michael Clarke's Lab at University of Michigan Medical School, Ann Arbor

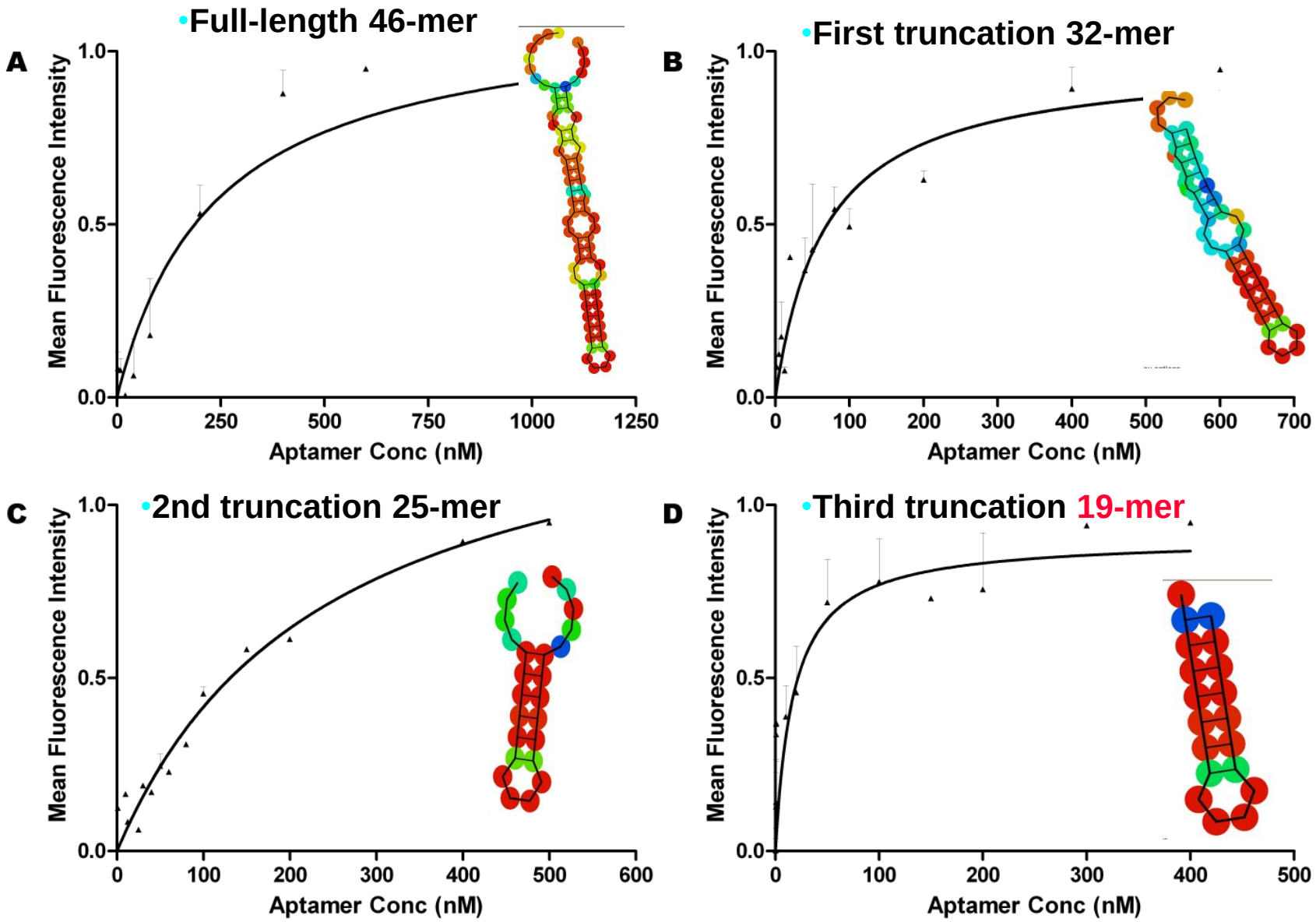
Cited 3740 times

EpCAM is a clinically validated/evaluated cancer marker

1. **Marker for: cancer stem cells (Breast, Liver, Colon Cancer)**
2. **and metastatic cancer cells (FDA-approved CellSearch, BrCa)**
3. **Overexpression associated with poor prognosis (triple-negative BrCa)**

Post-SELEX enginerring of EpCAM RNA aptamer

Fig 4.



Aptamer-siRNA chimera design

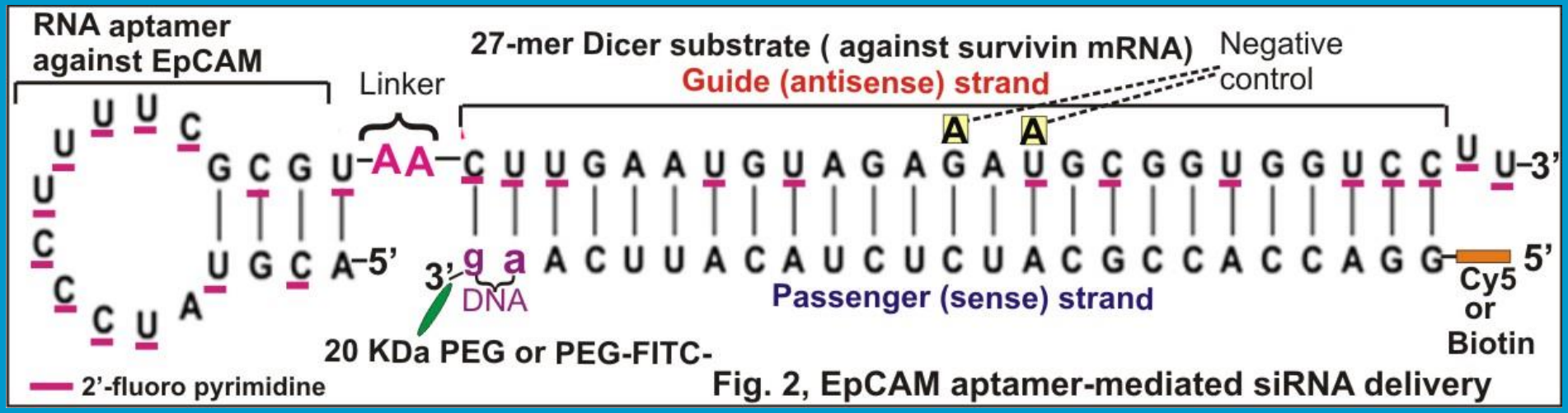
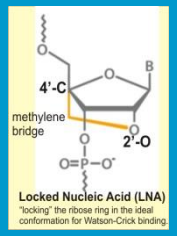
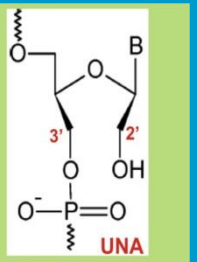


Fig. 2, EpCAM aptamer-mediated siRNA delivery





Unlocked Nucleic Acid
The ribose ring is cleaved between the 2'- and 3'-carbons. A highly flexible structure, used to modulate duplex characteristics

