Ets-1 identifying polynucleotide sequence for targeted delivery of anti-cancer drugs

Indian Patent Application No. 1623/DEL/2014

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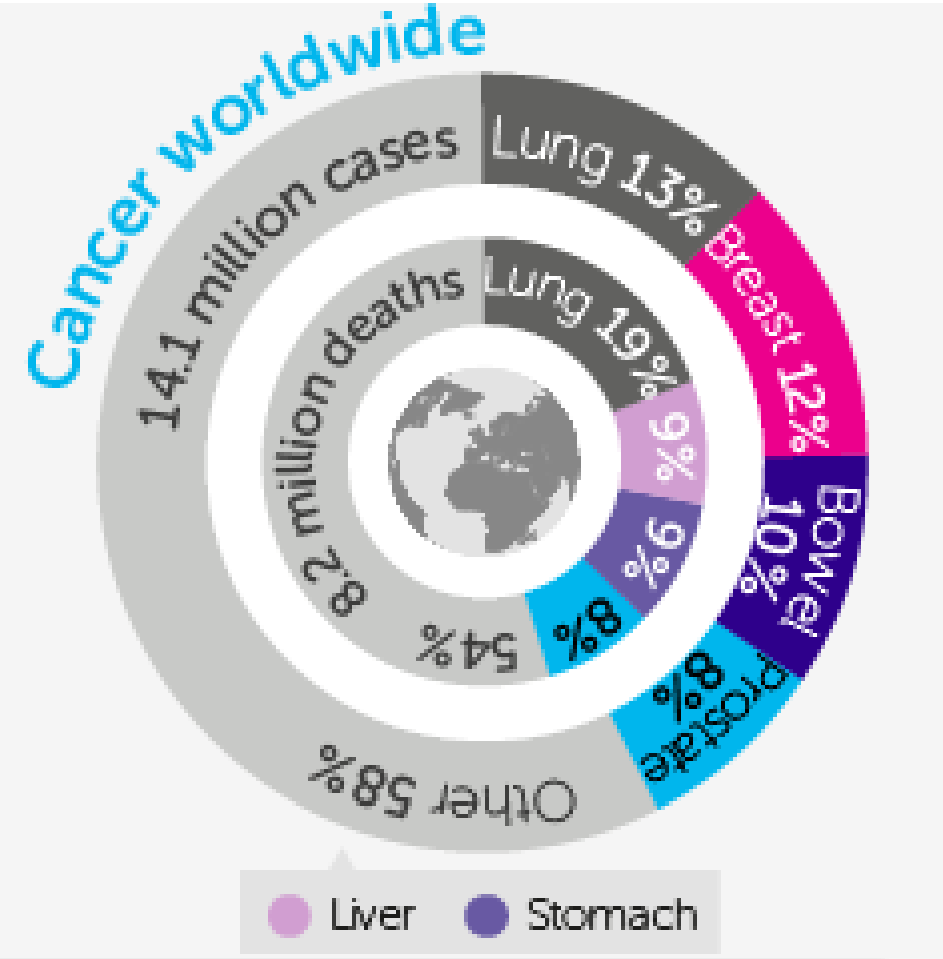
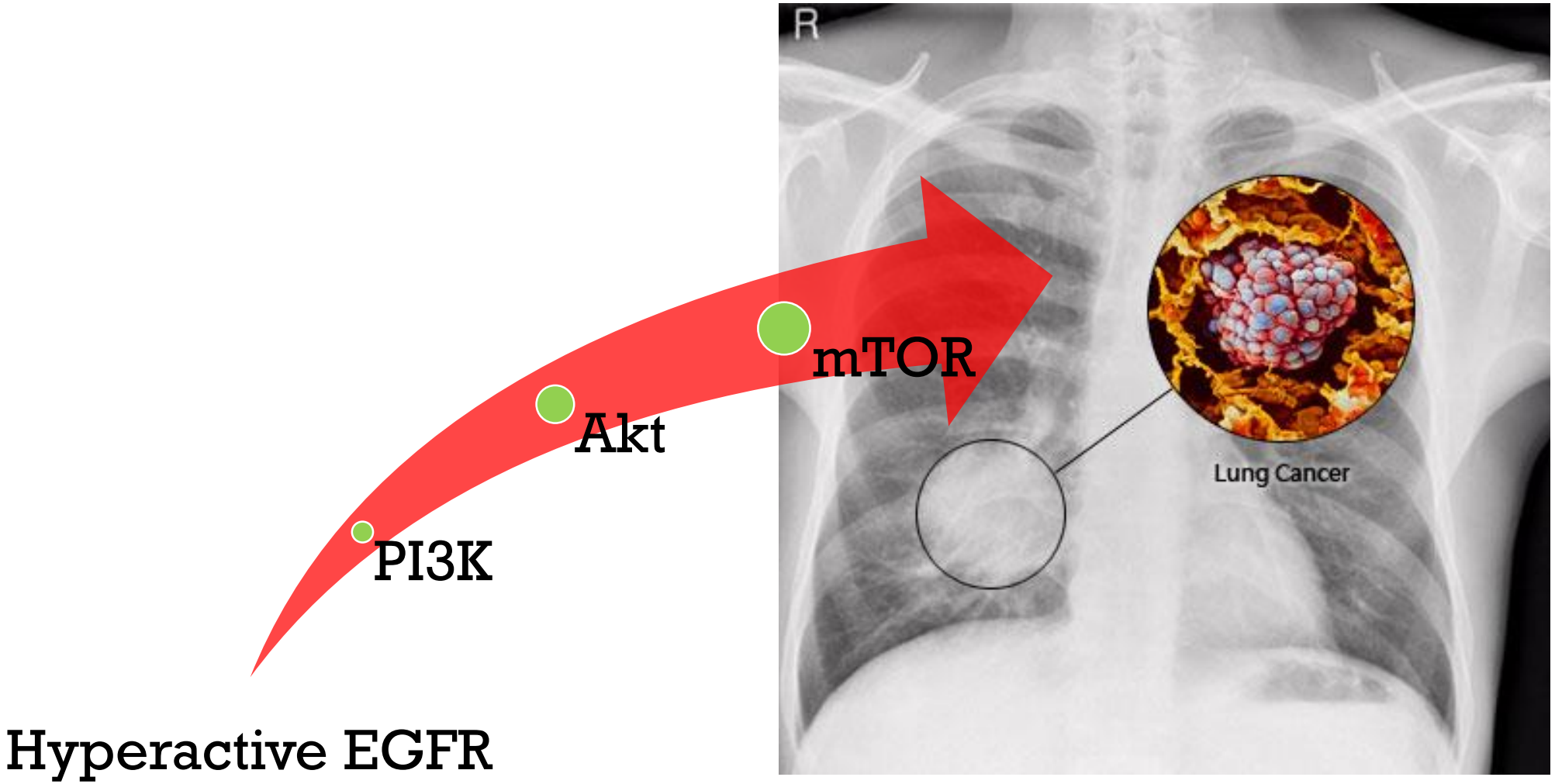
Research Philosophy

- Lung Cancer accounts for the majority of cancer associated mortality worldwide
- Blame or sympathy???
- Association with smoking- Not completely accurate portrayals
- About a quarter of lung cancer patients have never smoked in their life
- Unlucky combination of genetics and environmental factors



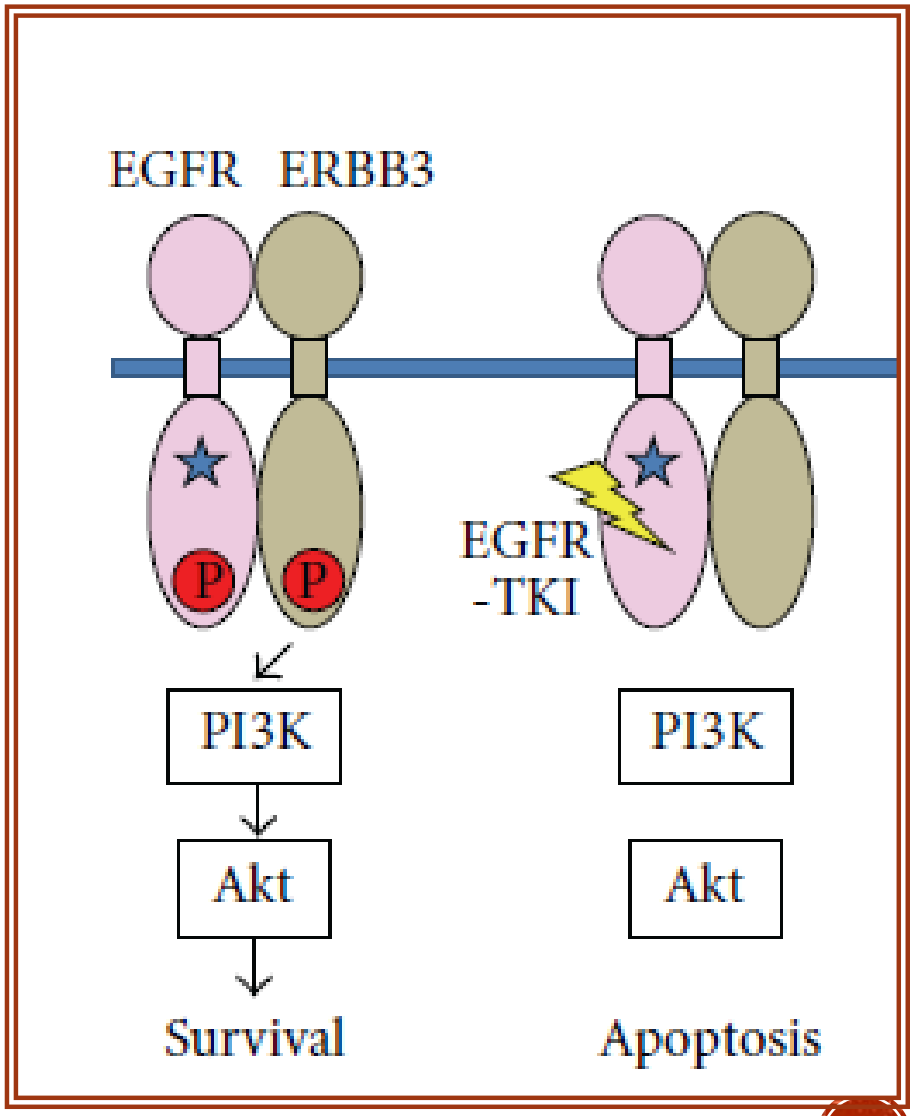
Cancer Research UK

Looking into the Molecular Activity….



THE SOLUTION: TYROSINE KINASE INHIBITORS

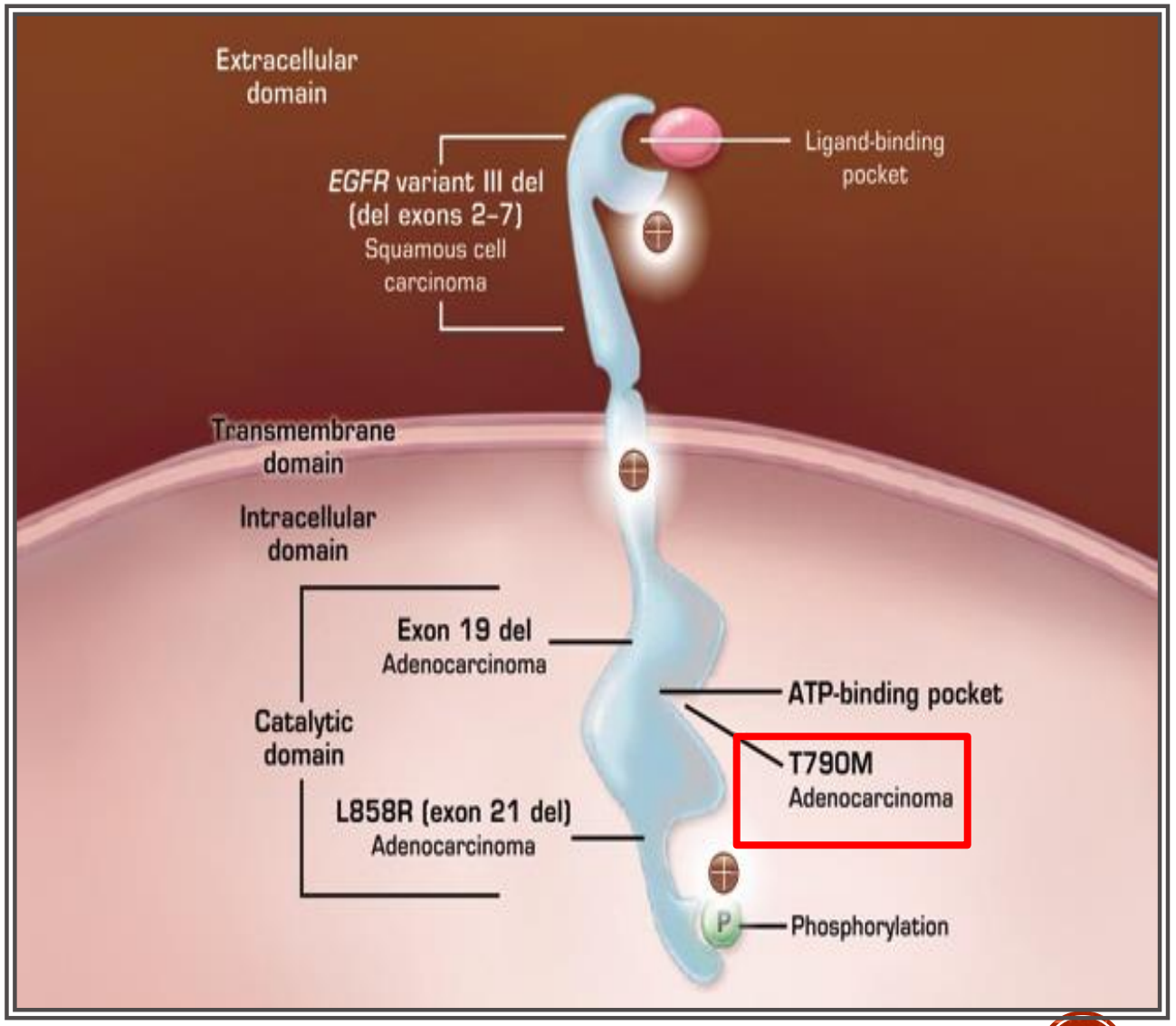
- Tyrosine kinase inhibitors: Gefitinib, Erlotinib, Lapatinib…
- Gefitinib: orally administered drug, approved for marketing in May 2003 for patients with non-small cell lung cancer (NSCLC)
- The approved indication was for the treatment of patients who were refractory to established cancer treatments (both a platinum drug and docetaxel)



Maemondo *et al*. (2010) Gefitinib or Chemotherapy for Non–SmallCell Lung Cancer with Mutated EGFR; N Engl J Med 2010;362:2380-8

MORE PROBLEM!!!! - T790M MUTATION

- T790 : Gatekeeper Residue
- Gefitinib treatment leads to selection of T790M mutant cells which leads to clinical gefitinib resistance because of selective proliferation of T790M mutant cells (Inukai *et al*. 2006)
- Substitution of Threonine with bulkier Methionine hinders the binding of TKIs
- Another reason behind resistance may be increased ATP affinity (Yun *et al*. 2008)



Inukai M, et al. Presence of epidermal growth factor receptor gene T790M mutation as a minor clone in non-small cell lung cancer. Cancer Res 2006; 66: 7854-7858

Yun CH, et al. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc Natl Acad Sci 2008; 105: 2070-2075

National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: non-small cell lung cancer, version 2/2012. http://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf

DRUG LOADED NANOPARTICLES

- Why drug loaded nanoparticles???