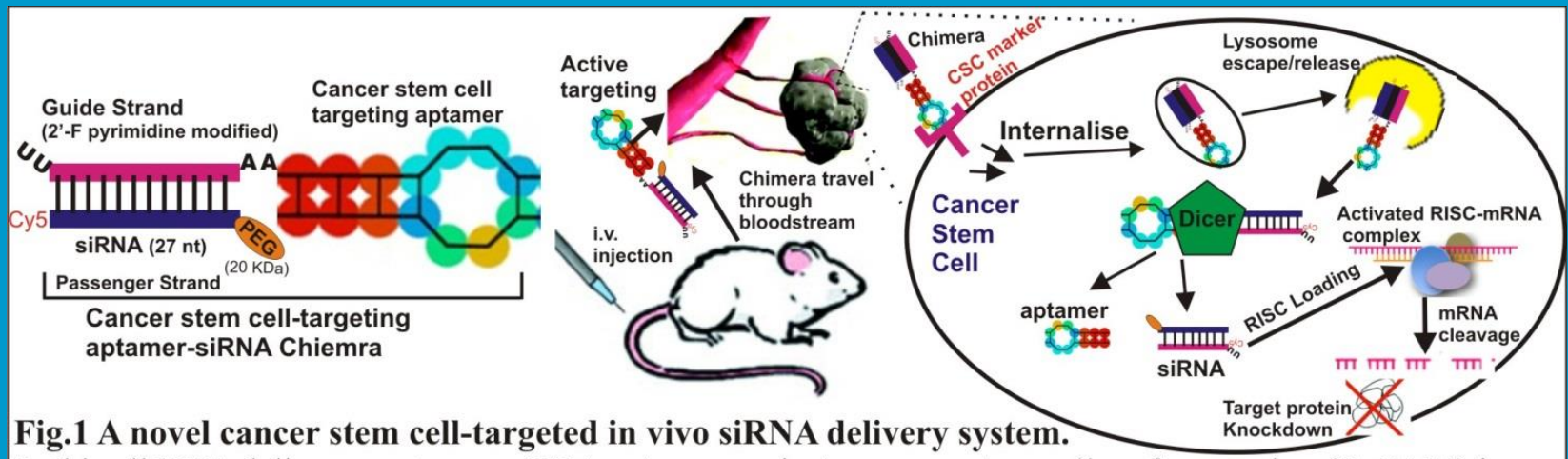
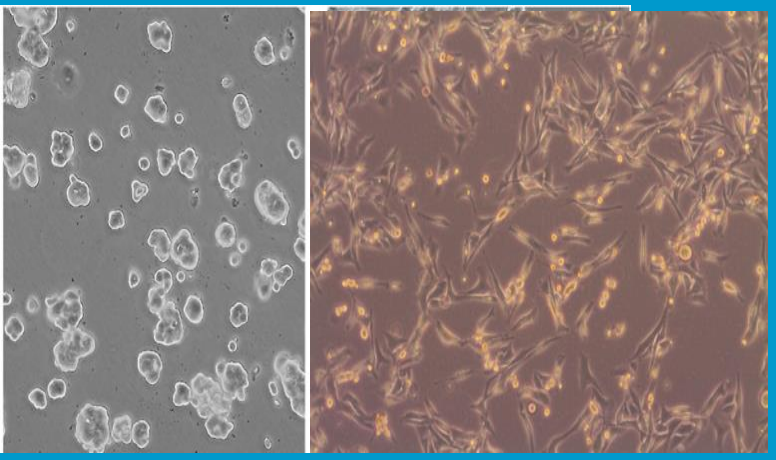


Fig.1 A novel cancer stem cell-targeted in vivo siRNA delivery system.
In this all RNA delivery system, an RNA aptamer against a cancer stem cell surface marker (EpCAM) is covalently linked to smartly engineered siRNA. The chimera is injected i.v. to target cancer stem cells via both passive and active targeting. Upon binding to cancer stem cells, the chimera is efficiently internalized, gaining access to the cytoplasm, processed by Dicer, loaded to RISC, thus leading to mRNA degradation.

A doxorubicin-resistant breast cancer cell line MCF-7/Adr

Wild-type MCF-7

Doxorubicin-resistant variant MCF-7/ADR



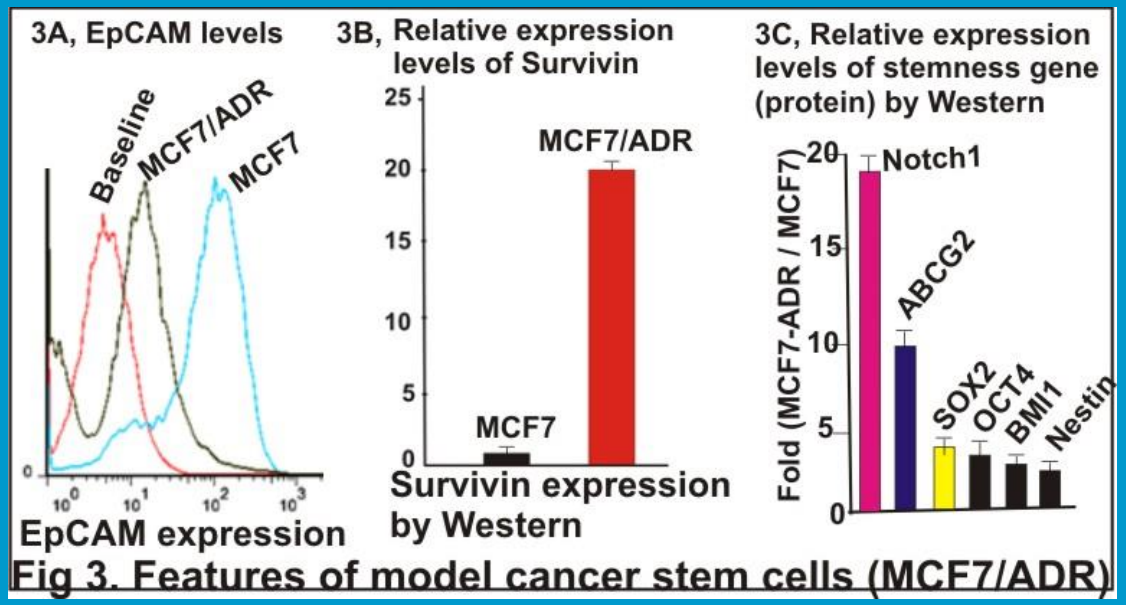
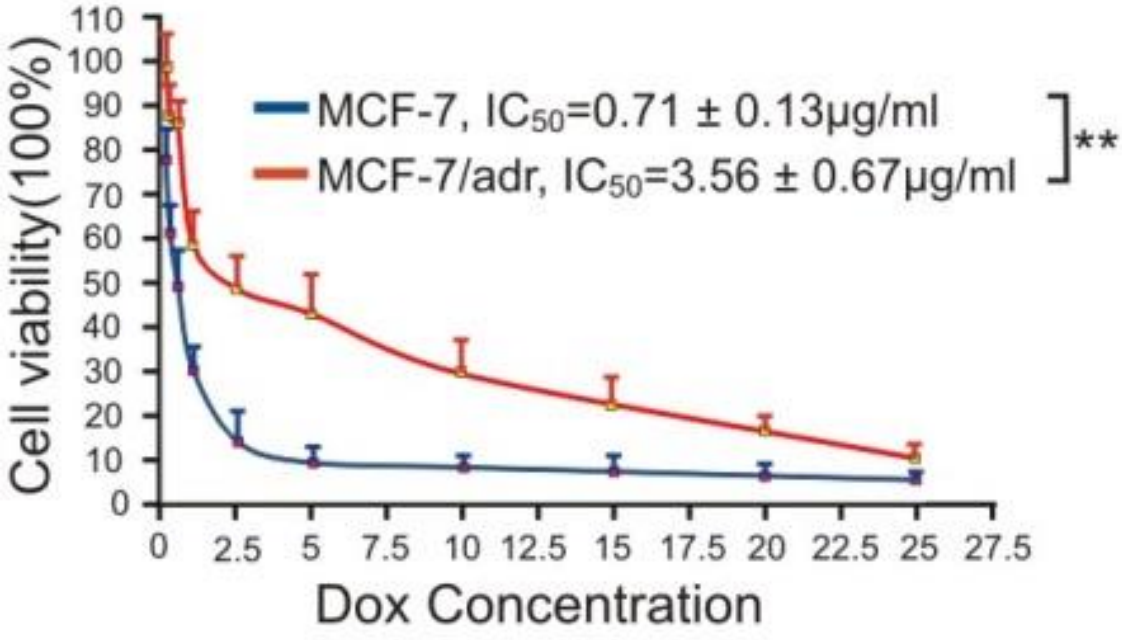