✓Prolonged systemic circulation lifetime

✓ Sustained drug release kinetics

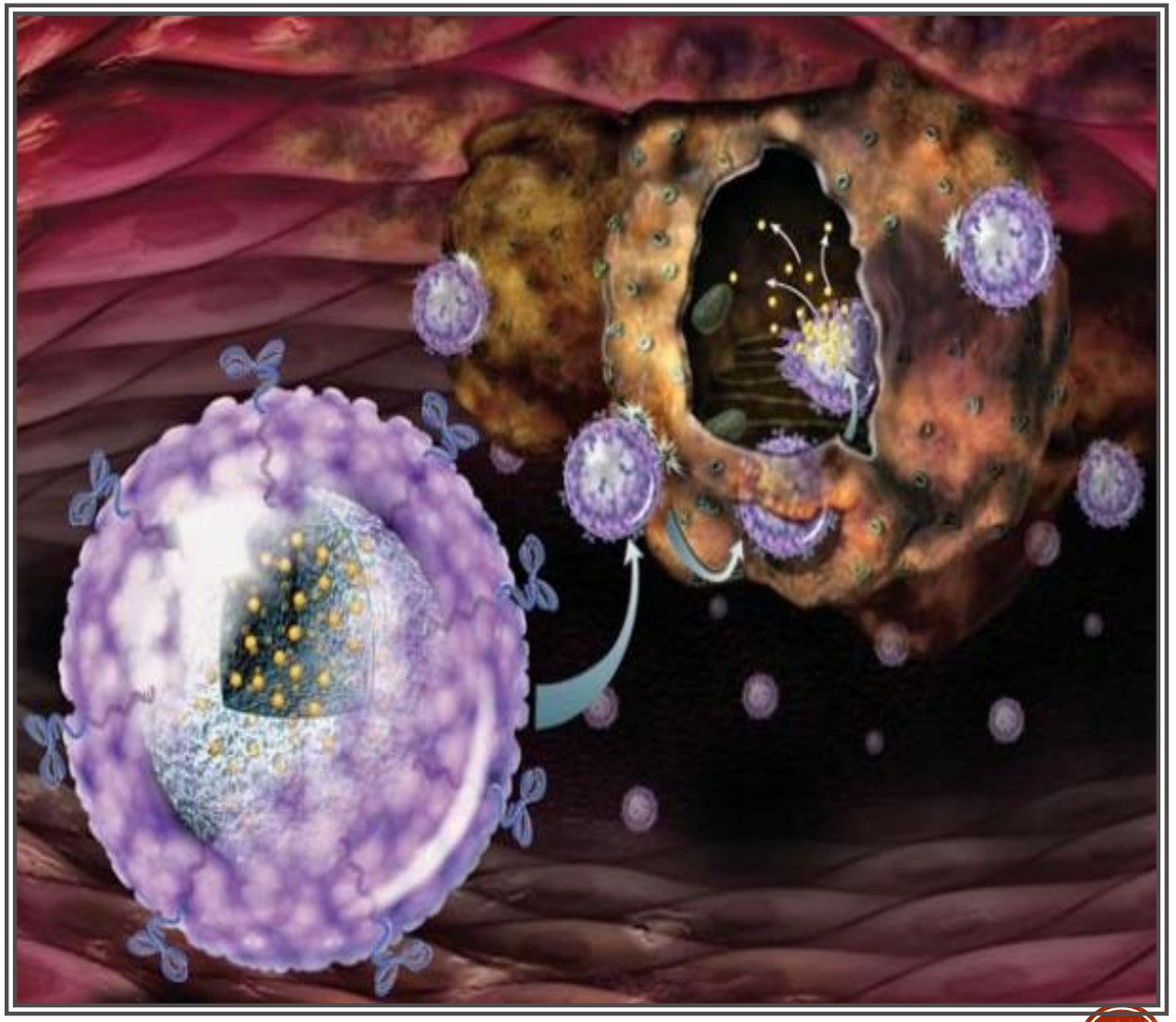
✓Better tumor accumulations through both passive and active mechanisms

- Various nanoparticle based drug delivery systems :-

✓ Abraxane (Breast cancer)

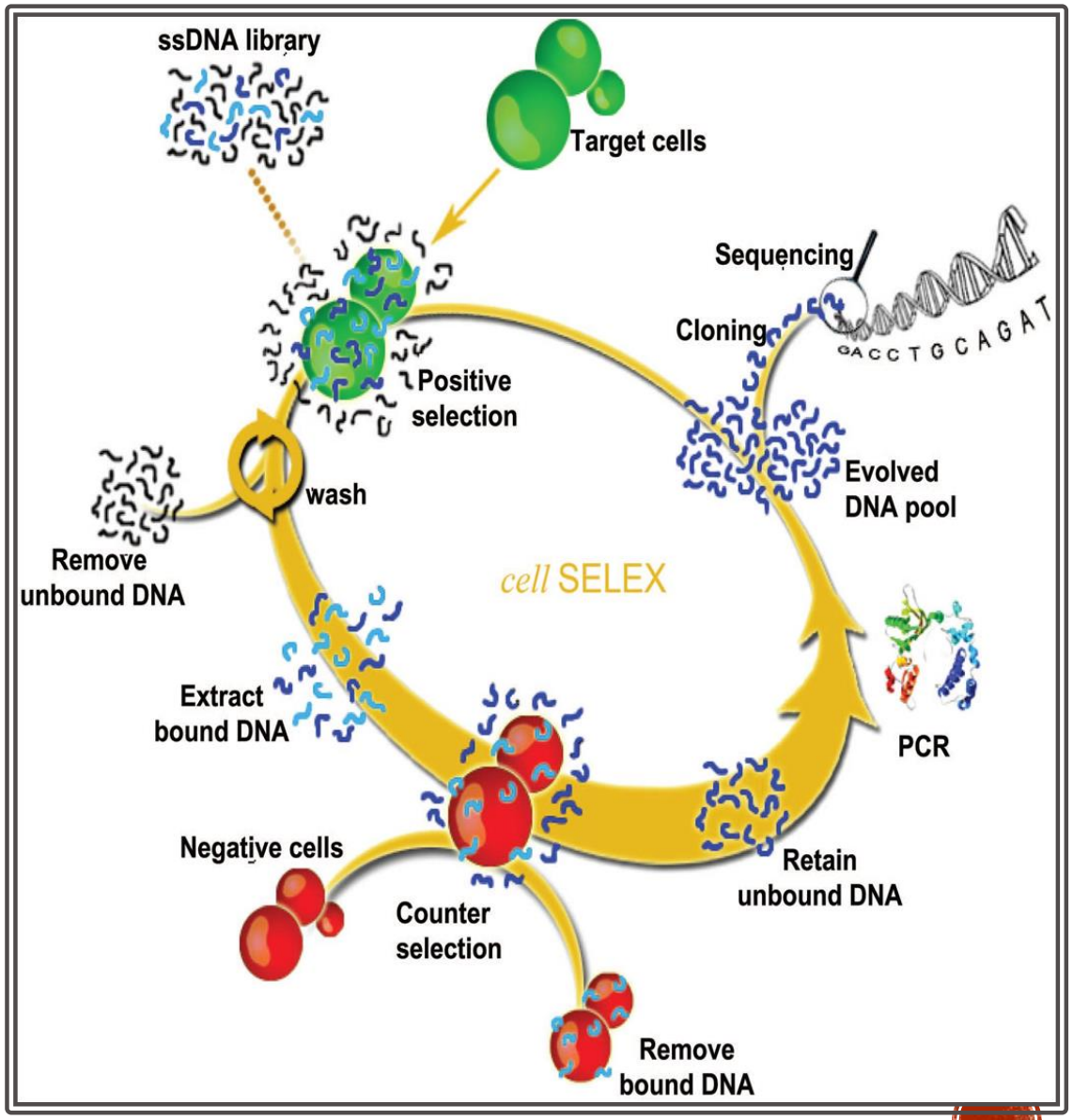
✓Genexol (Breast cancer)

✓Oncaspar (leukemia)



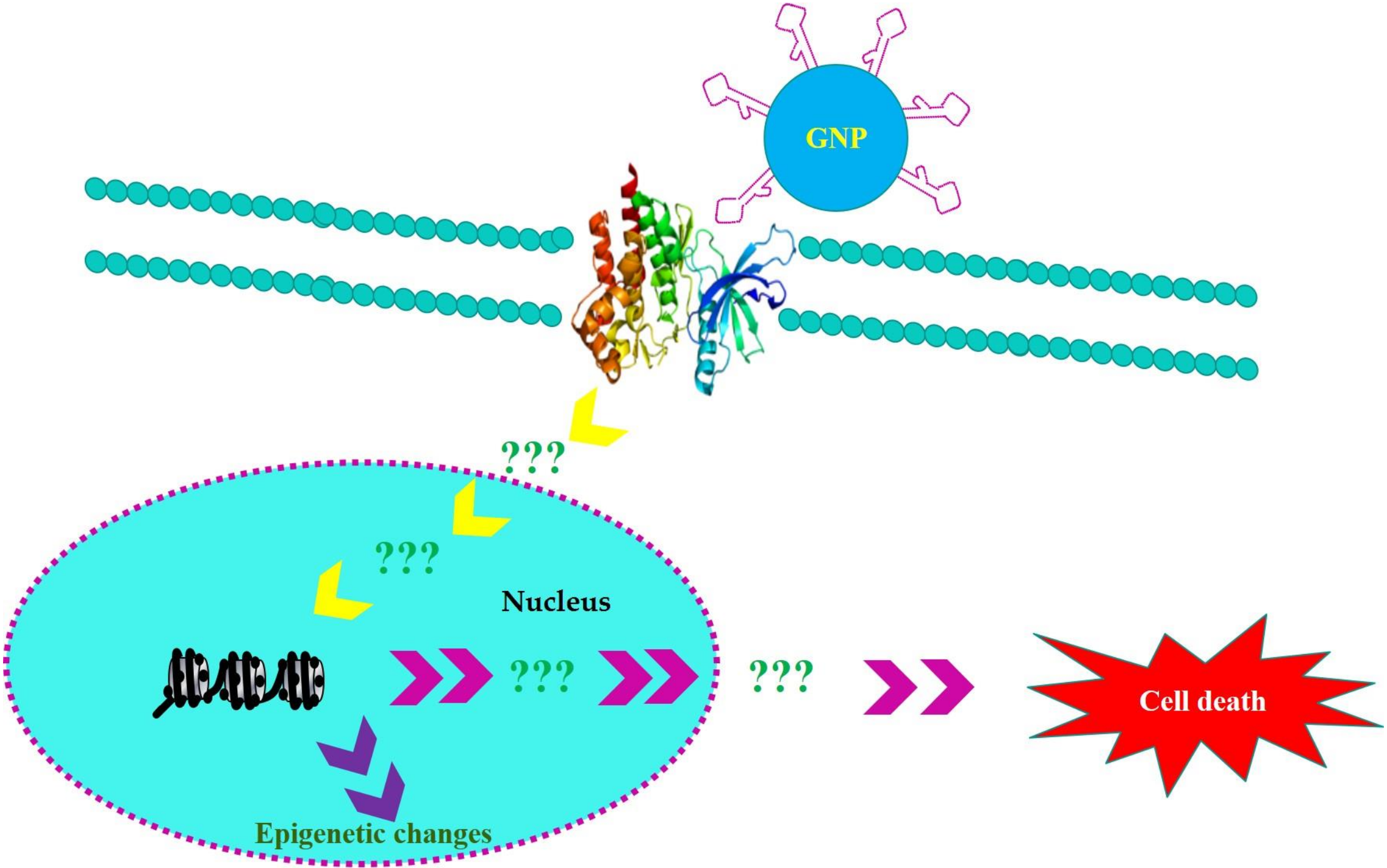
APTAMERS

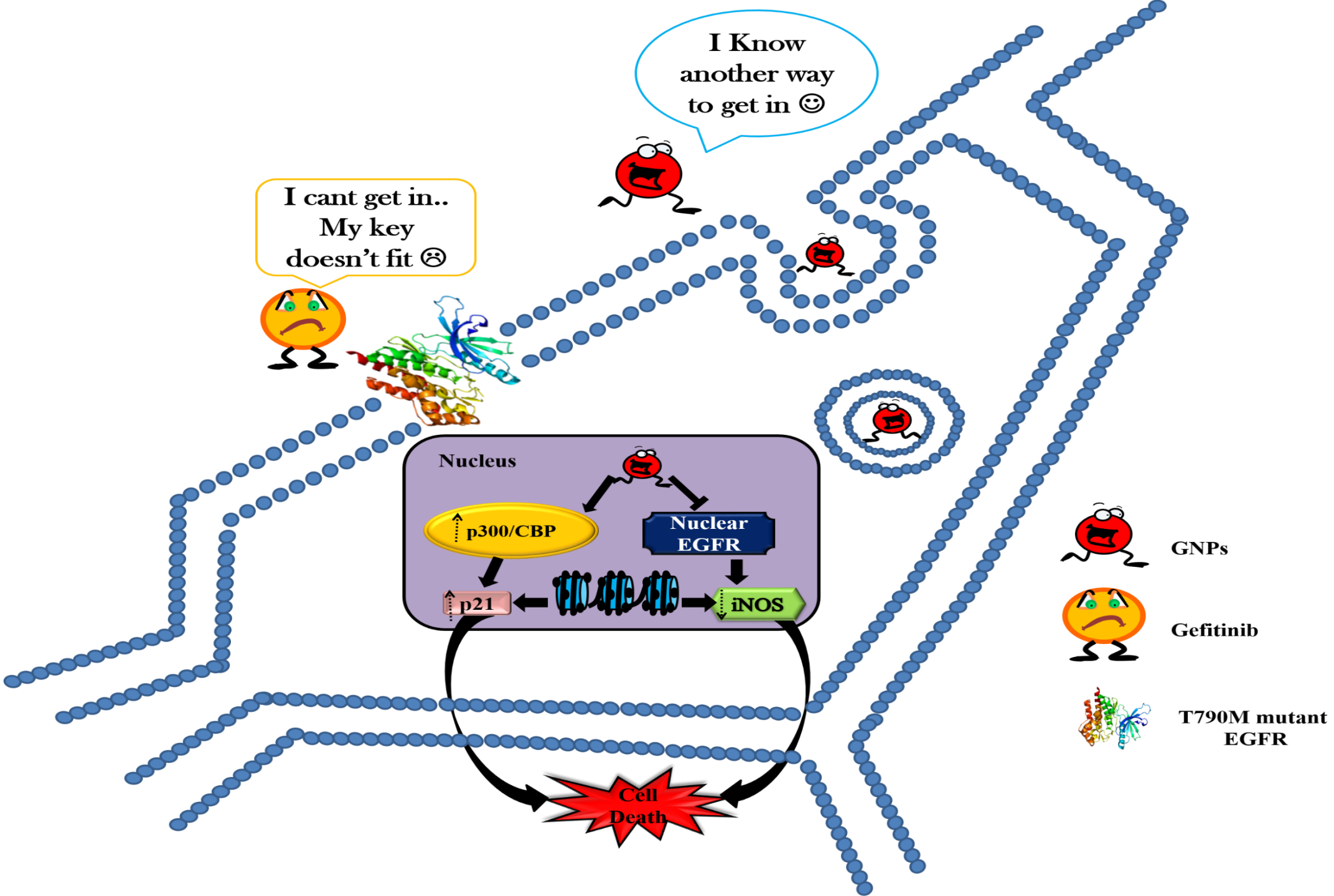
- Aptus + meros = Aptamers
- SELEX (Systemic Evolution of Ligands by Exponential Enrichment) (Larry Gold and Andrew Ellington)
- Lately, Cell-SELEX has taken over the conventional method of aptamer selection
- Aptamer Vs Antibodies
- Aptamers for drug delivery (PSMA)
- **Aptamer translated to clinic: Pegaptanib (Macugen®)**



Ellington AD and Szostak JW. In vitro selection of RNA molecules that bind specific ligands. Nature 1990; 346: 818-822

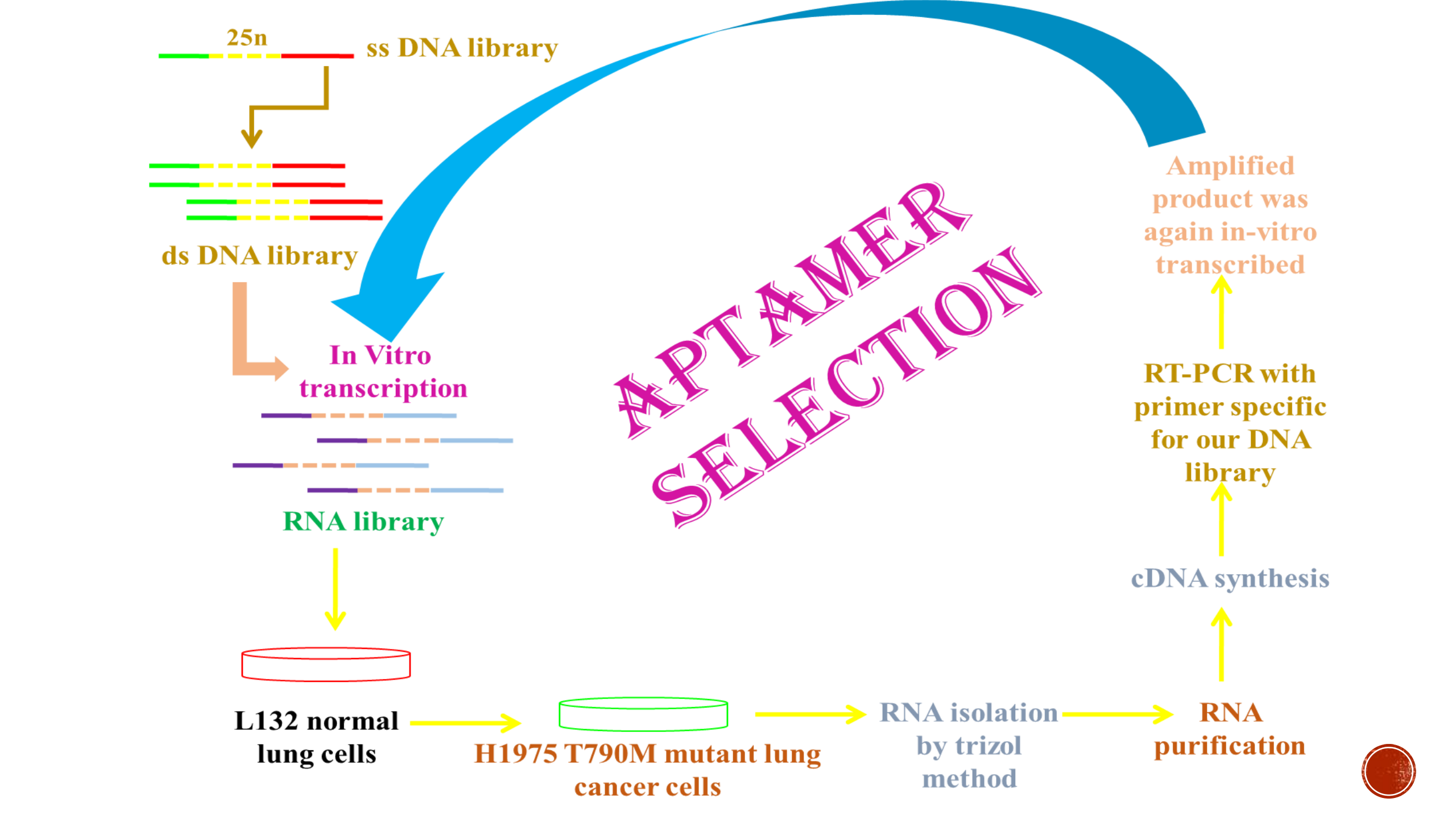
Aptamer therapeutic	Company	Medical condition	Current status
Pegaptanib (Macugen)	Eyetech Inc/Pfitzer	Age related macular degeneration/diabetic macular edema/proliferative diabetic retinopathy	In market
E10030	Ophthotech Corp	Neovascular age related macular degeneration	Awaiting Phase III
ARC1905	Ophthotech Corp	Neovascular age related macular degeneration	Phase I
RB006	Regado Biosciences, Inc	Coronary artery disease	Awaiting Phase III
ARC1779	Archimex Corp	von Willebrand's disease	Awaiting Phase III
NU172	Nuvelo/ ARCA Biopharma	Coronary artery disease	Phase II
ARC19499	Archimex	Hemophilia	Phase I/II, status is uncertain
AS1411	Antisoma PLC	Renal cell carcinoma/non-small cell lung cancer	Awaiting Phase III
NOX-A12	Noxxon Pharma	Tumor	Phase II, recruting patients
NOX-E36	Noxxon Pharma	Type II diabetes mellitus/ renal impairment/ nephropathy/lupus nephritis	Phase IIa