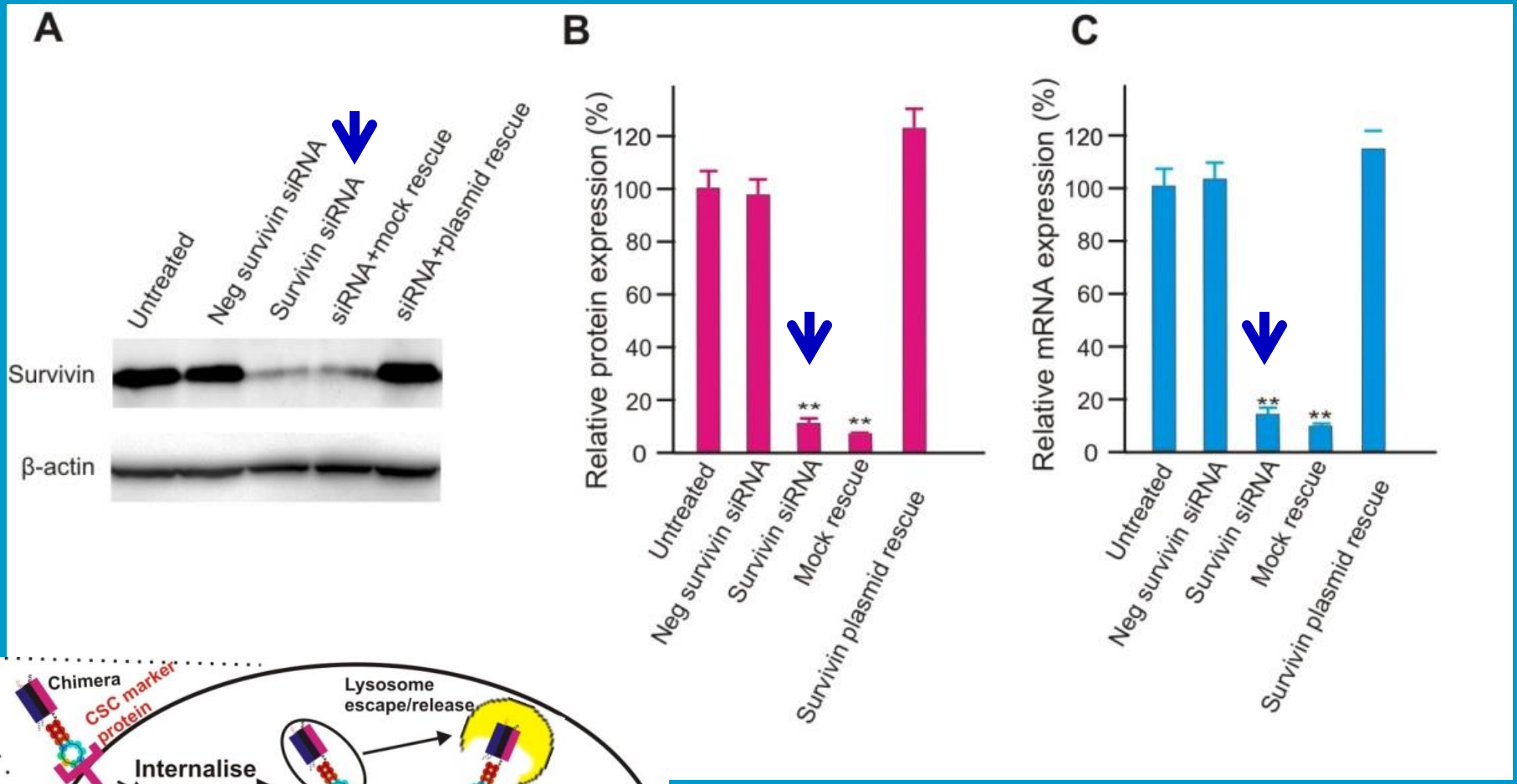
Fig 3. Features of model cancer stem cells (MCF7/ADR)

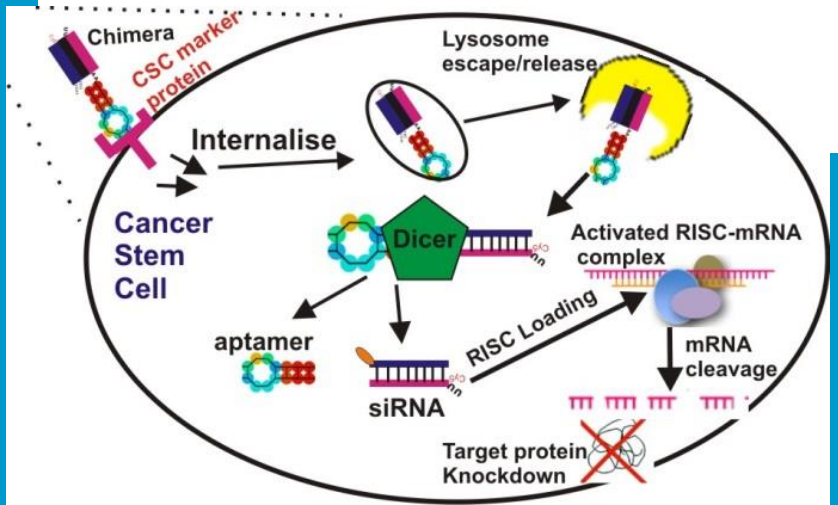


5-fold increase in IC_{50}

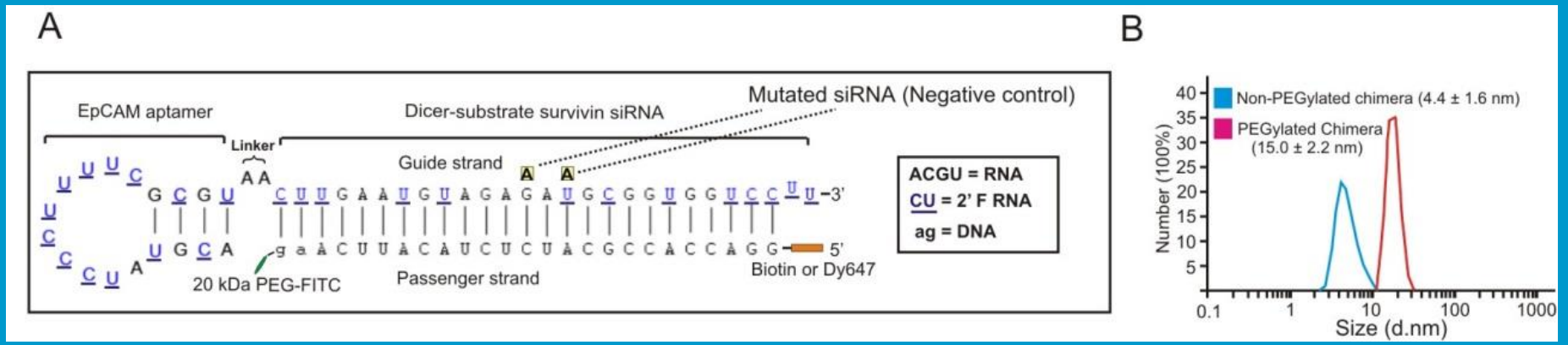
A model of induced chemoresistance

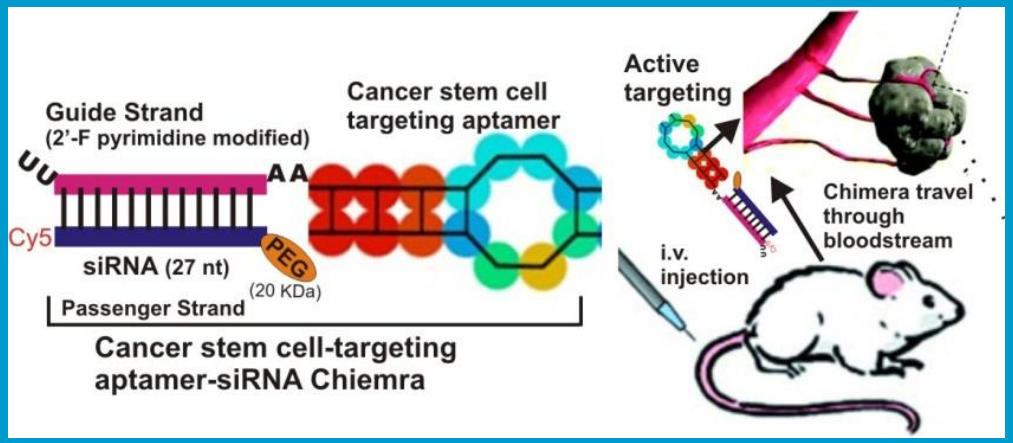
Aptamer-siRNA chimera efficiently silence survivin in vitro (

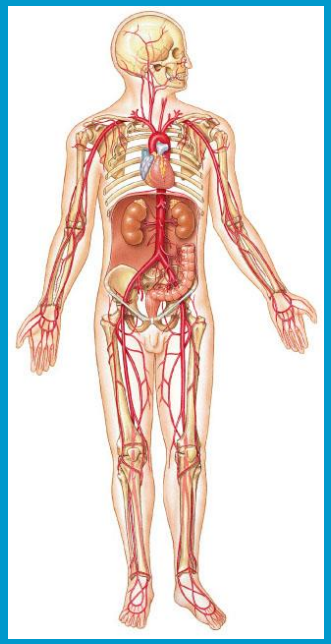




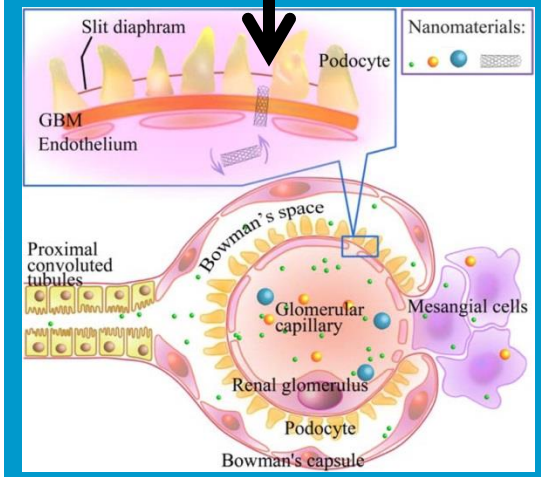
Aptamer-siRNA chimera targeting CSC in vitro (CSC marker analysis)