GNP
???
???
Nucleus
???
???
Epigenetic changes
Cell death

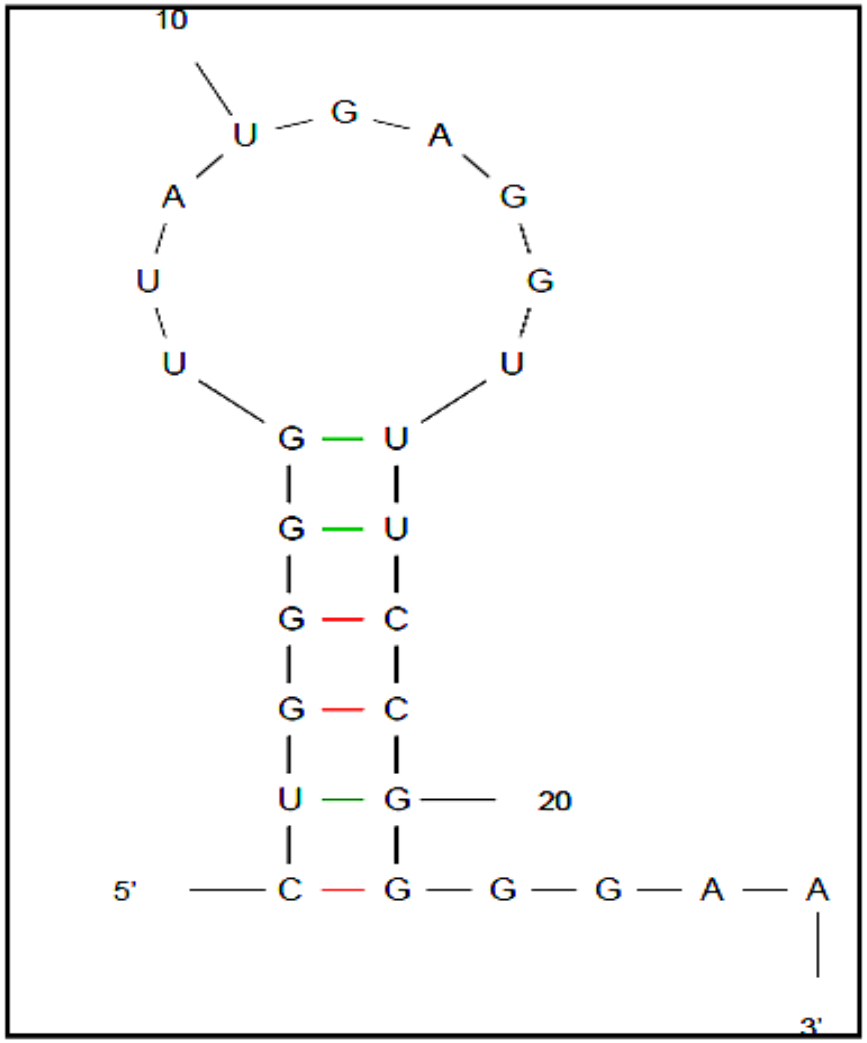

I Know another way to get in ☺
I cant get in.. My key doesn't fit ☹
Nucleus
p300/CBP
Nuclear EGFR
p21
iNOS
Cell Death
GNPs
Gefitinib
T790M mutant EGFR

Bare Nanoparticle = Letter without an address

Aptamers: Defining the destination of NPs

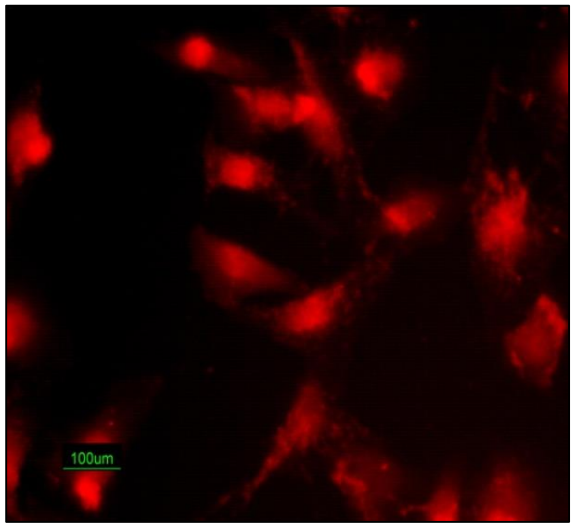
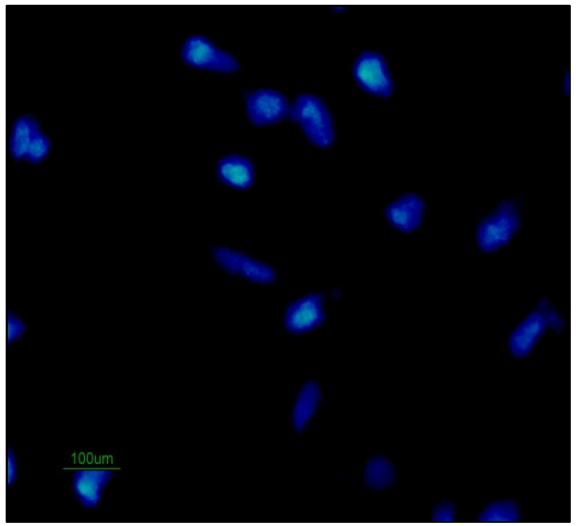
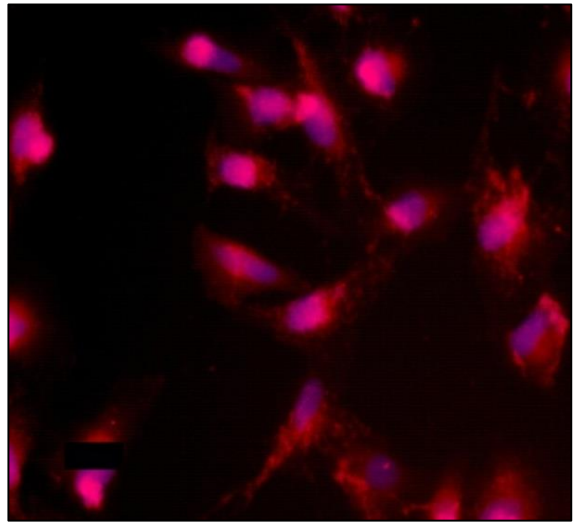
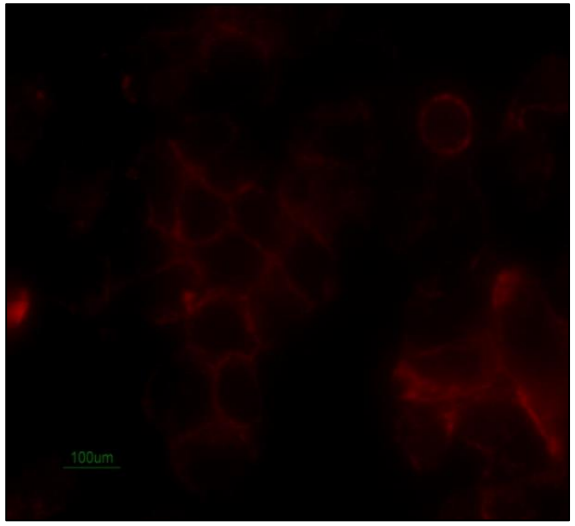
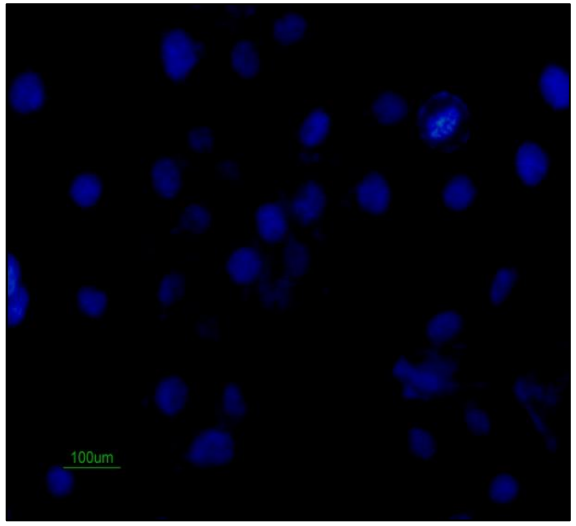
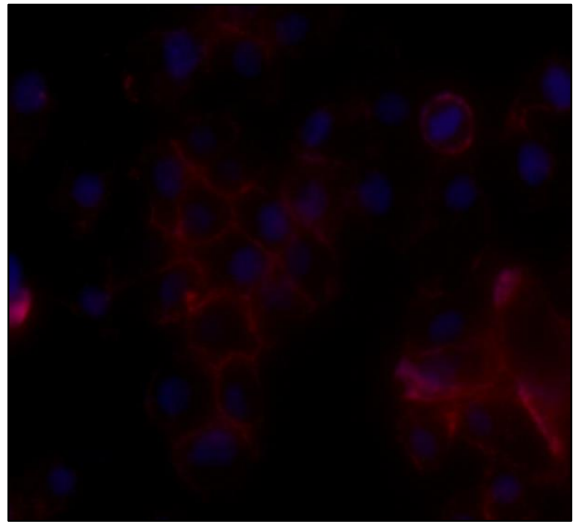

25n
ss DNA library
ds DNA library
In Vitro transcription
RNA library
APTAMER SELECTION
Amplified product was again in-vitro transcribed
RT-PCR with primer specific for our DNA library
cDNA synthesis
RNA purification
RNA isolation by trizol method
H1975 T790M mutant lung cancer cells
L132 normal lung cells

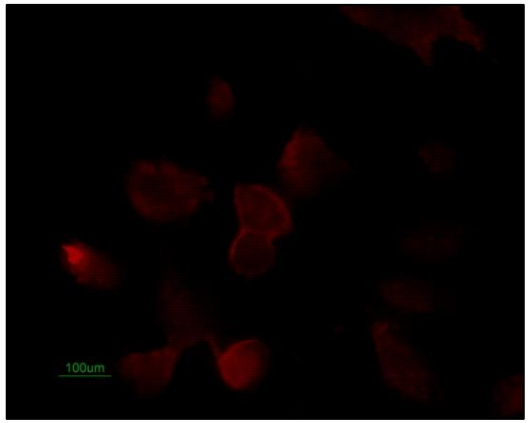
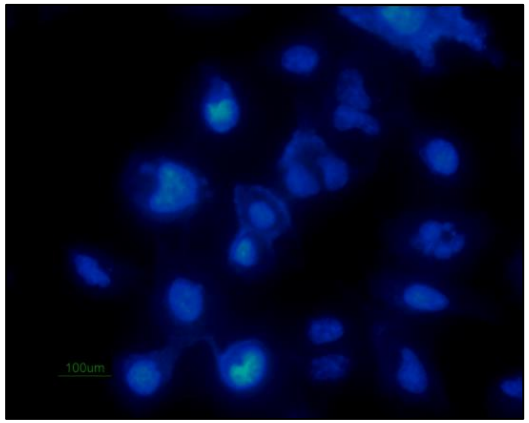
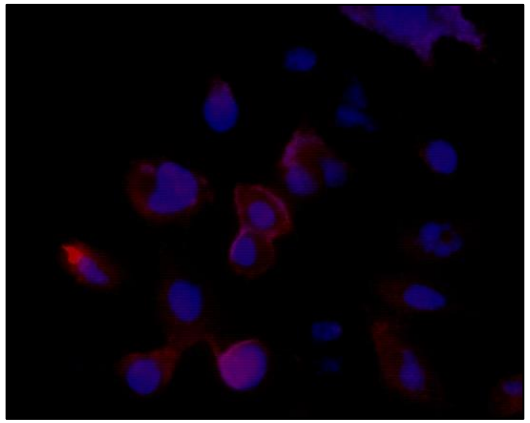
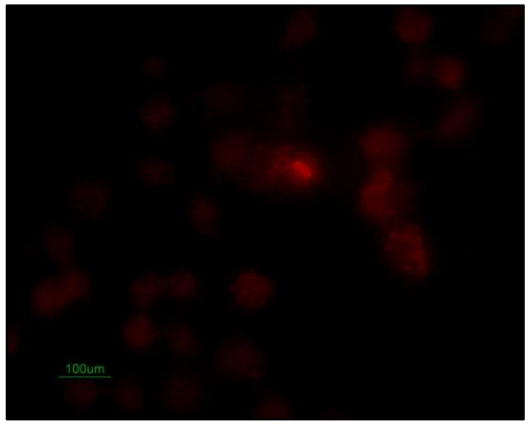
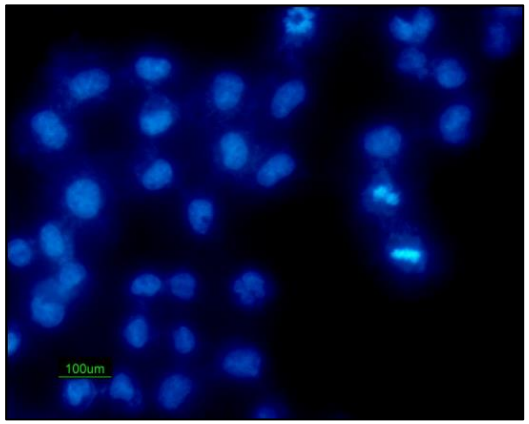
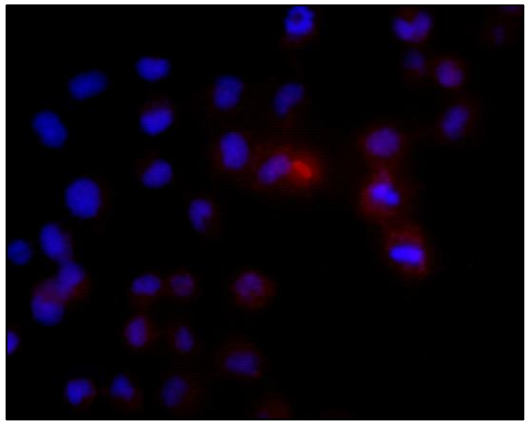
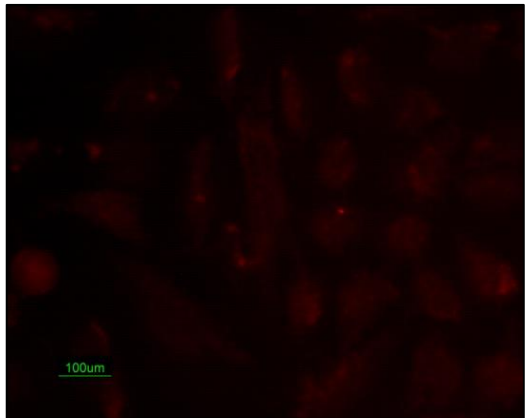
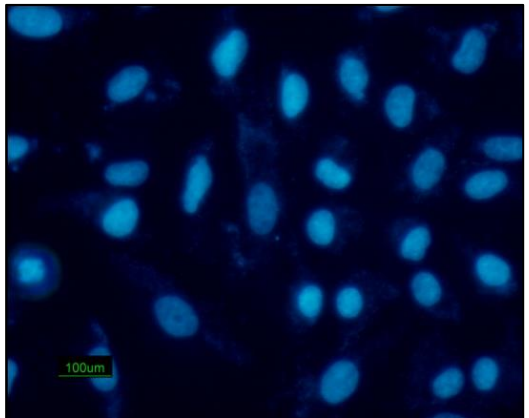
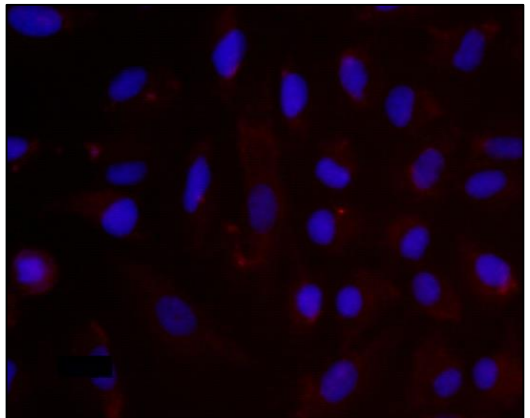
Predictive secondary structures of internalized aptamer

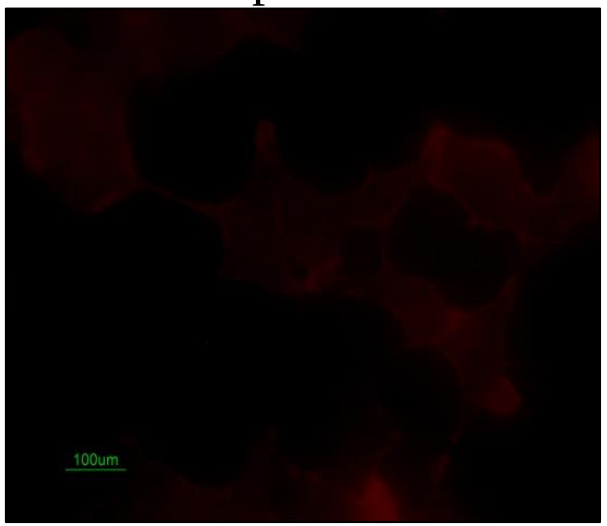
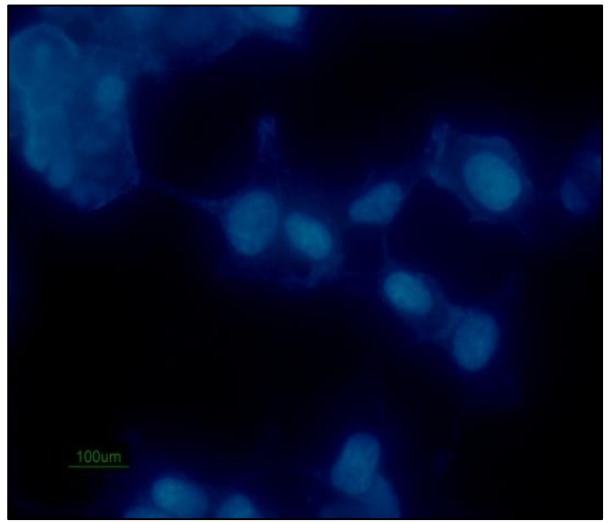
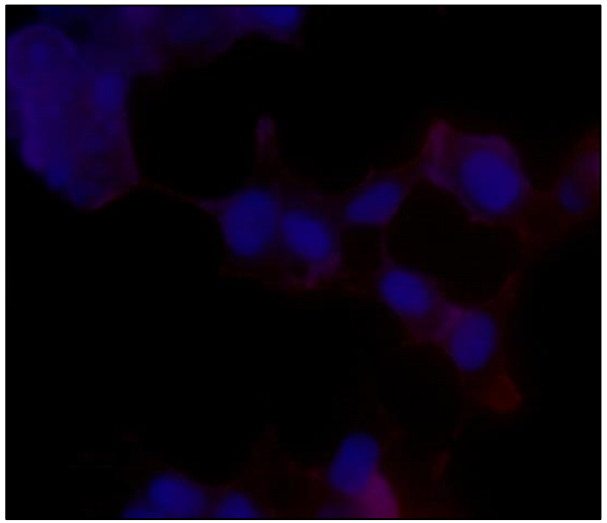
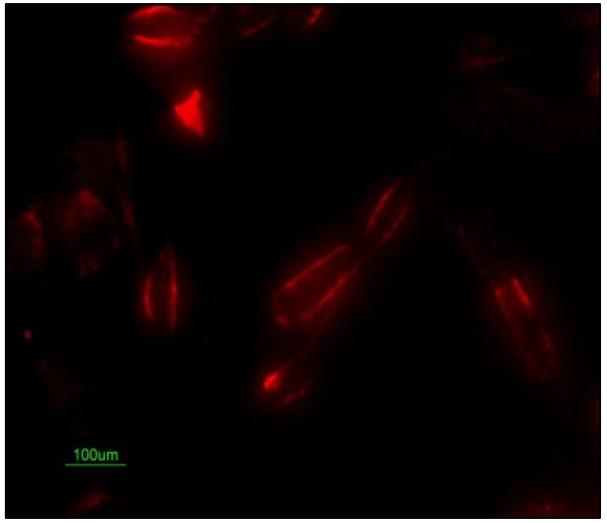
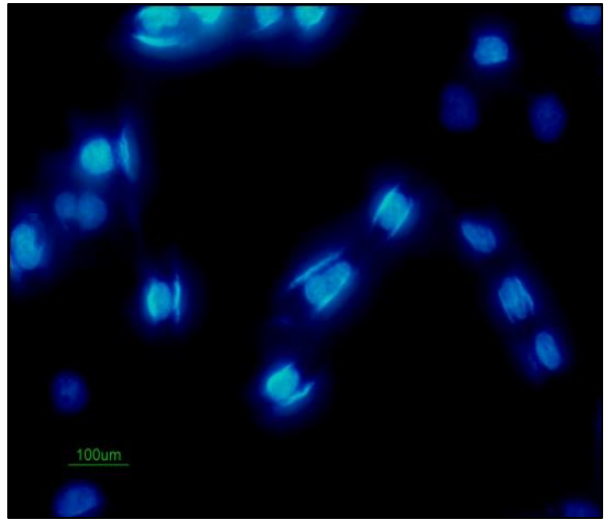
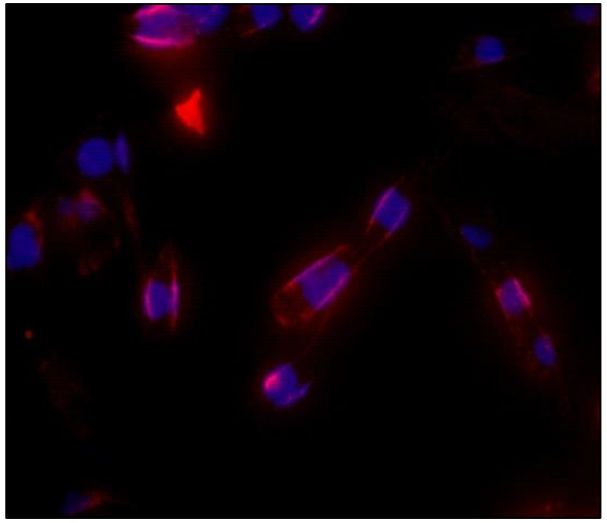


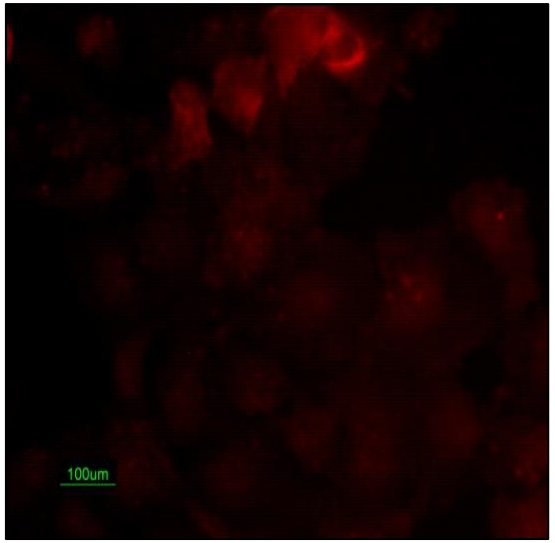
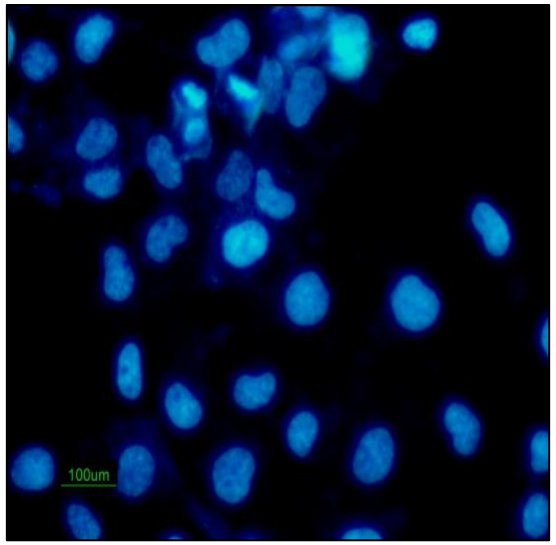
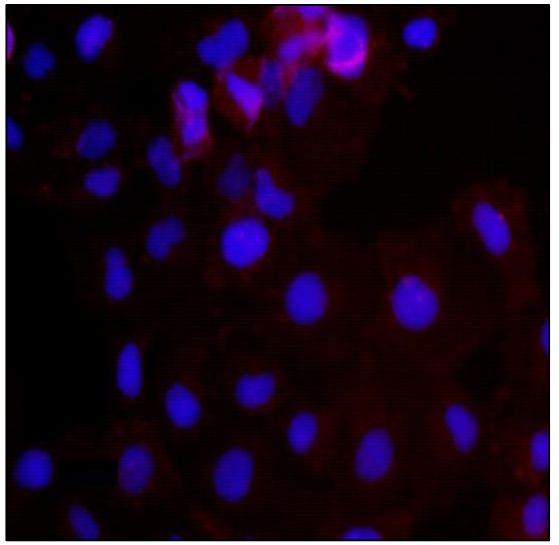
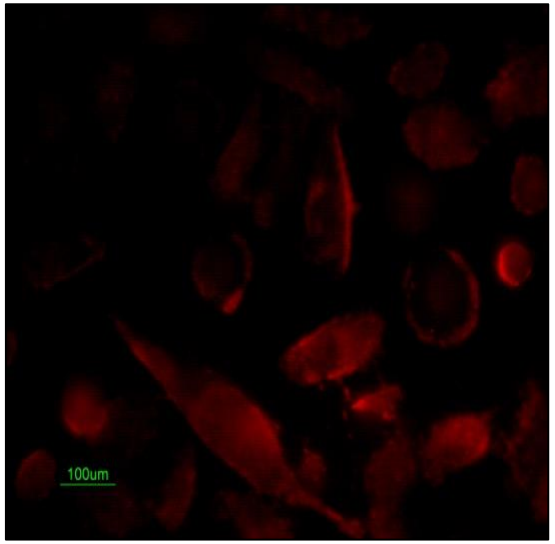
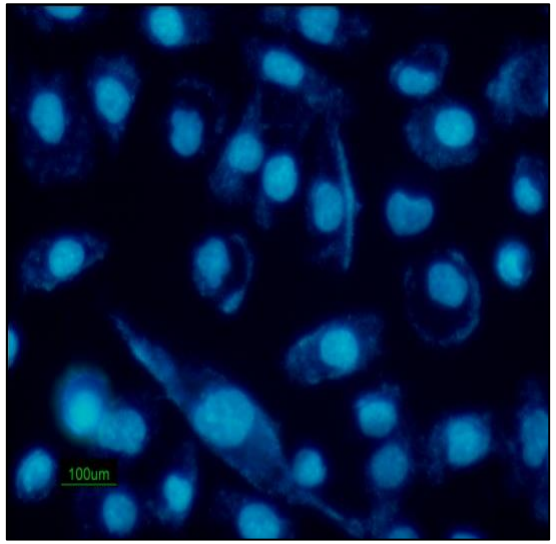
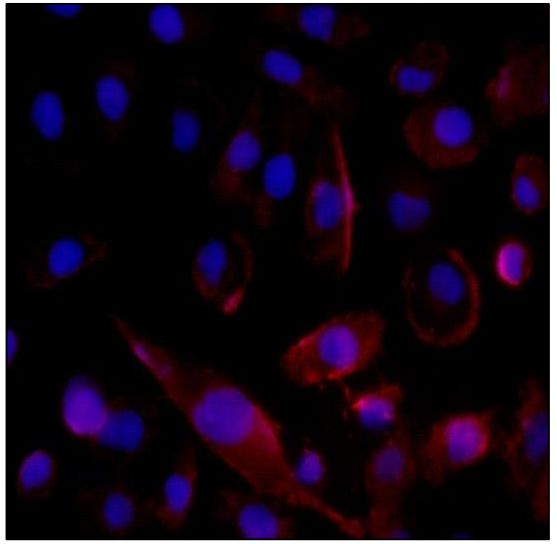
Truncated aptamers show enhanced internalization specificity as the non-specific antigen binding site has been removed from these sequences.

Selected aptamer exhibits high affinity towards H1975 lung cancer cells

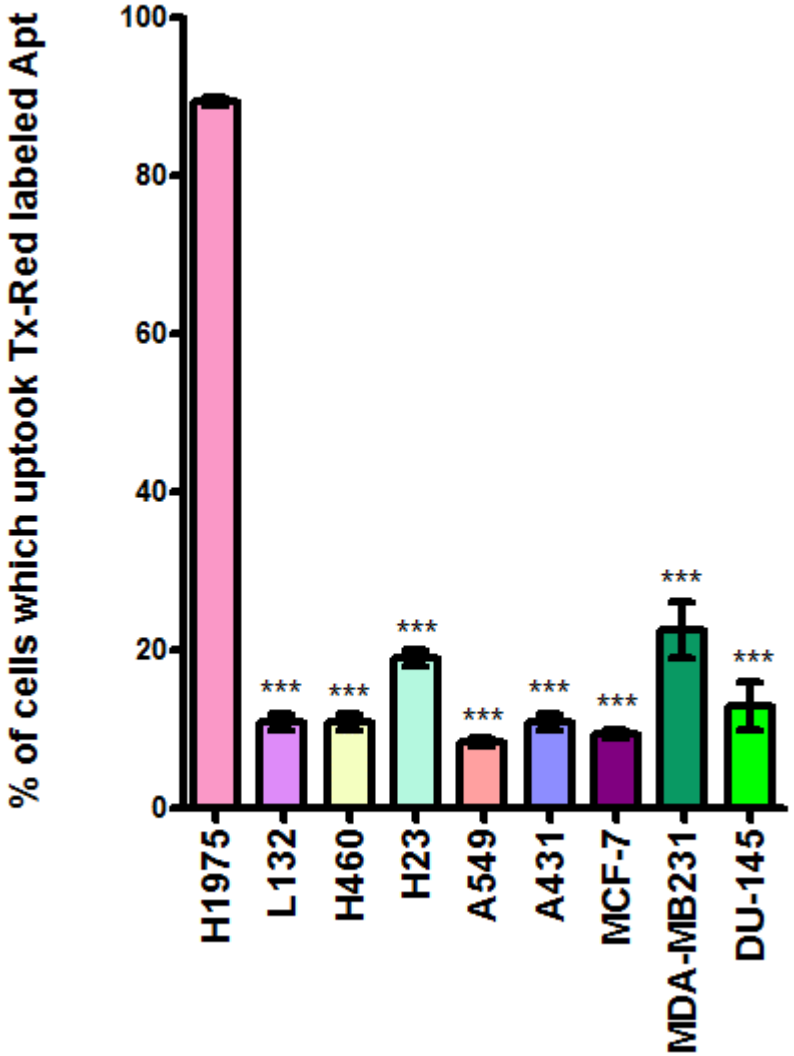
	Texas Red Labeled Aptamer	DAPI stained DNA	Merged
H1975			
L132			

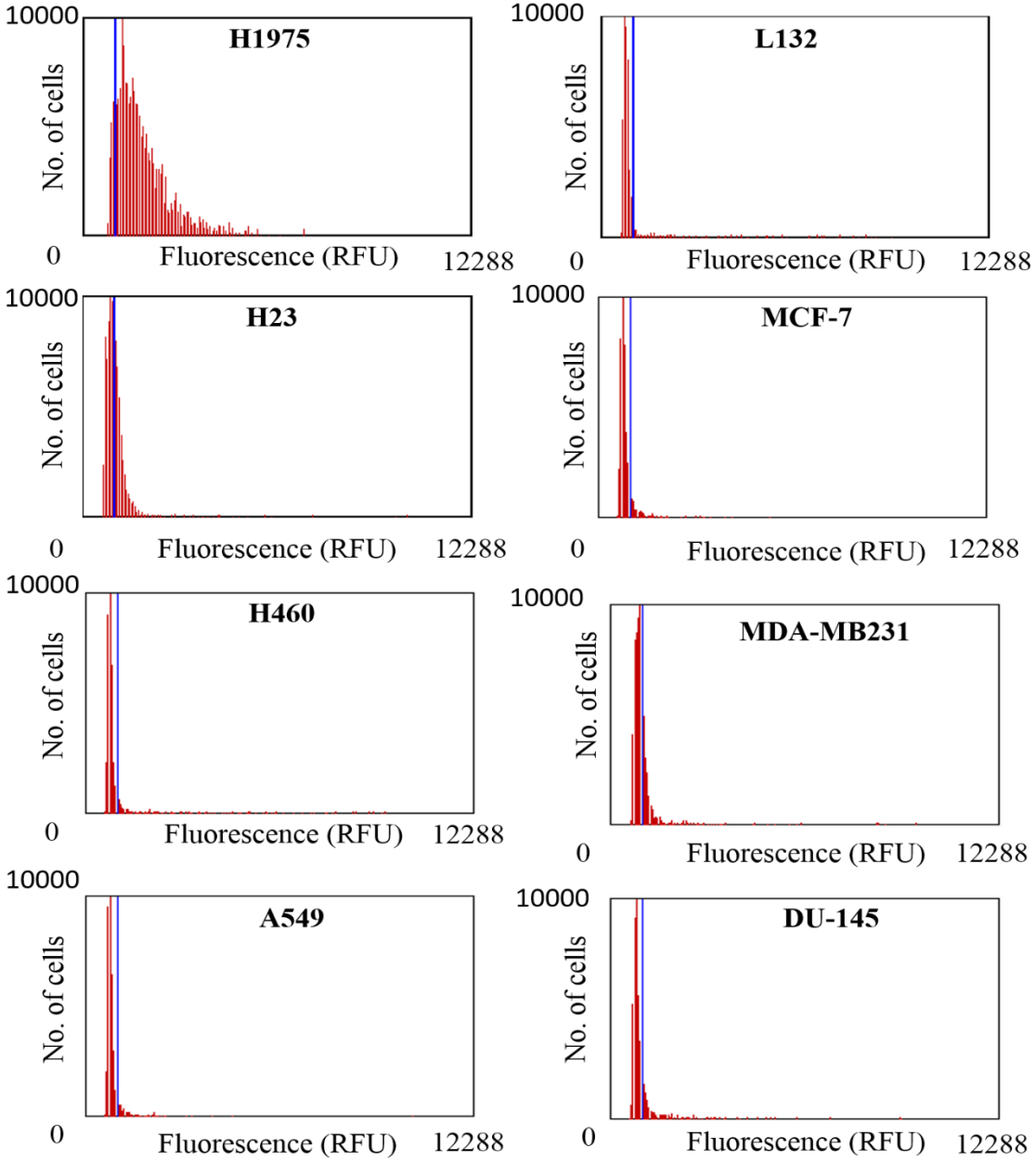
	Texas Red Labeled Aptamer	DAPI stained DNA	Merged
H23			
H460			
A549			