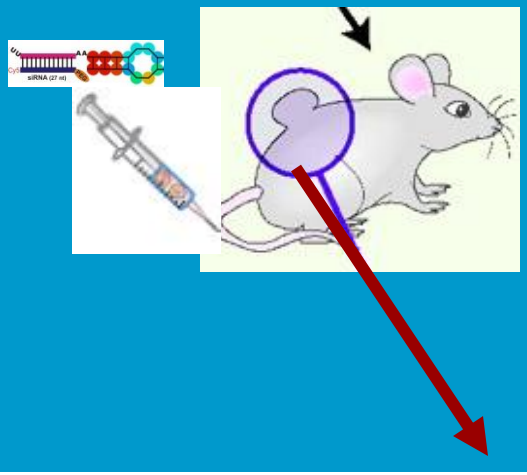




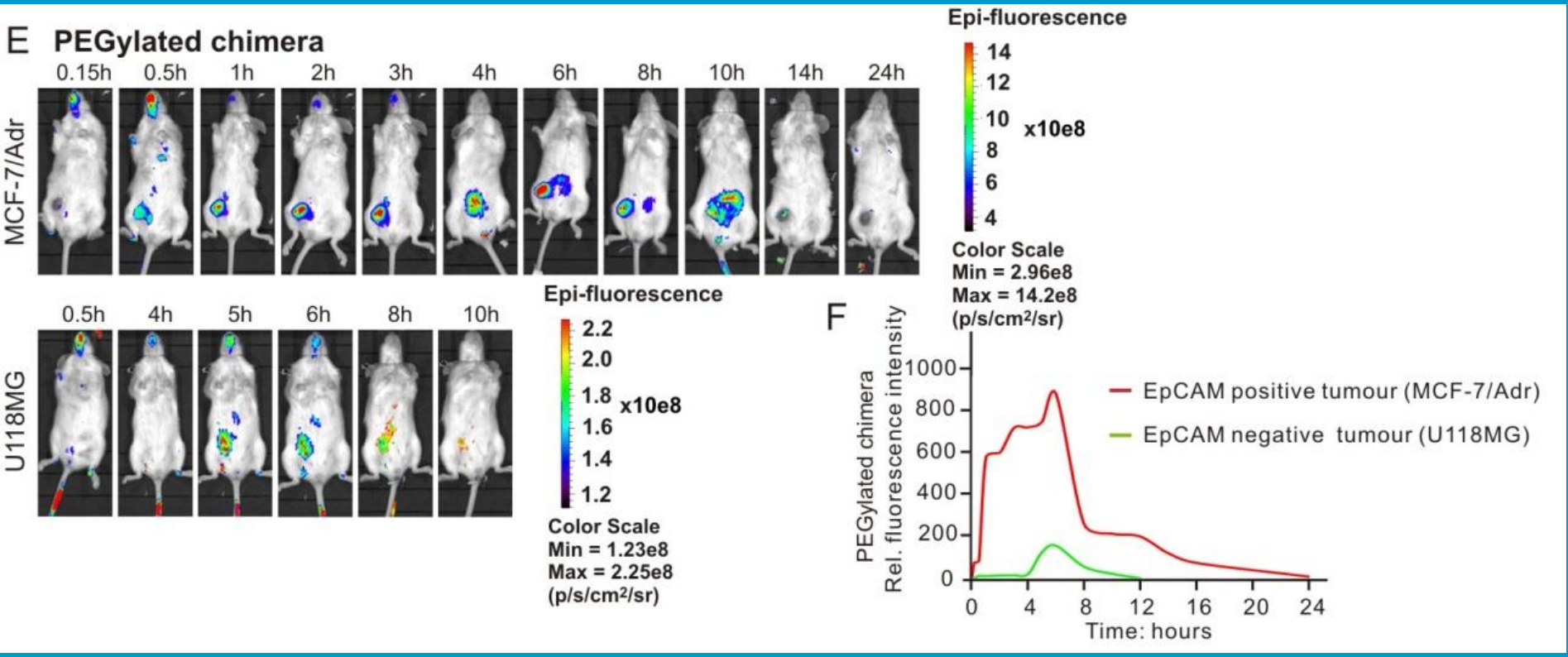
Renal filtration: 10 nm



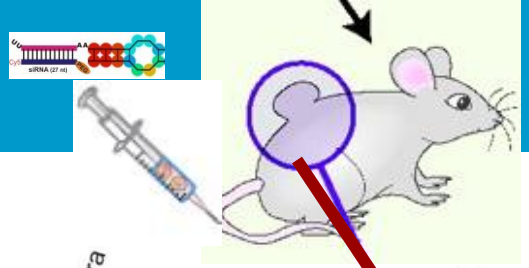
In vivo cancer stem cell-targeted delivery of siRNA

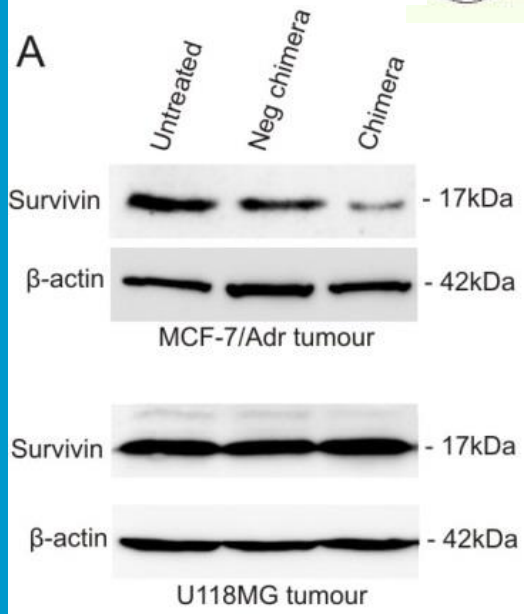


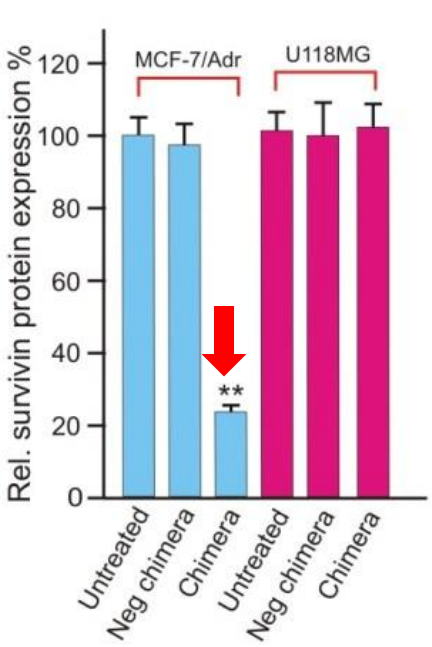
Tumour PK and Biodistribution of aptamer-siRNA