	Texas Red Labeled Aptamer	DAPI stained DNA	Merged
MCF-7			
MDA-MB231			

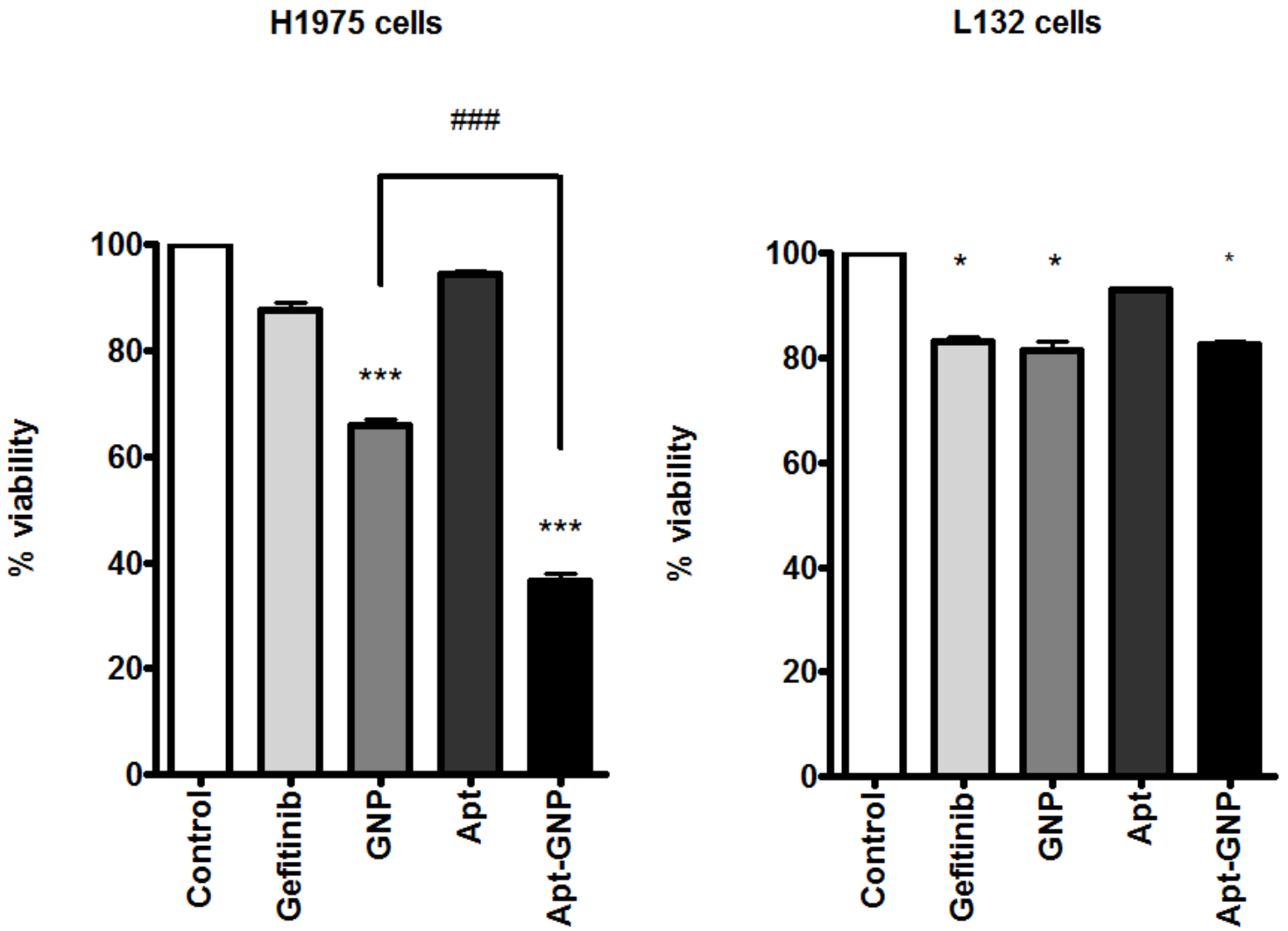
	Texas Red Labeled Aptamer	DAPI stained DNA	Merged
A431			
DU-145			

Quantification of Internalized Aptamer:

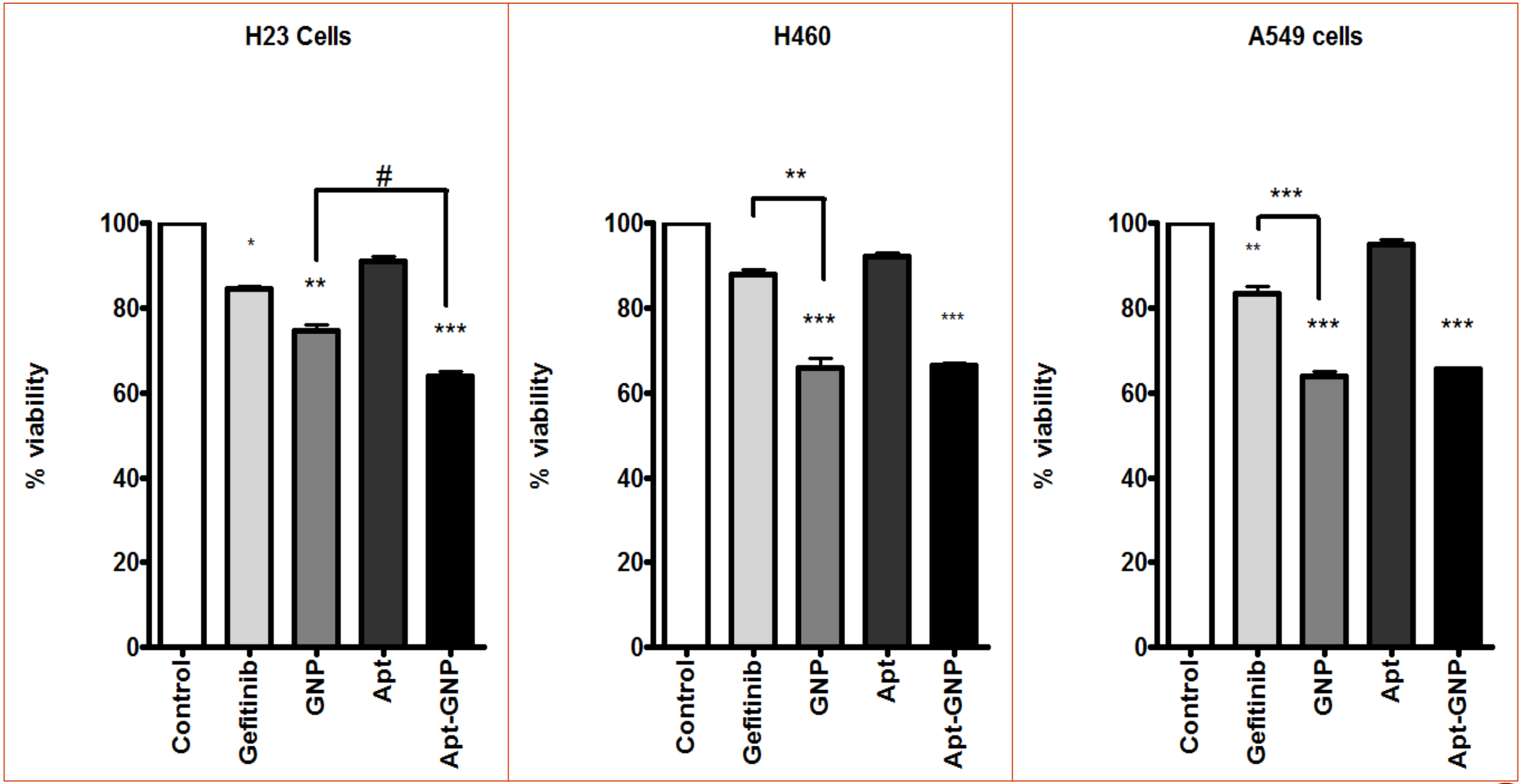


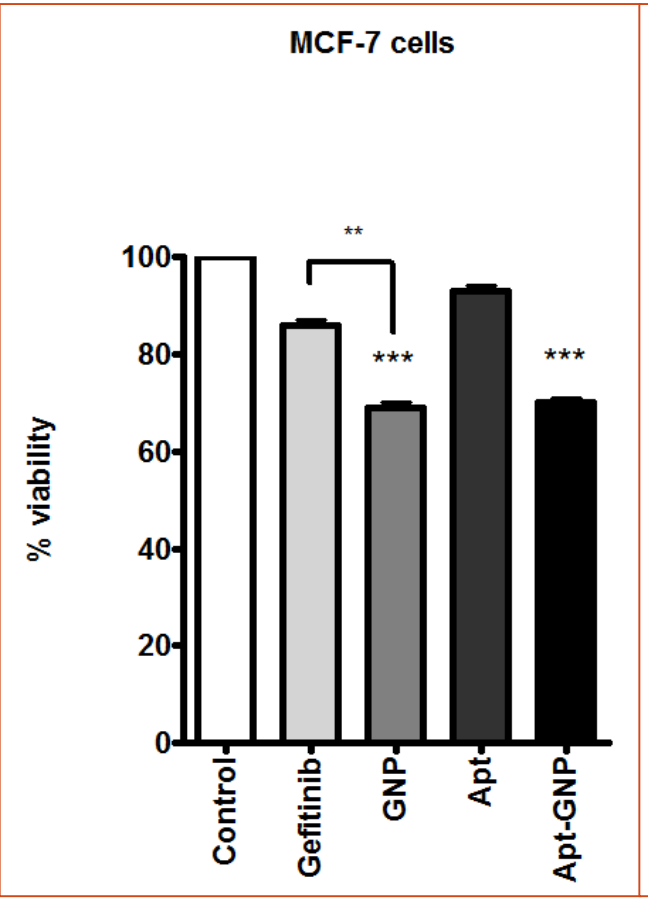


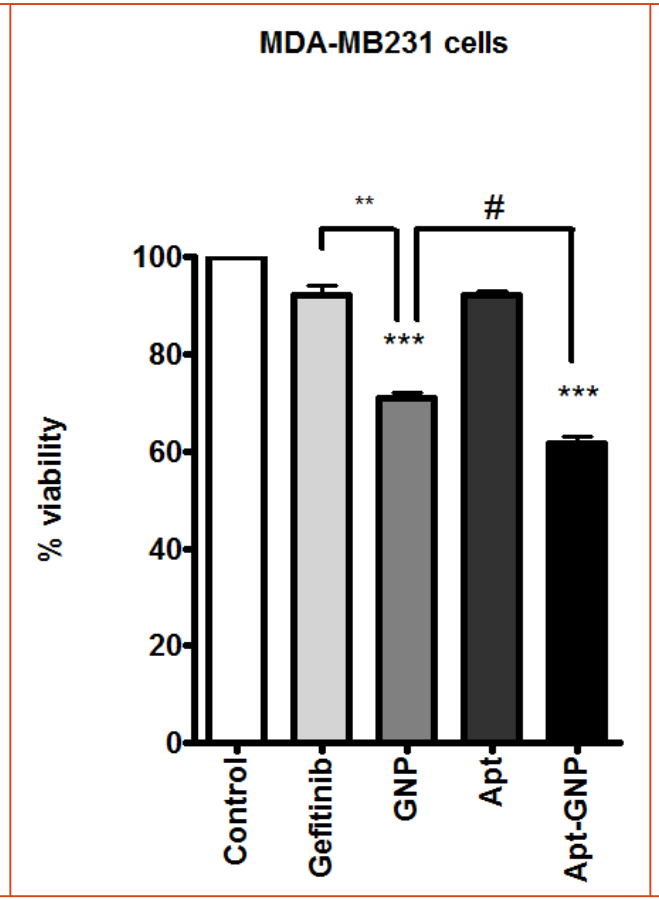
Aptamer conjugated to GNPs shows high cytotoxic effect specifically in H1975 cells

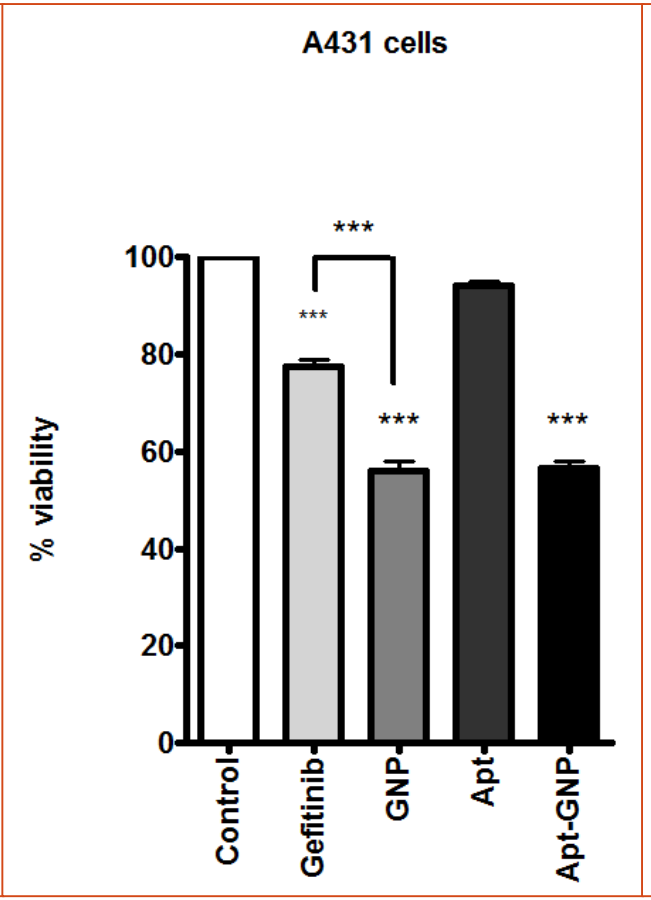


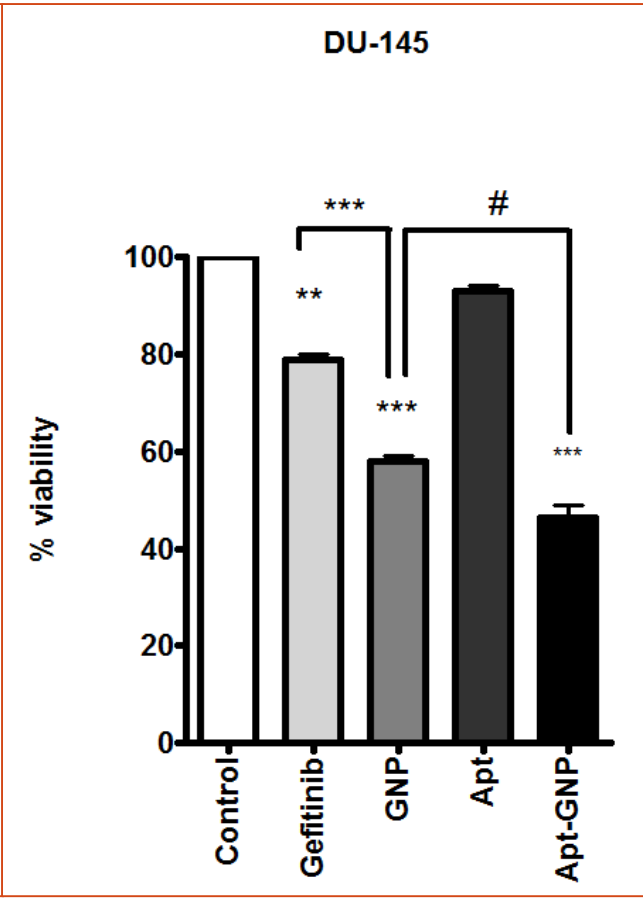
All values are represented as Mean ± SEM (n=3); ***p<0.001, *p<0.05 Vs Control and ###p<0.001, #p< 0.05 Vs GNP


H23 Cells
% viability
100
80
60
40
20
0
*
**
#
***
Control
Gefitinib
GNP
Apt
Apt-GNP
H460
% viability
100
80
60
40
20
0
**
***
***
Control
Gefitinib
GNP
Apt
Apt-GNP
A549 cells
% viability
100
80
60
40
20
0
**
***
***
***
Control
Gefitinib
GNP
Apt
Apt-GNP

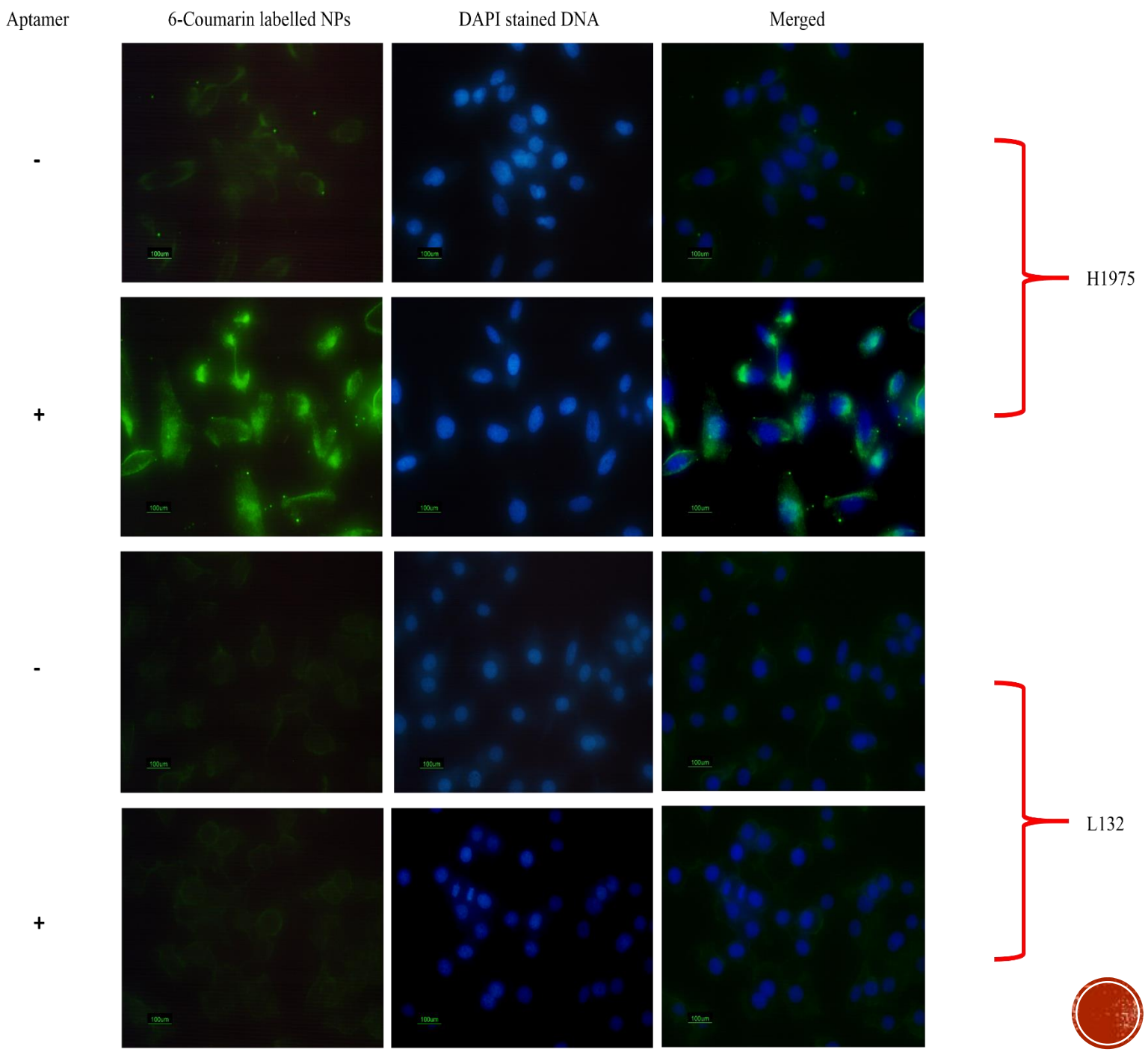

MCF-7 cells
% viability
100
80
60
40
20
0
**
***
***
Control
Gefitinib
GNP
Apt
Apt-GNP


MDA-MB231 cells
% viability
100
80
60
40
20
0
**
#
***
***
Control
Gefitinib
GNP
Apt
Apt-GNP


A431 cells
% viability
100
80
60
40
20
0
***
***
***
***
Control
Gefitinib
GNP
Apt
Apt-GNP

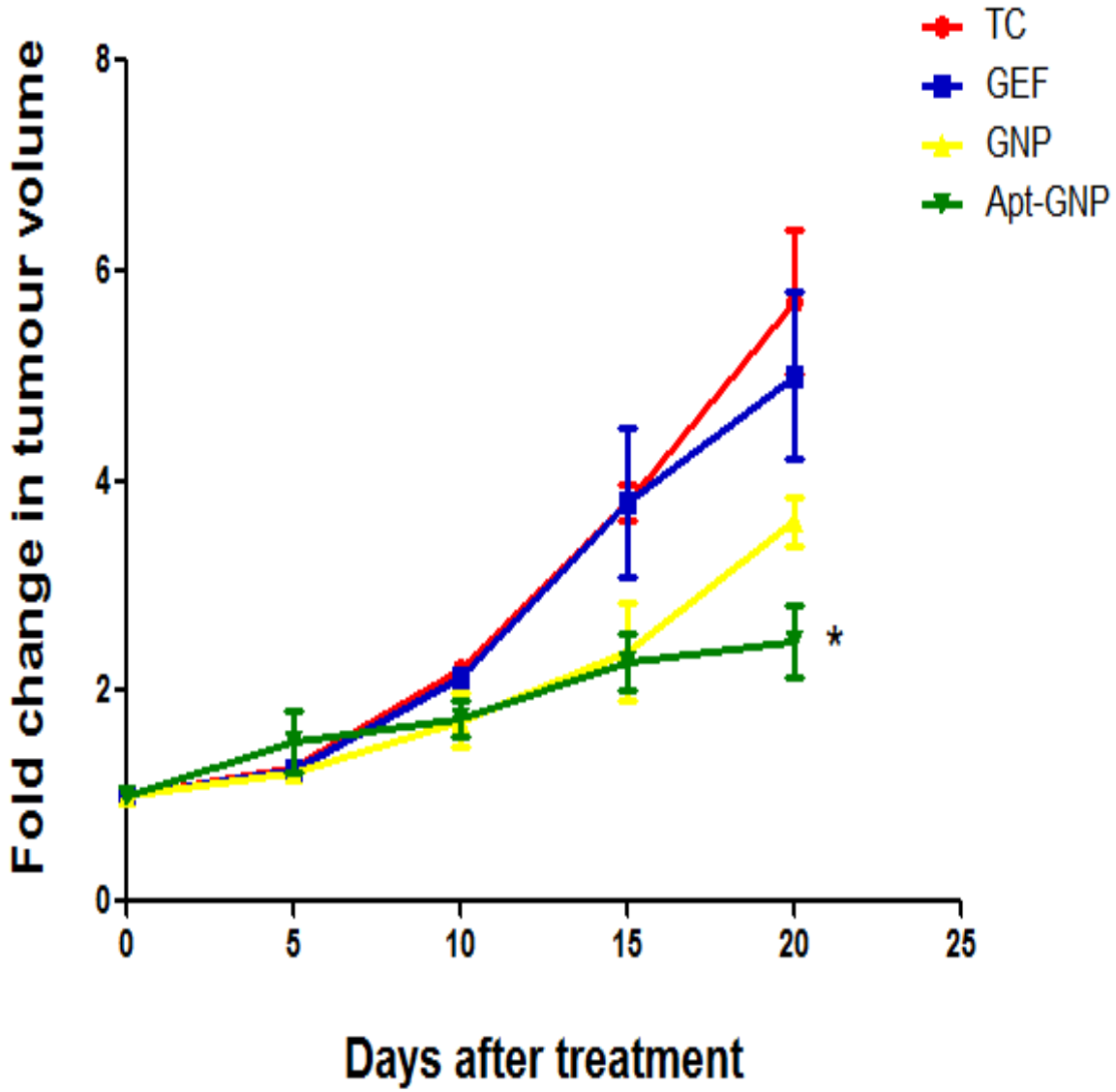

DU-145
% viability
100
80
60
40
20
0
**
***
#
***
Control
Gefitinib
GNP
Apt
Apt-GNP

Uptake of apt-6-coumarin loaded NP bioconjugate

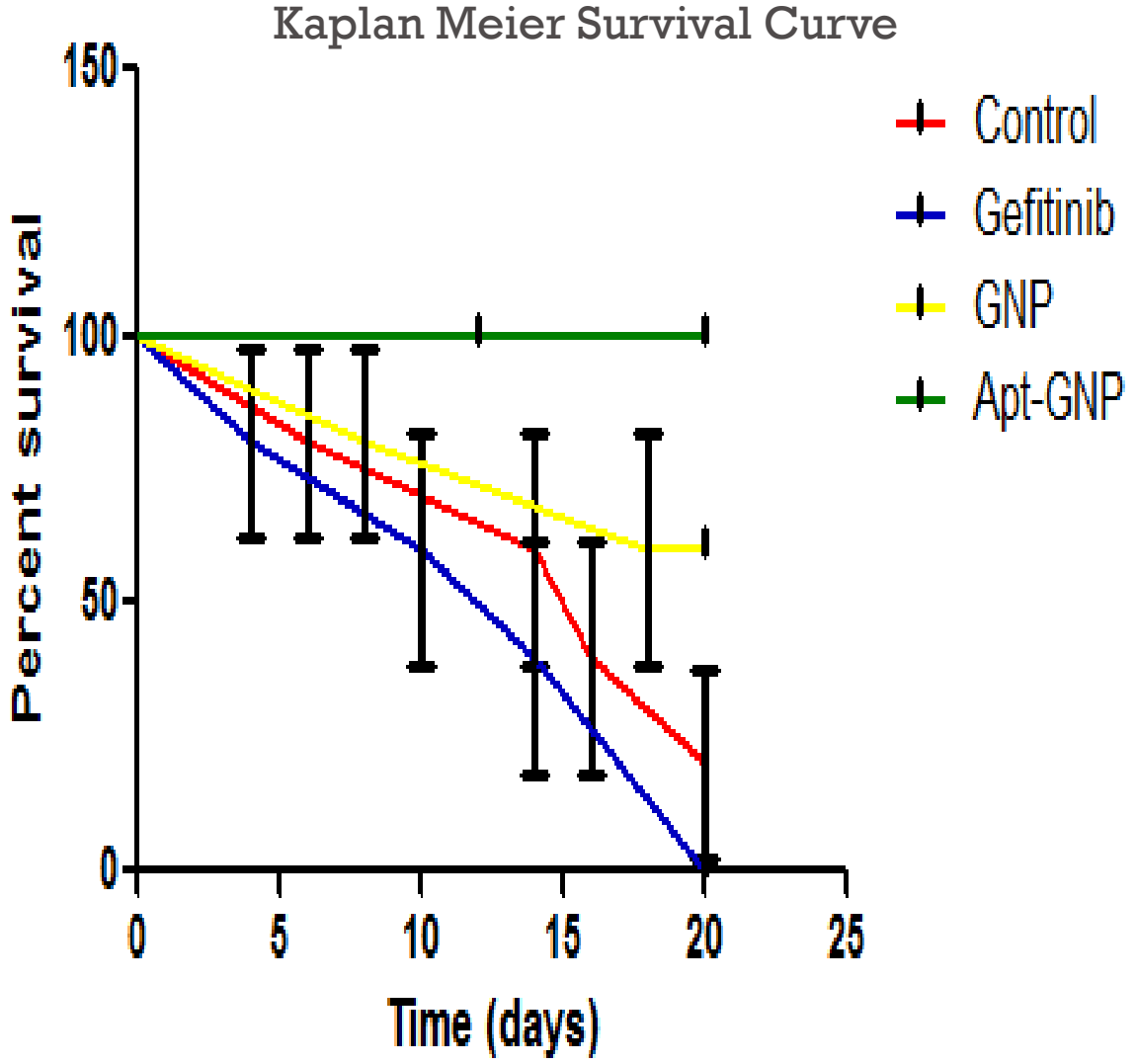


Aptamer-np bio-conjugates exhibit high efficacy in H1975 xenograft model of lung cancer

A



B

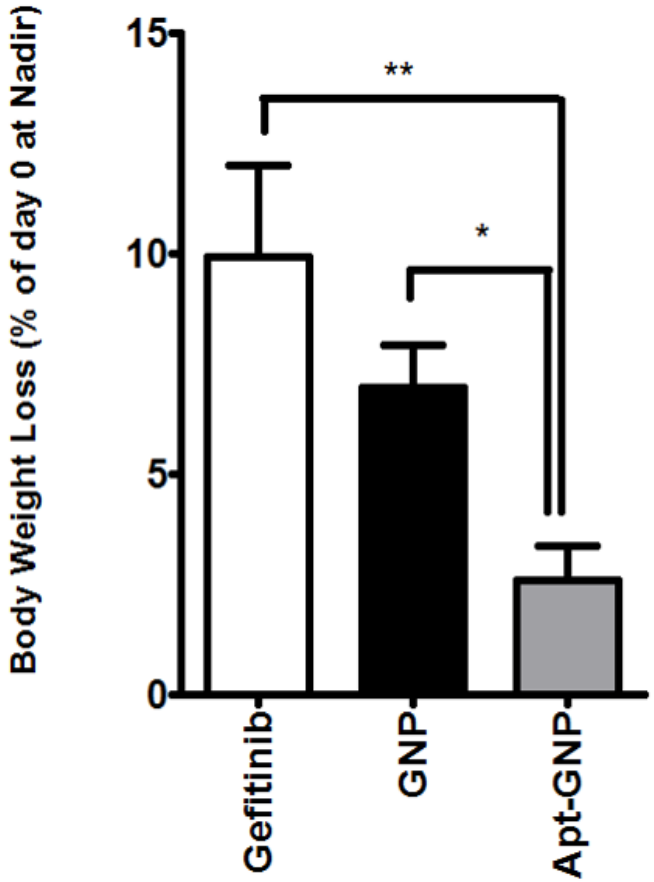


D

Representative tumours excised out of mice at end point

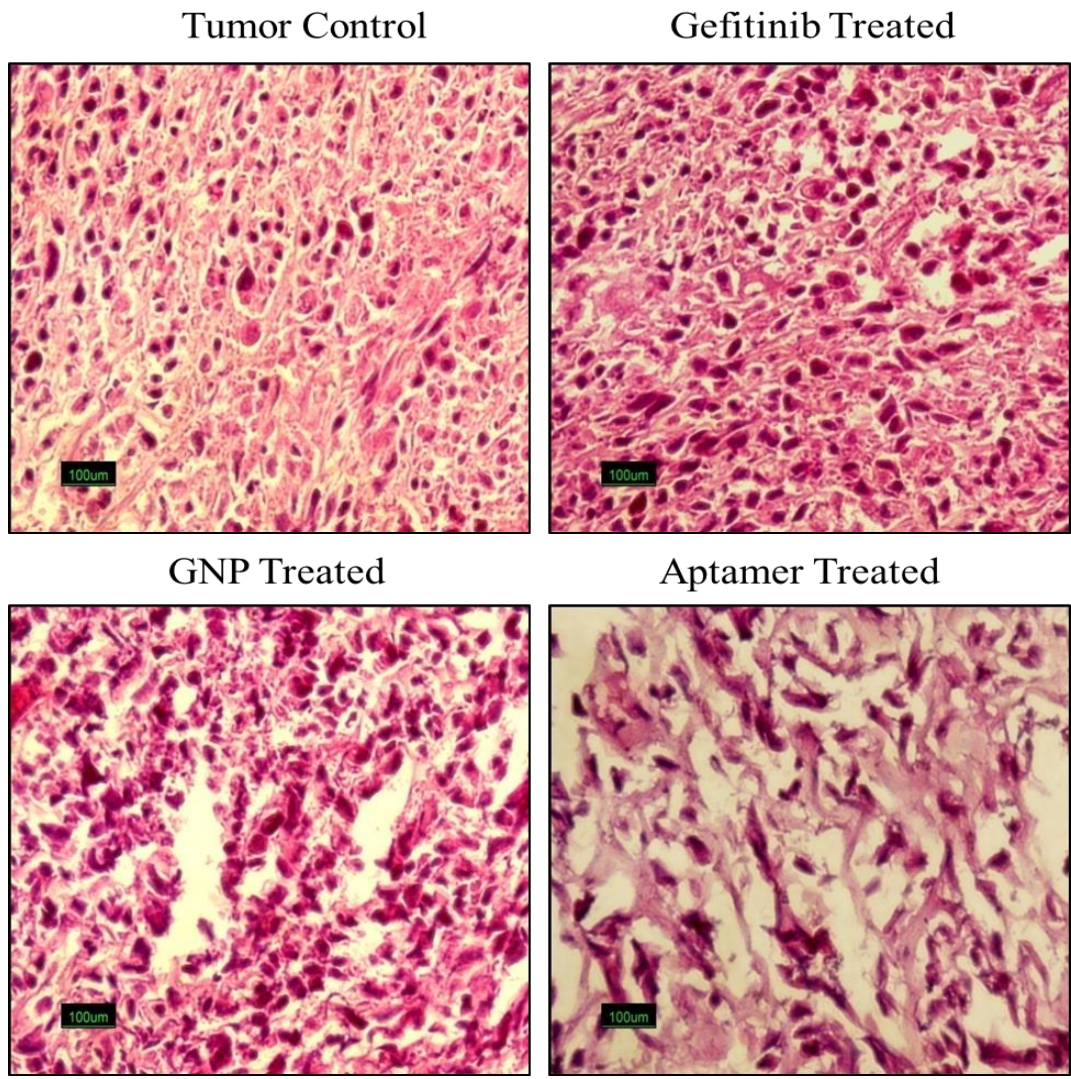
E

Body weight Loss of animals



F

Histopathological analysis of tumours



Why Apt-GNPs show higher anti-cancer effect????

- Instant binding and internalization of GNPs within tumour will ensure that whole of the drug is released within the tumour itself

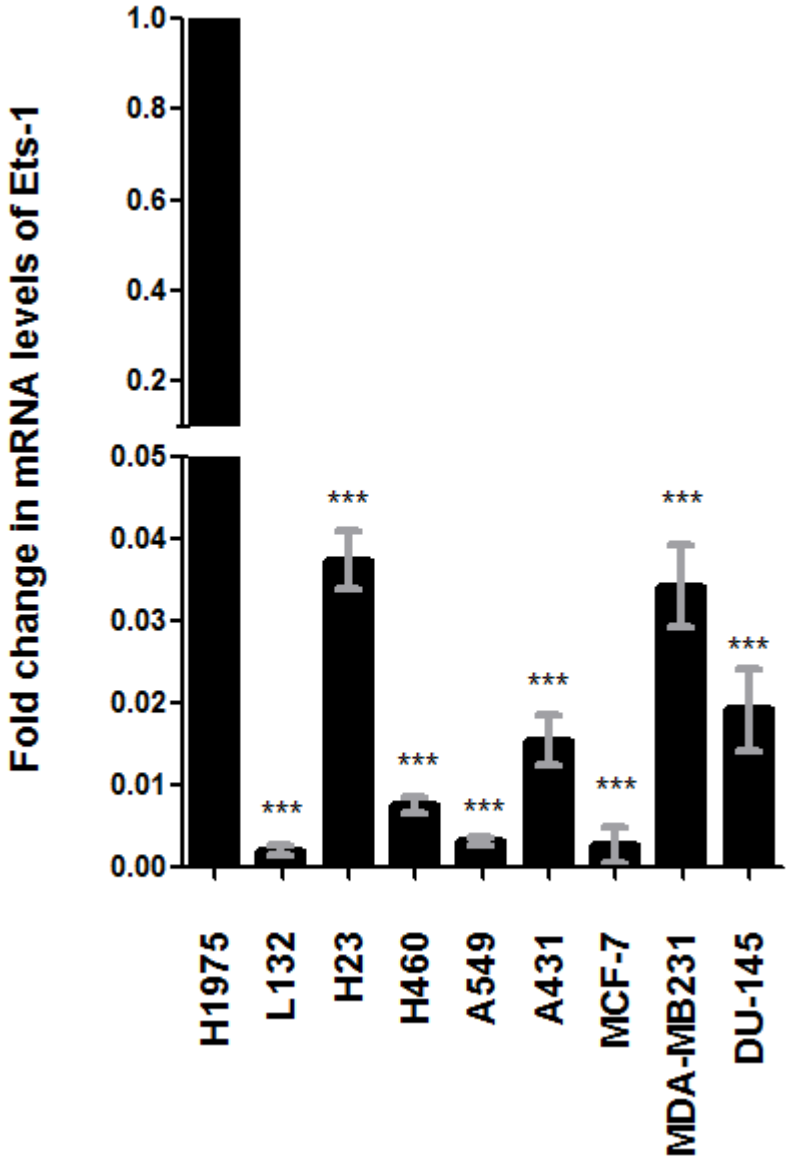
- Presence of Apt on the surface of NPs alters the surface charge or size of the conjugated system and leading to a lower rate of lymphatic or systemic clearance

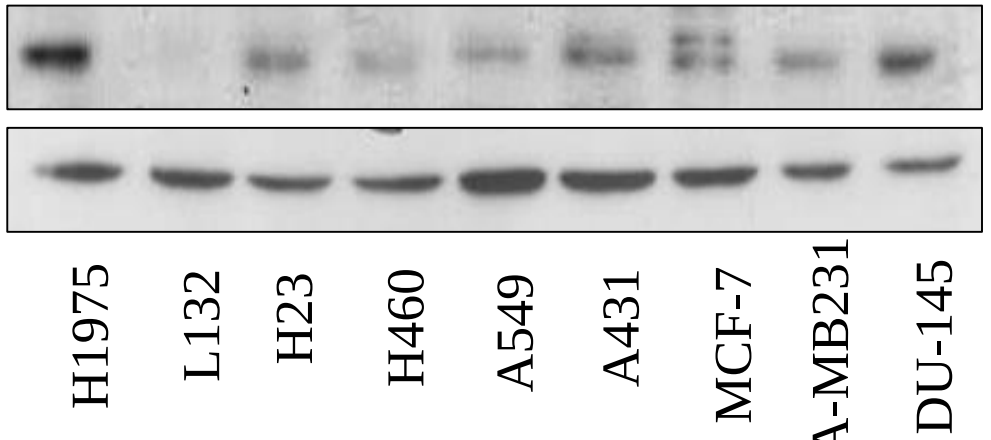

BIOINFORMATIC
ANALYSIS???