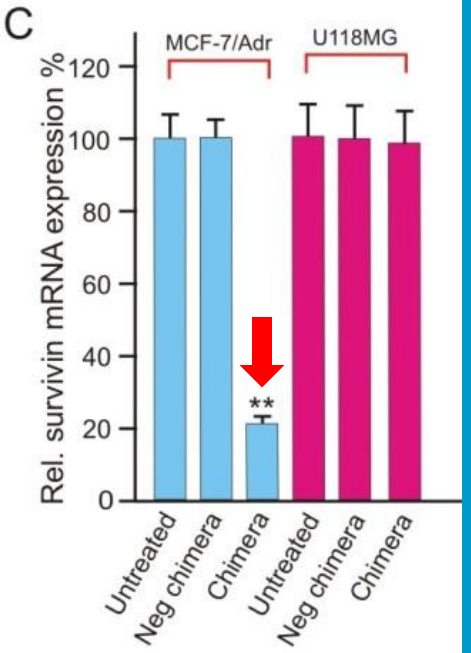


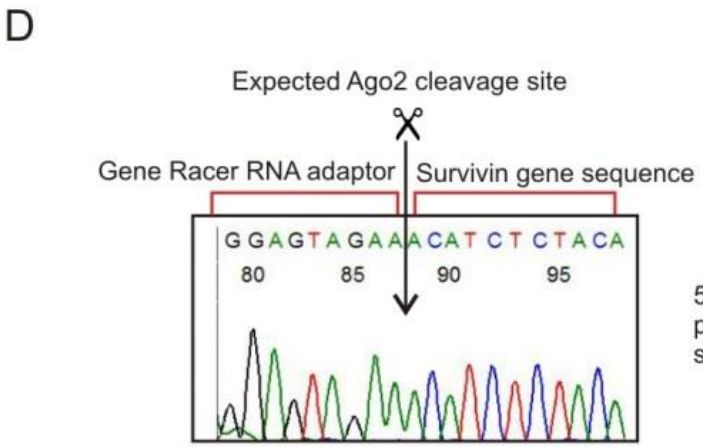
Effective in vivo knockdown

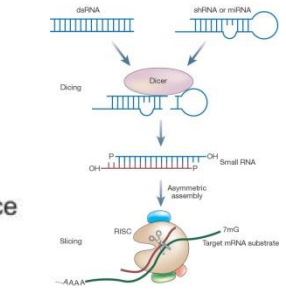




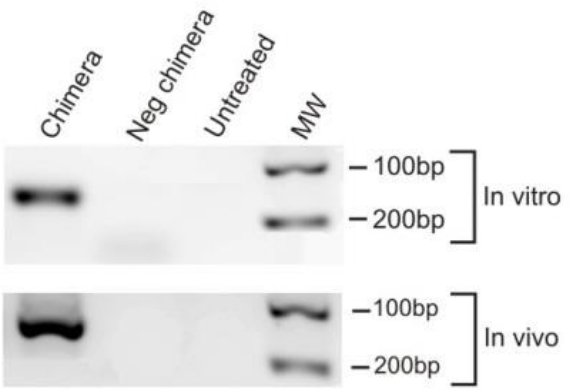




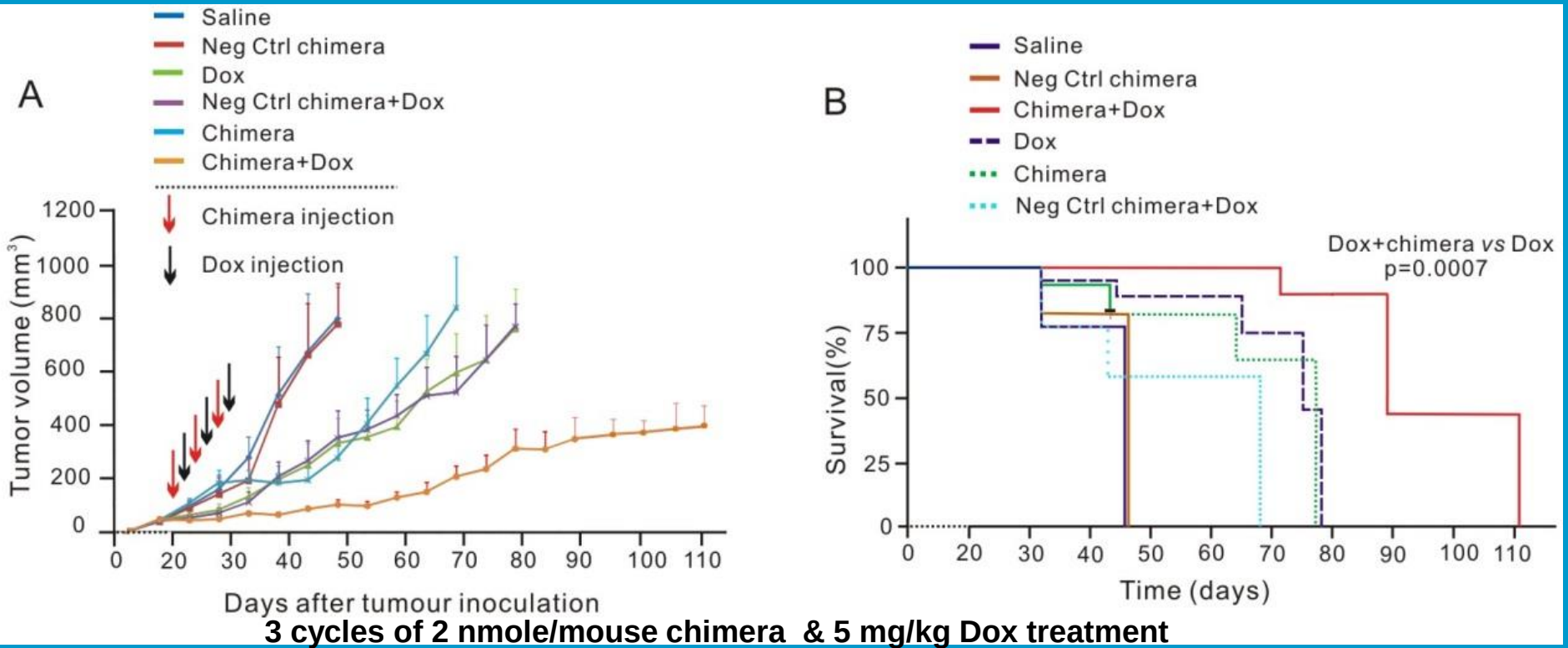




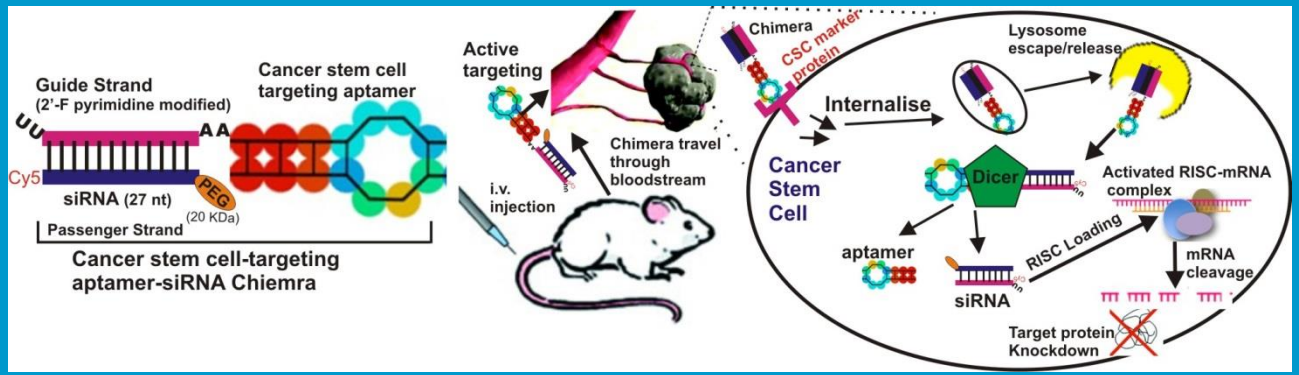
5' RACE PCR products Expected size = 141bp



In vivo silencing of survivin gene leads to enhanced suppression of tumour growth and marked improved survival



3 cycles of 2 nmole/mouse chimera & 5 mg/kg Dox treatment



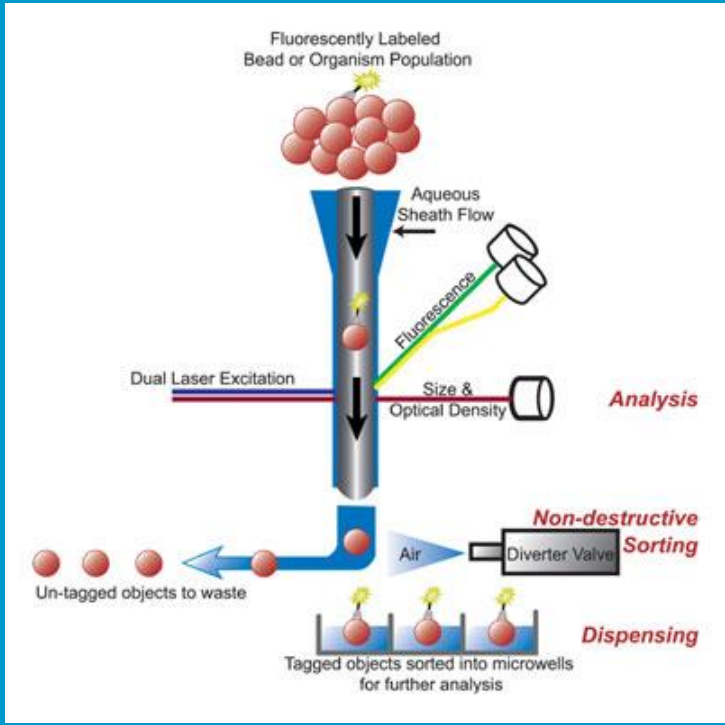
Mechanism of action:

3) Elimination of cancer stem cells (reduction of % of CSC marker-positive cells)

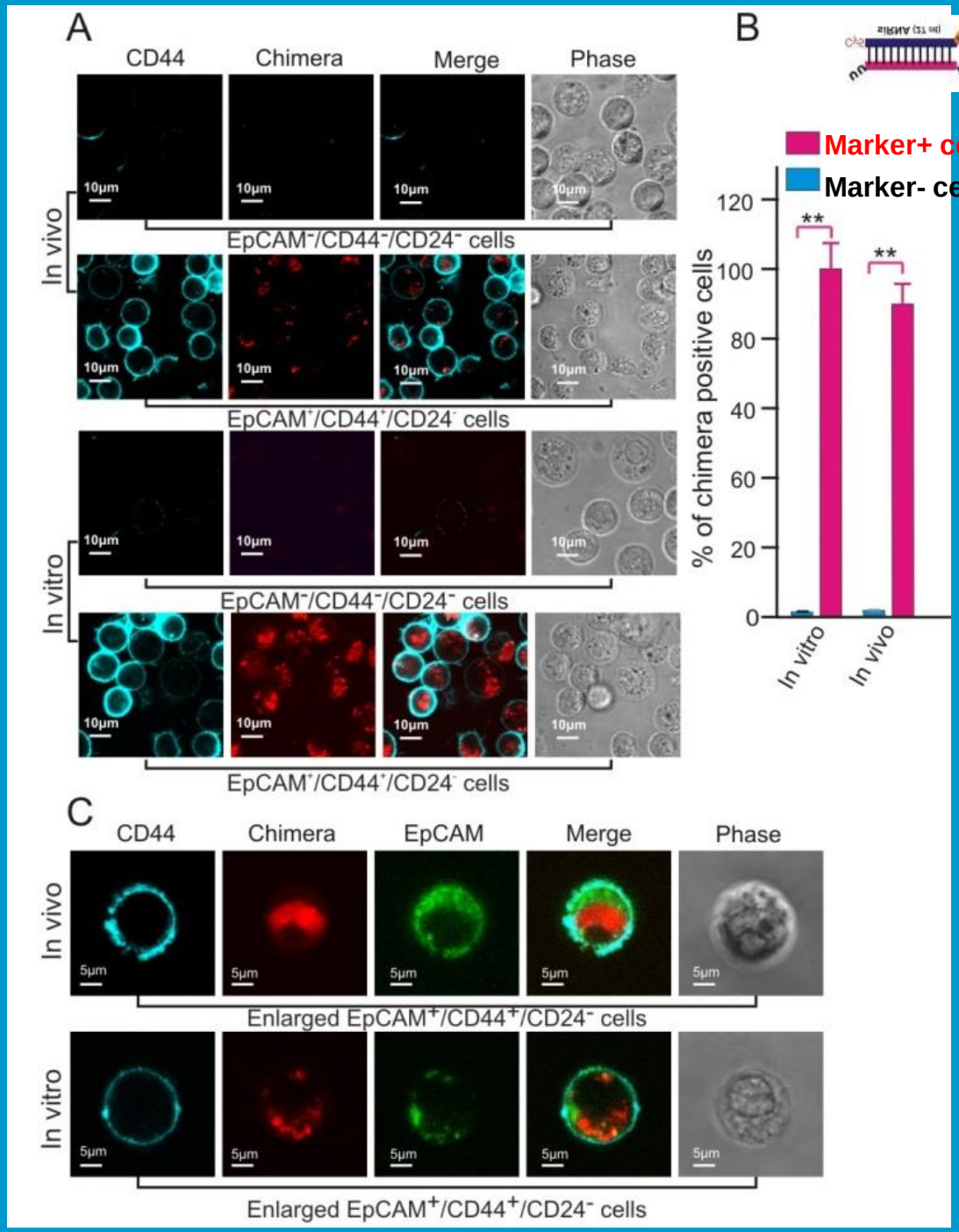
D

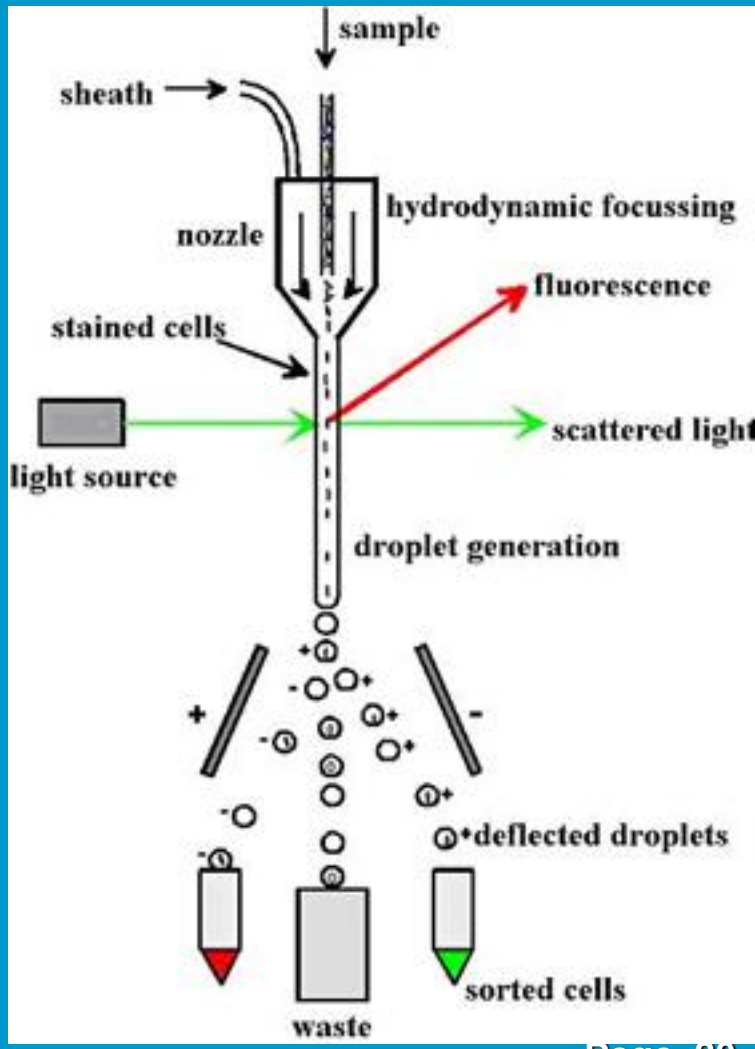
Percentage of tumorigenic EpCAM$^+$/CD44$^+$/CD24$^-$ population in MCF-7/Adr tumors

	Untreated	Dox	Neg chimera	Neg chimera + Dox	Chimera	Dox+chimera
% of EpCAM+/ CD44+/CD24- cells	11.82 ± 1.93	11.66 ± 2.10	12.87 ± 2.31	11.92 ± 2.52	7.82 ± 1.33 *	1.87 ± 0.29 **