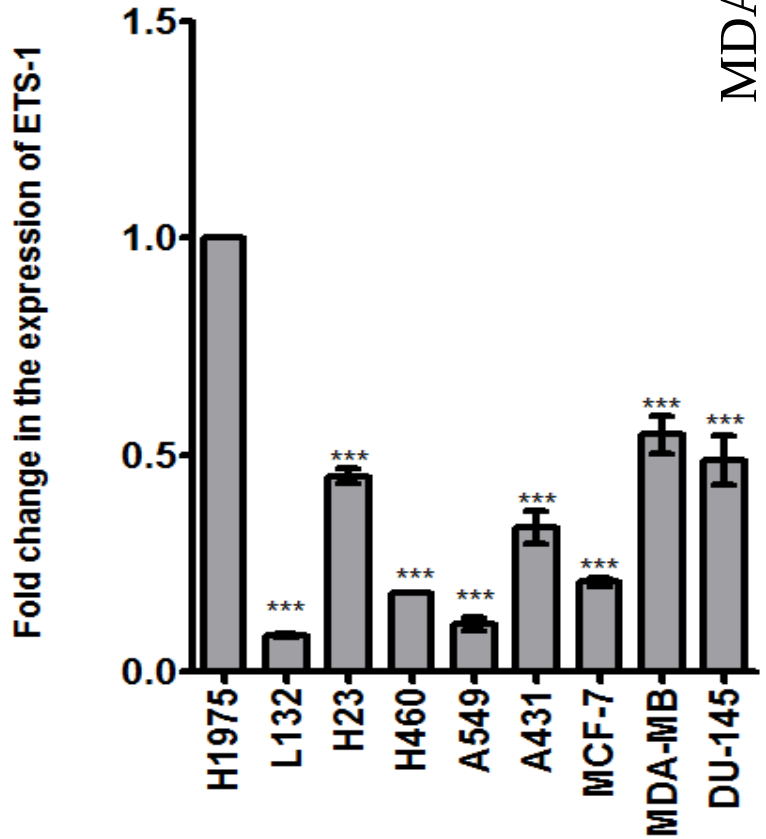
Ets-1: The target of selected aptamer as identified by MATCH software

- Ets comprises a family of transcription factors whose genesis lies in E26, an avian erythroblastosis virus, which carries v-ets oncogene
- High levels of Ets-1 in lung, squamous cell carcinoma have been linked with higher incidence of lymph node metastasis
- ETS-domain proteins bind to sequences which have central GGA motif
- To confirm that our sequence is binding specifically to H1975 cells due to the presence of Ets-1 we checked the levels of Ets-1 mRNA in both H1975 and L132 cells.

Ets-1 transcription factor is highly expressed in H1975 cells

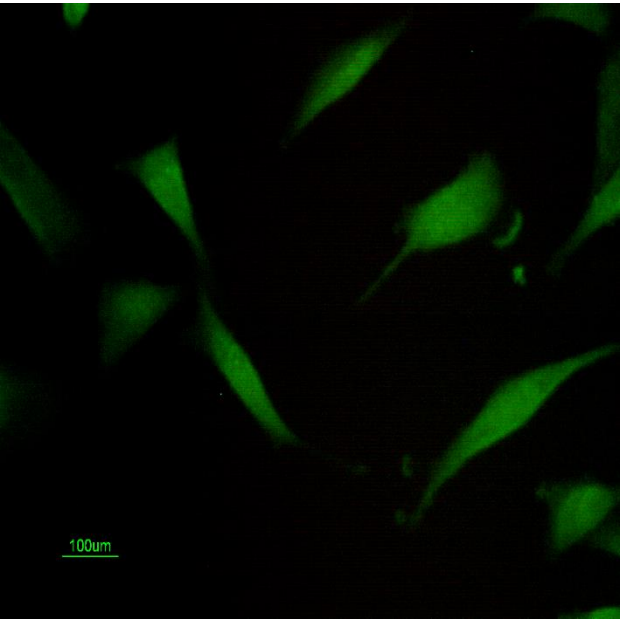




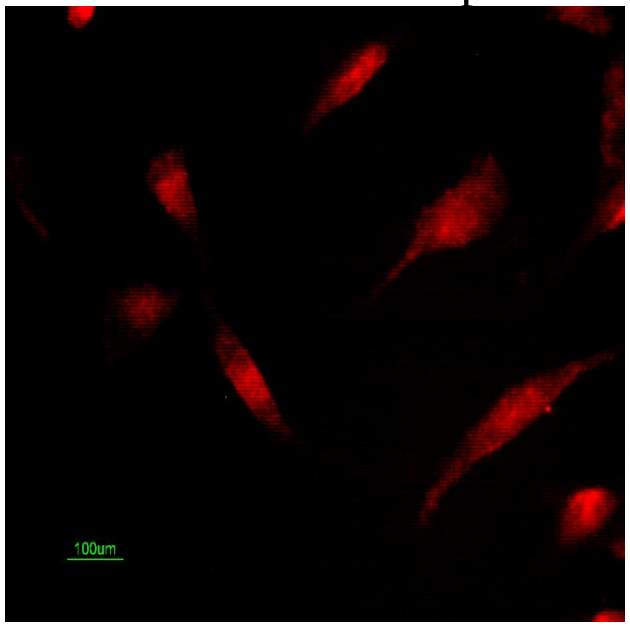


Texas red labelled aptamers co-localize with Ets1 protein in H1975 cells

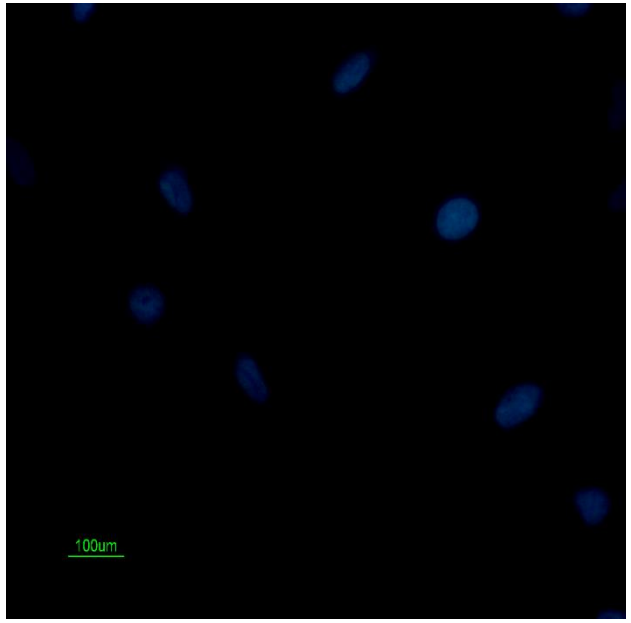
Ets-1



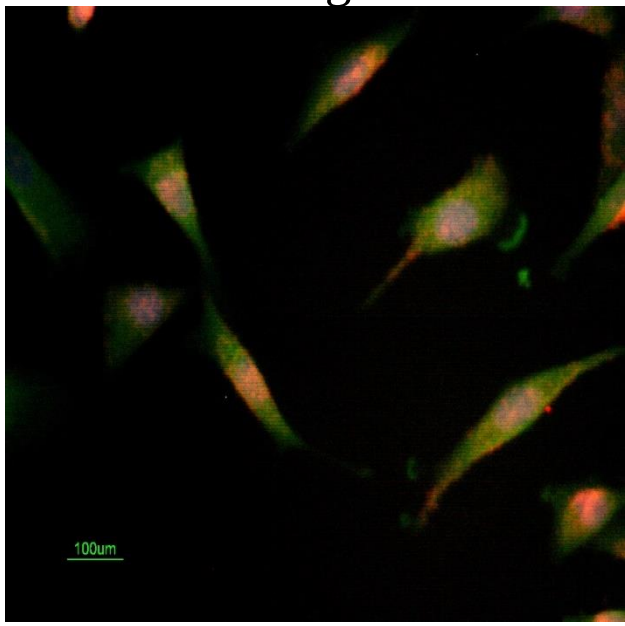
Texas Red labelled Aptamer



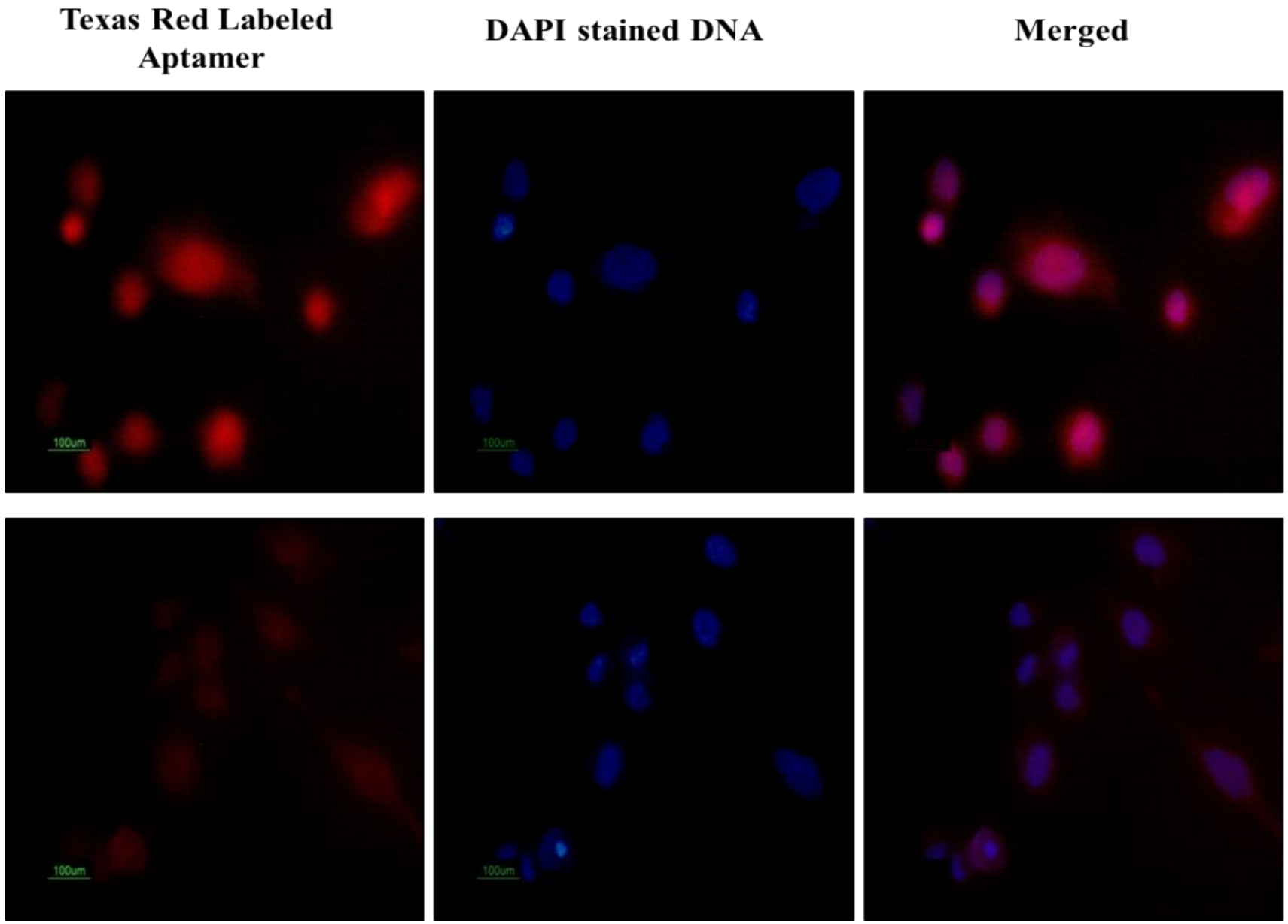
DAPI

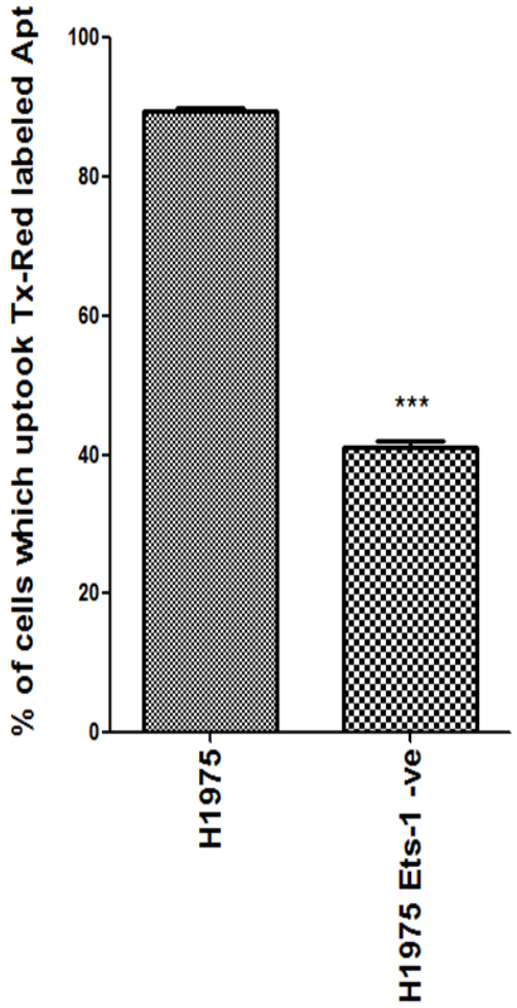


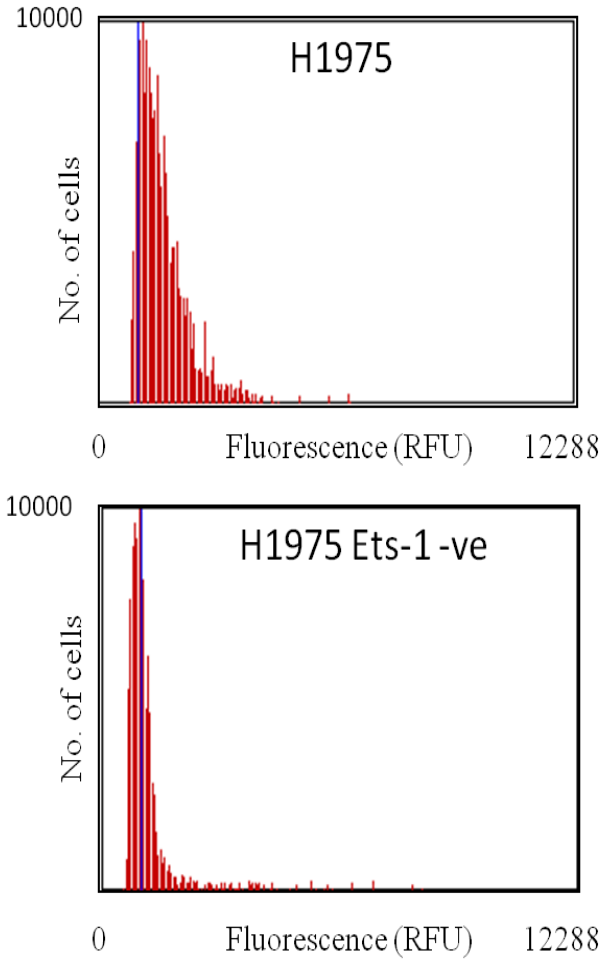
Merged

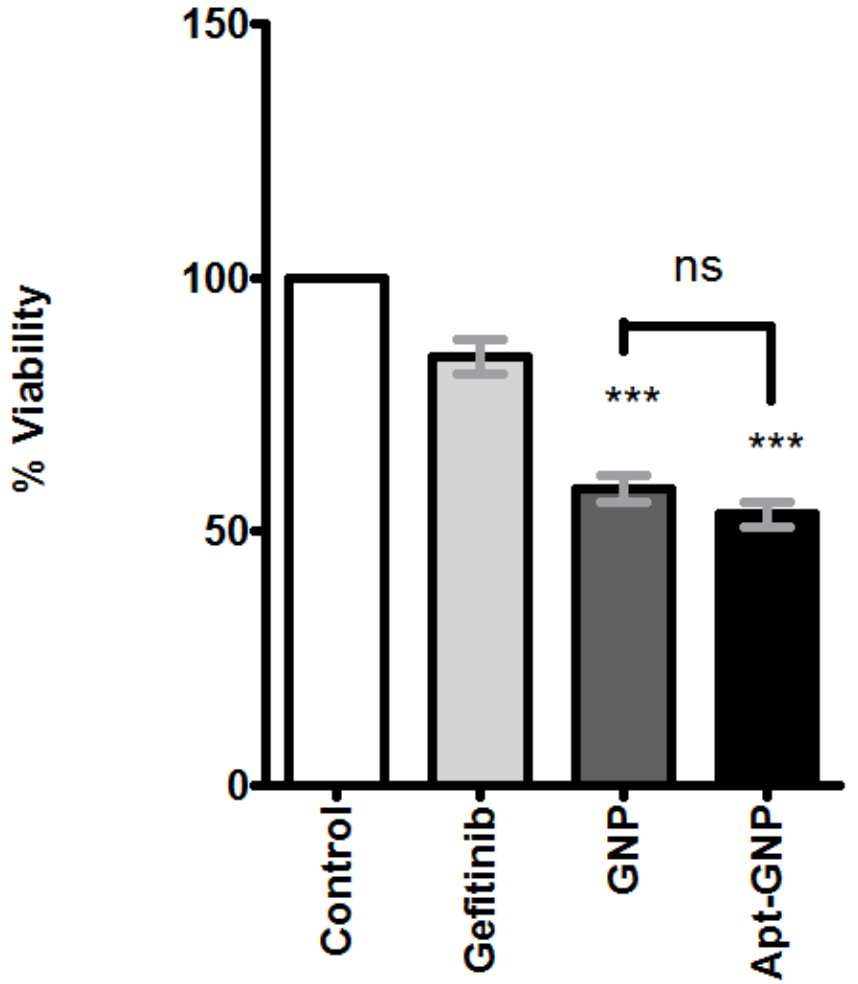


Ets-1 depleted H1975 cells show lower aptamer uptake

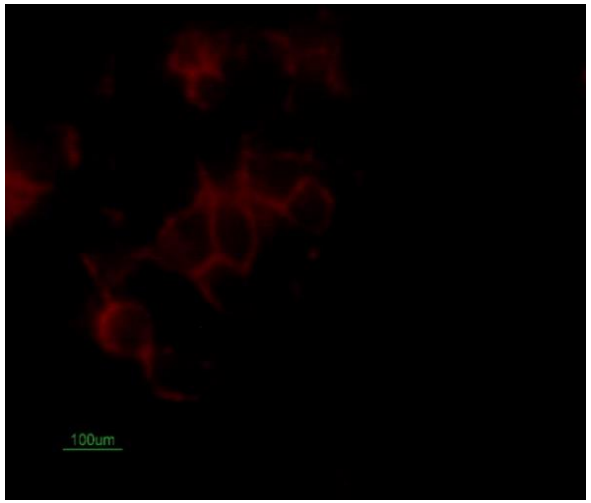
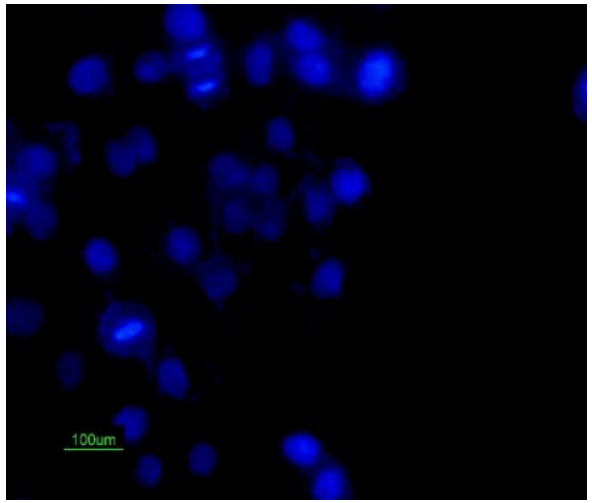
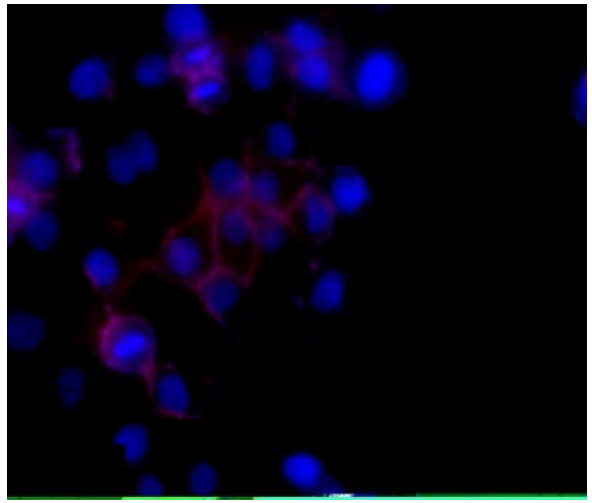
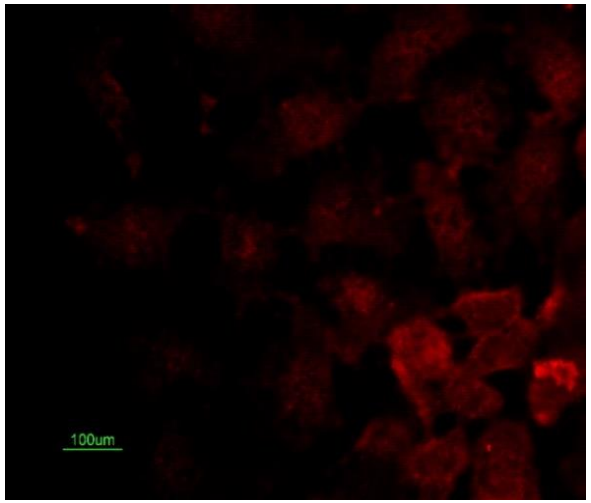
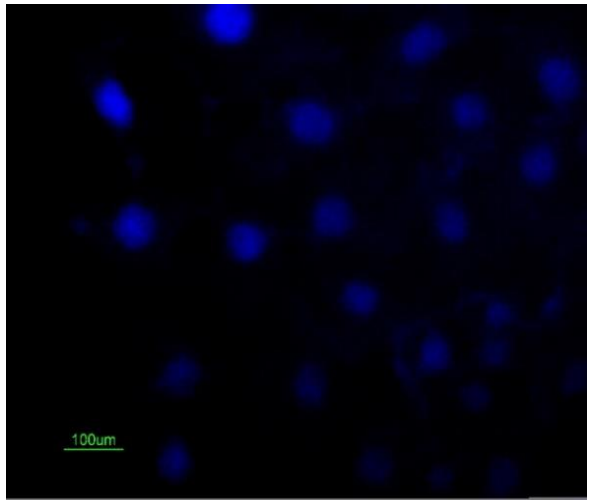
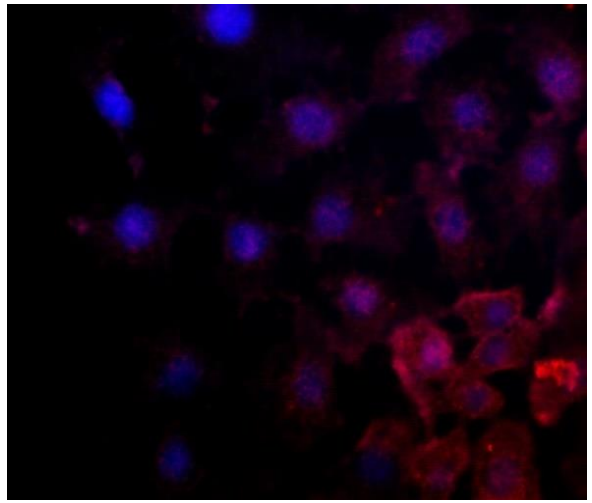


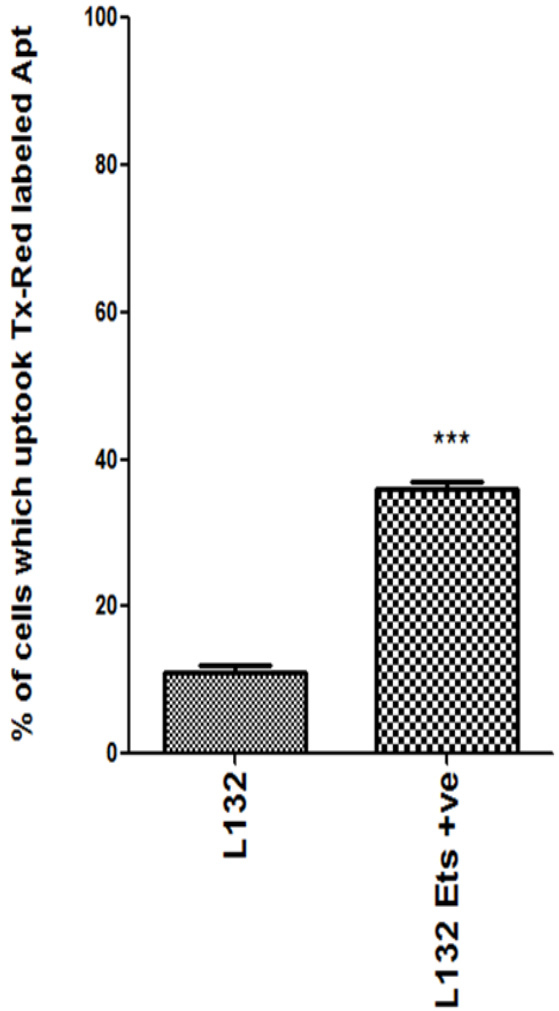

% of cells which uptook Tx-Red labeled Apt
100
80
60
40
20
0
***
H1975
H1975 Ets-1 -ve

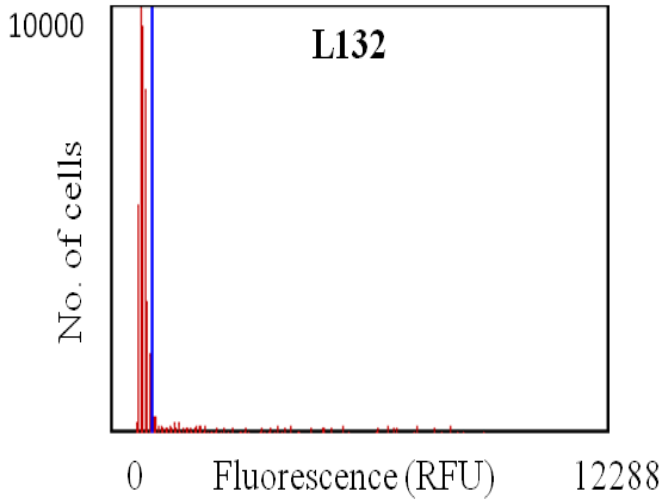

10000
H1975
No. of cells
0
Fluorescence (RFU)
12288
10000
H1975 Ets-1 -ve
No. of cells
0
Fluorescence (RFU)
12288

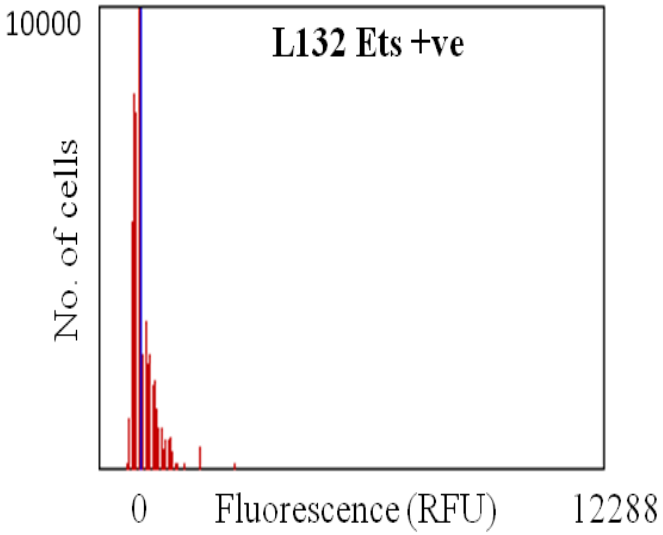

% Viability
150
100
50
0
ns
***
***
Control
Gefitinib
GNP
Apt-GNP

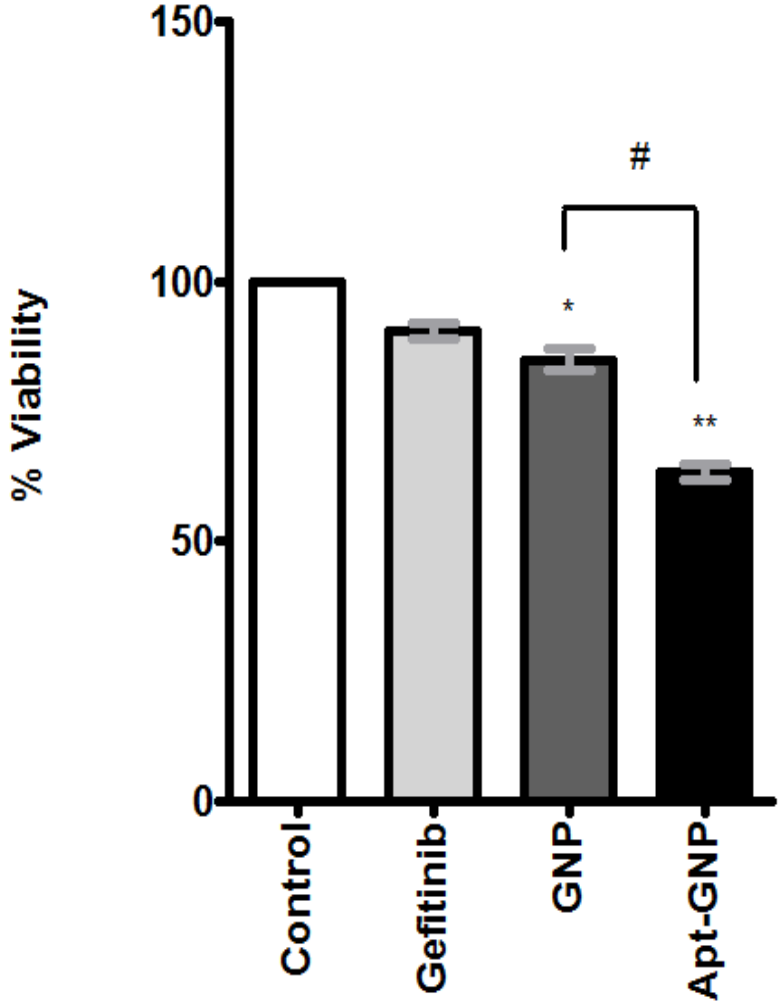
Over-expression of Ets-1 in L132 cells renders them identifiable by Aptamers

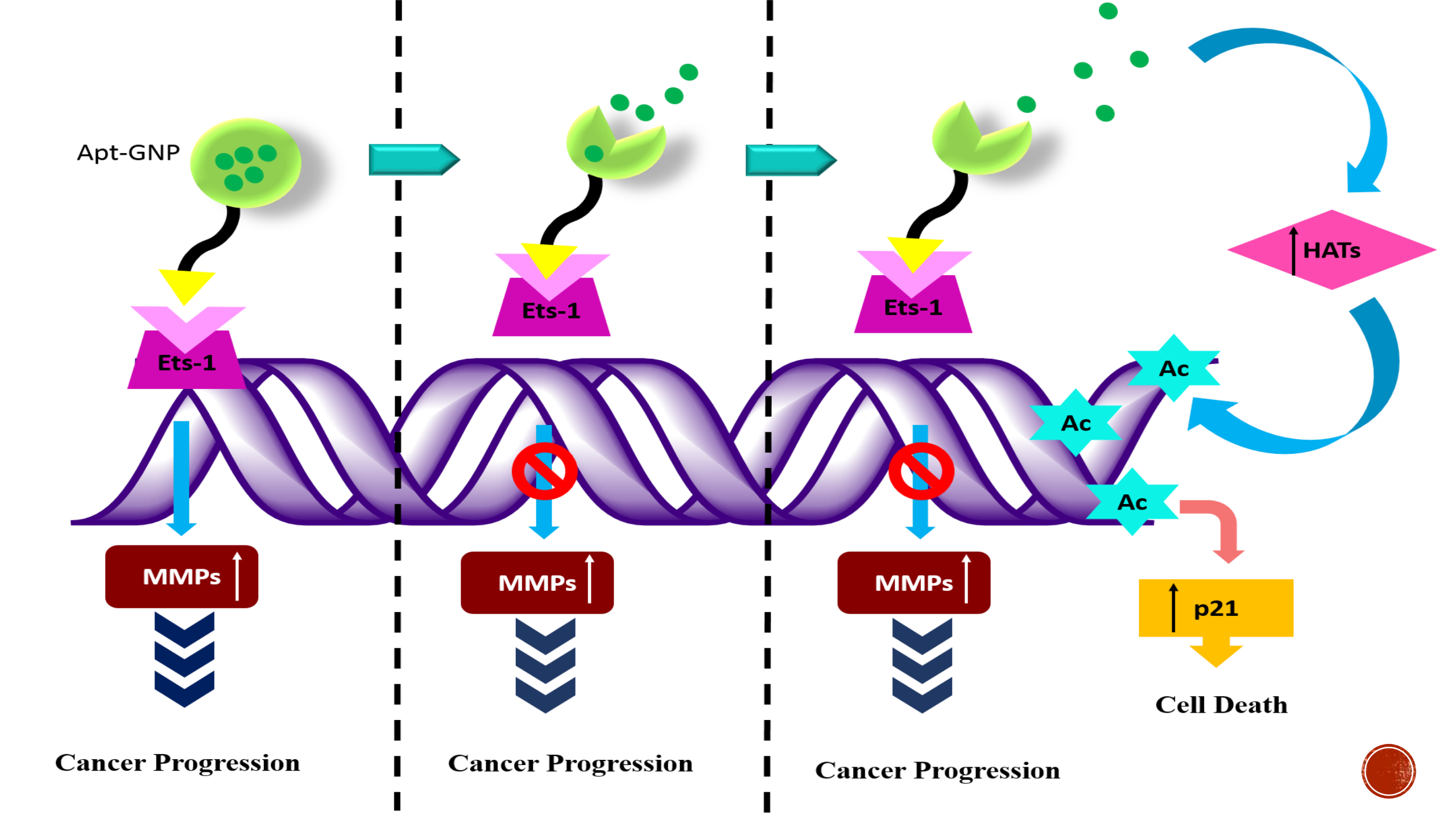
	Texas Red Labelled Aptamer	DAPI stained DNA	Merged
L132 cells			
L132 cells (Ets-1 +ve)			


% of cells which uptook Tx-Red labeled Apt
100
80
60
40
20
0
***
L132
L132 Ets +ve


10000
L132
No. of cells
0
Fluorescence (RFU)
12288


10000
L132 Ets +ve
No. of cells
0
Fluorescence (RFU)
12288


% Viability
150
100
50
0
#
*
**
Control
Gefitinib
GNP
Apt-GNP


Apt-GNP
Ets-1
Ets-1
Ets-1
HATs
Ac
Ac
Ac
MMPs
MMPs
MMPs
p21
Cell Death
Cancer Progression
Cancer Progression
Cancer Progression

ADVANTAGES....

- Higher retention in Ets-1 positive metastatic cancer cells
- Can aid in decreasing the therapeutic dose of anti-cancer drugs
- Better safety profile (lower side effects)
- Can be conjugated to various anti-cancer drugs
- Can be used for diagnostic purposes
- Cost effective


Thank You…