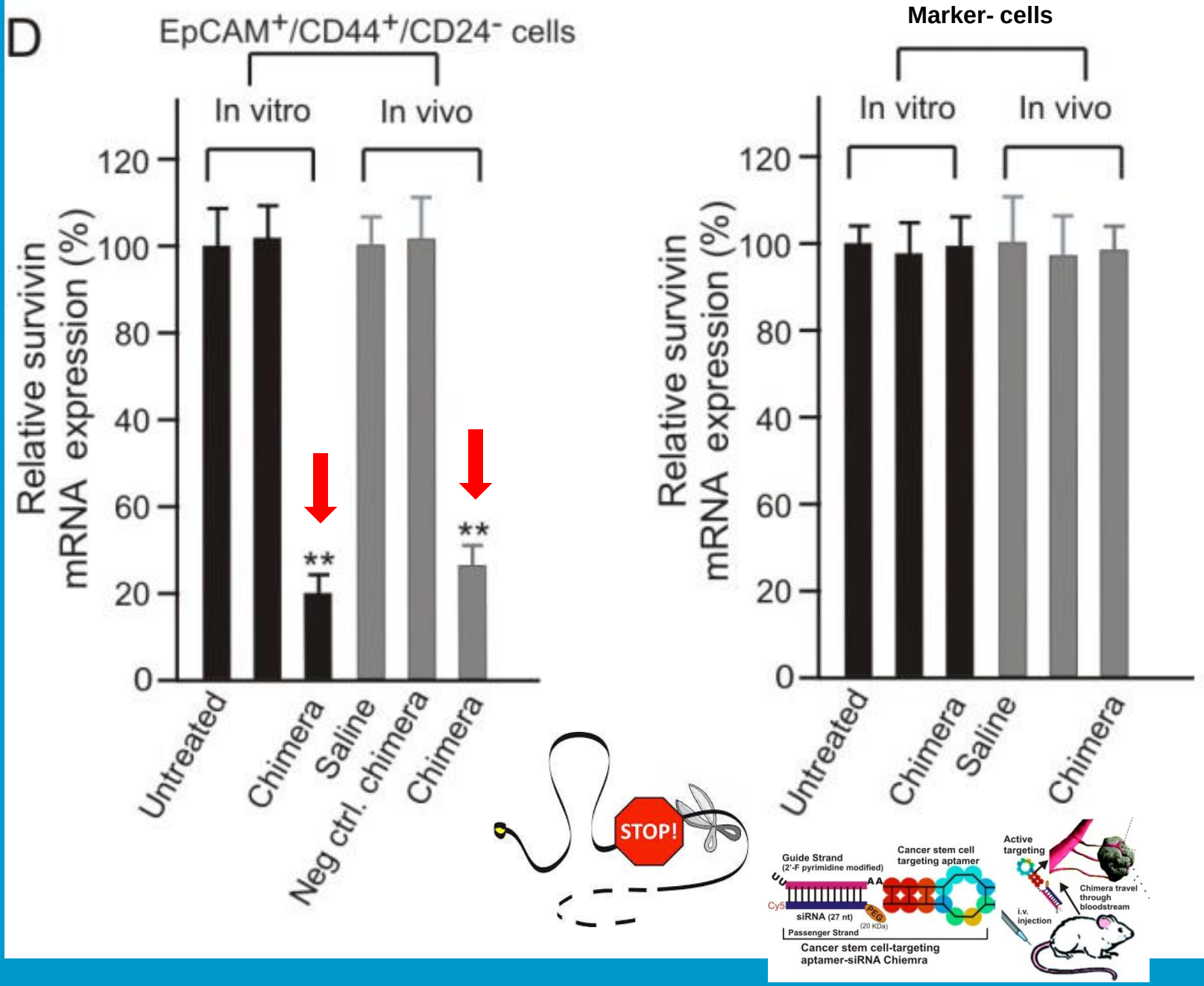


Mechanism of action:
5) In vivo targeting of CSC

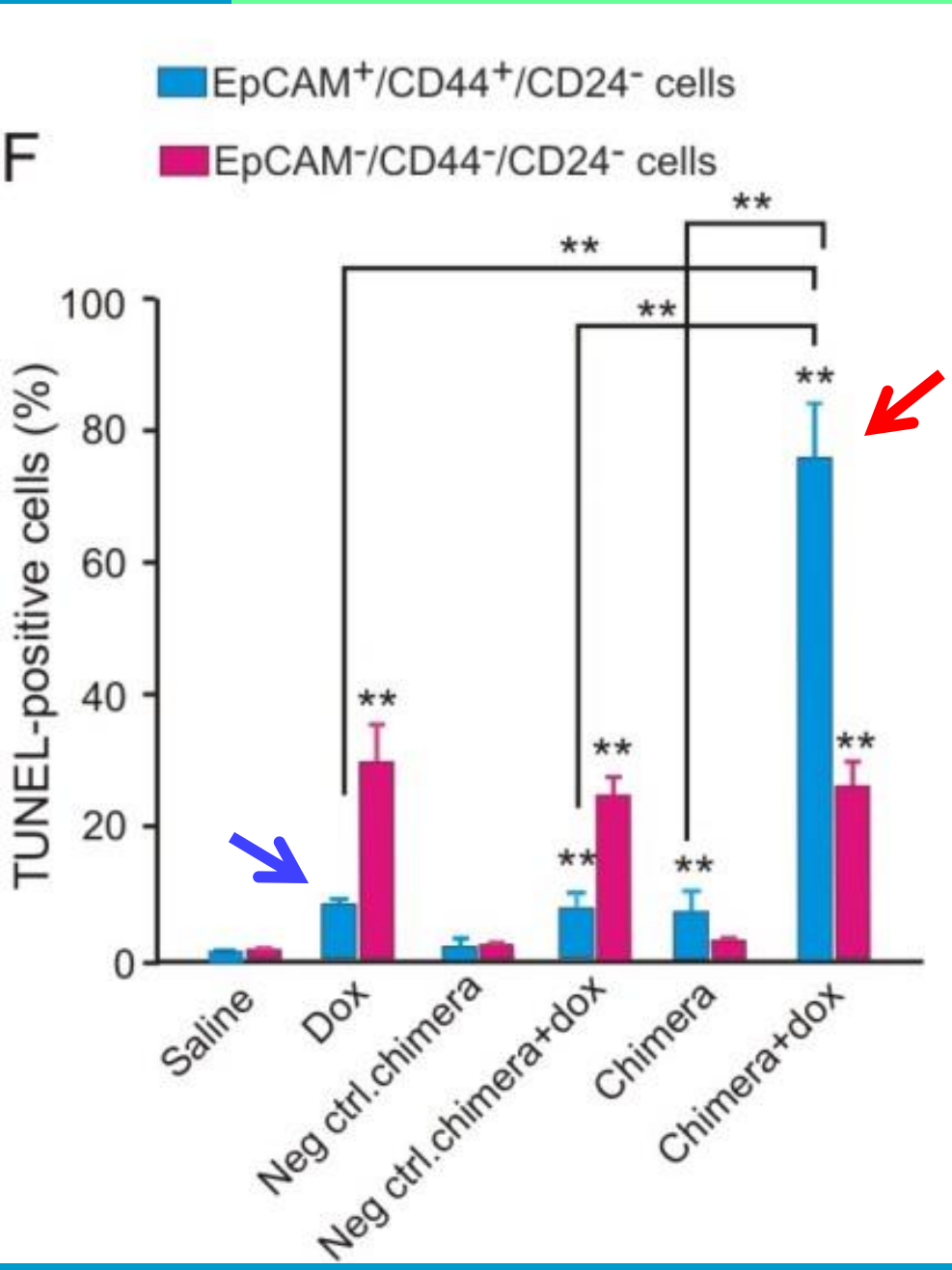


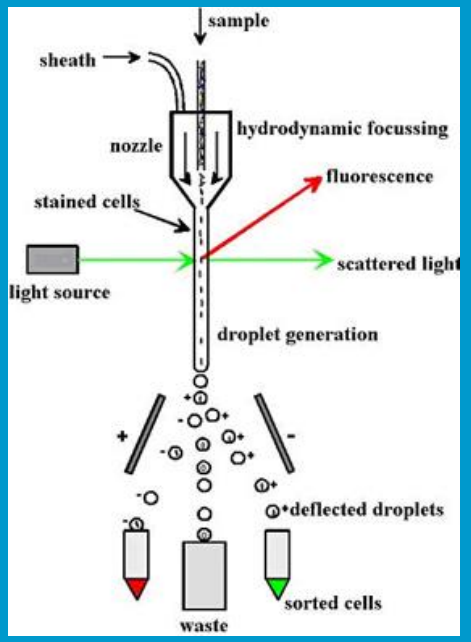


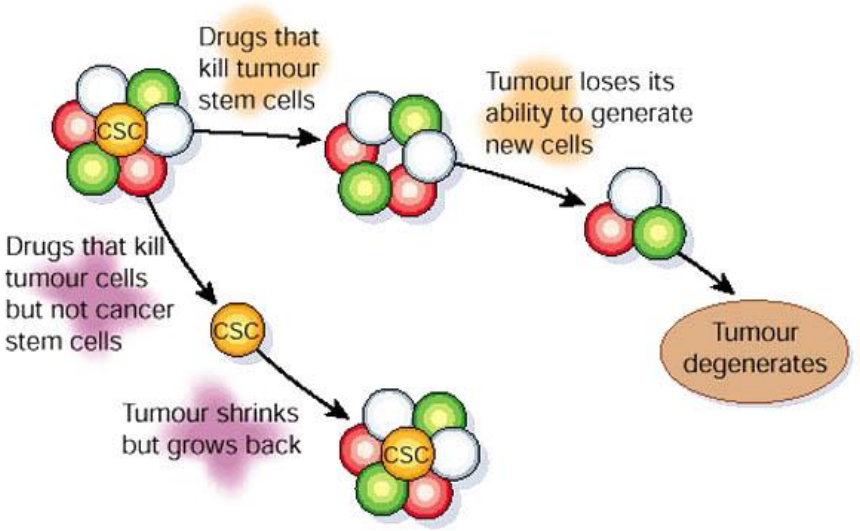
Mechanism of action: 6) In vivo silencing of survivin in CSC



Mechanism of action: 7) Reversal of chemoresistance in CSC







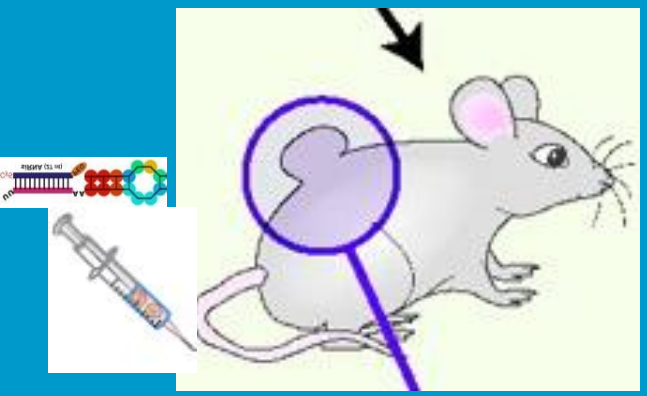
Mechanism of action:

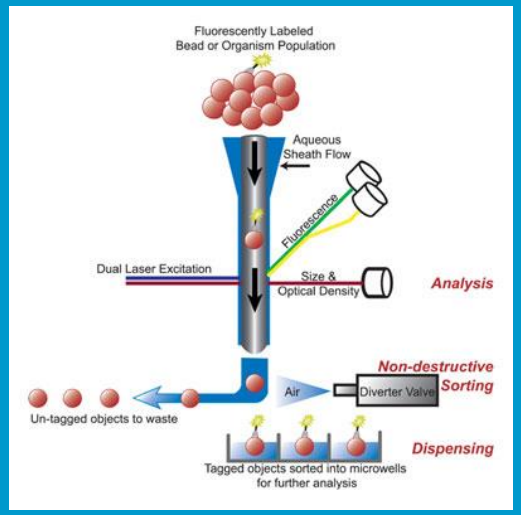
8) Diminished tumourigenicity of cancer stem cells after 3 cycles of treatment

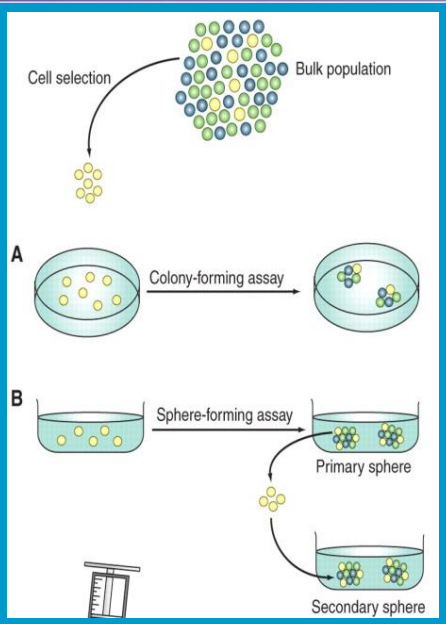
G

Mammosphere formation capacity of sorted EpCAM^{+}/CD44^{+}/CD24^{-} cells (%)

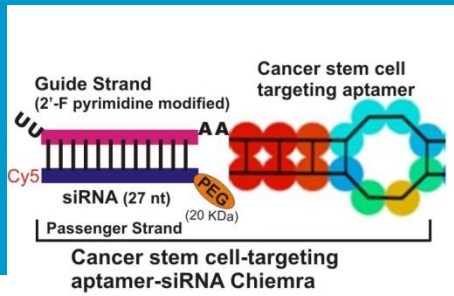
	Saline	Dox	Neg ctrl. chimera	Chimera	Dox+ chimera	Dox+Neg ctrl. chimera
Cells sorted from treated tumor						
Primary sphere	14.62±3.09	13.23±2.87	15.01±2.36	13.05±2.18	2.14±0.26**	13.89±2.97
Secondary sphere	19.66±3.01	19.21±2.89	17.58±2.54	15.56±3.05	3.09±0.64**	17.79±2.15
Cells sorted from in vitro treatment						
Primary sphere		15.58±2.25	15.62±1.53	10.10±1.98	1.25±0.32**	14.26±1.08
Secondary sphere		16.32±2.41	17.08±2.93	13.21±2.14	2.03±0.31**	15.96±2.33

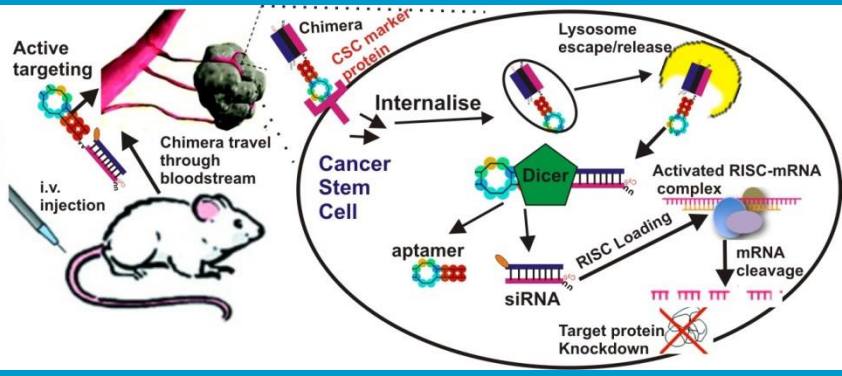


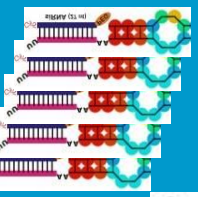


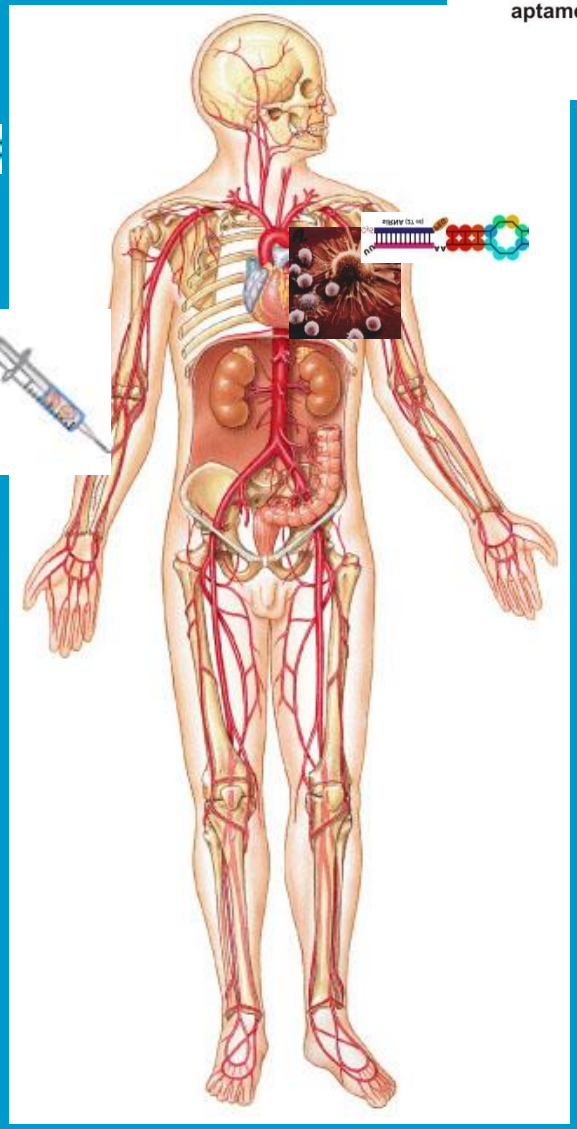


Conclusion









First CSC-targeted RNAi

Totally chemical synthesized for large-scale production

Excellent biodistribution profile

Can be used to knockdown any cancer genes in CSC

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