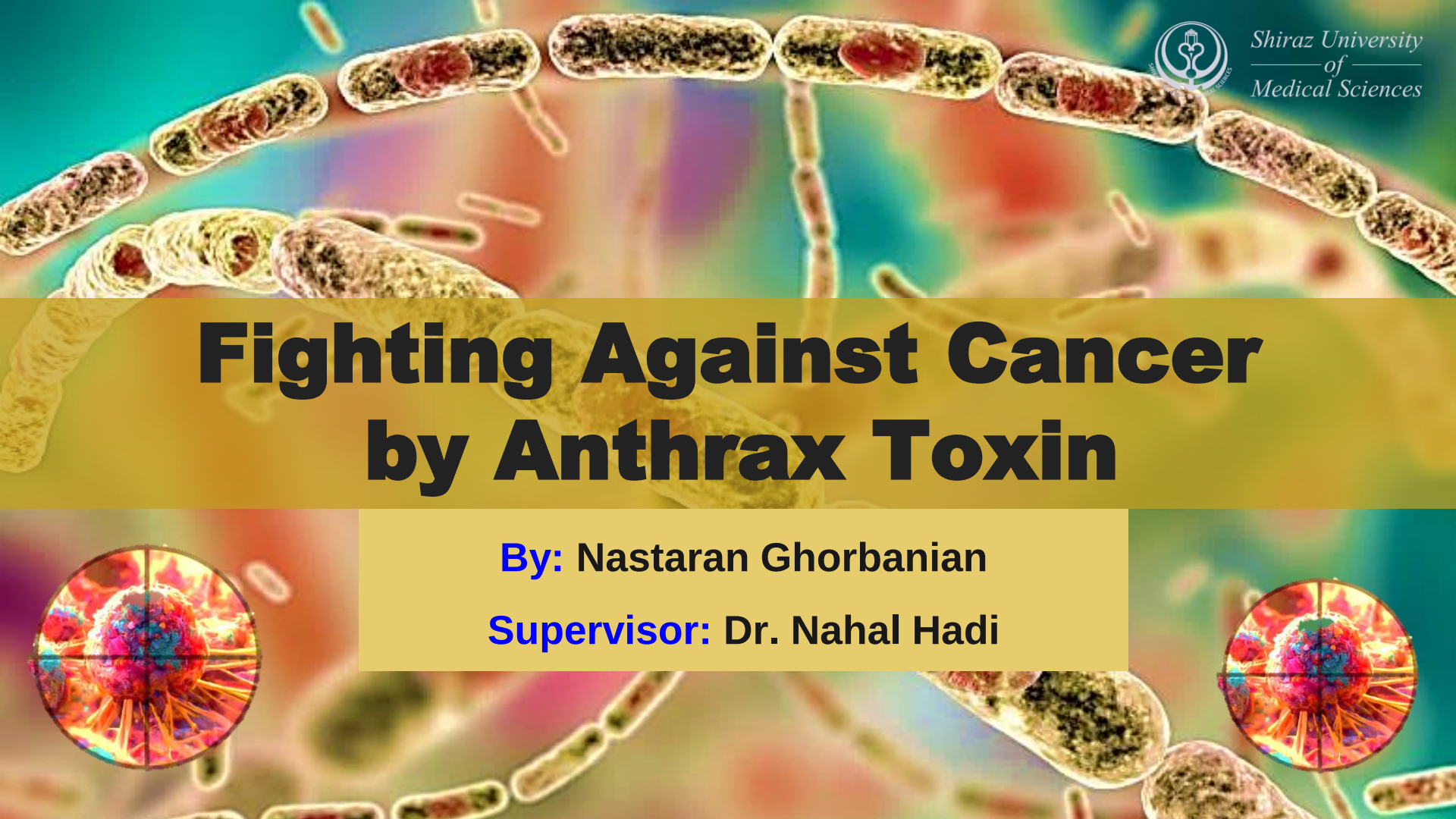


Shiraz University of Medical Sciences

Fighting Against Cancer by Anthrax Toxin

By: Nastaran Ghorbanian

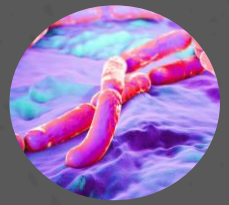
Supervisor: Dr. Nahal Hadi

Table of Contents

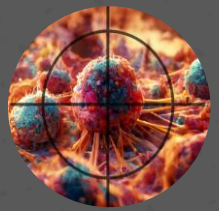
1. Introduction

- Definition of cancer
- Epidemiology of cancer
- Traditional treatments & their disadvantages

2. *Bacillus anthracis*



- Characteristics
- Mechanism of action of anthrax toxin

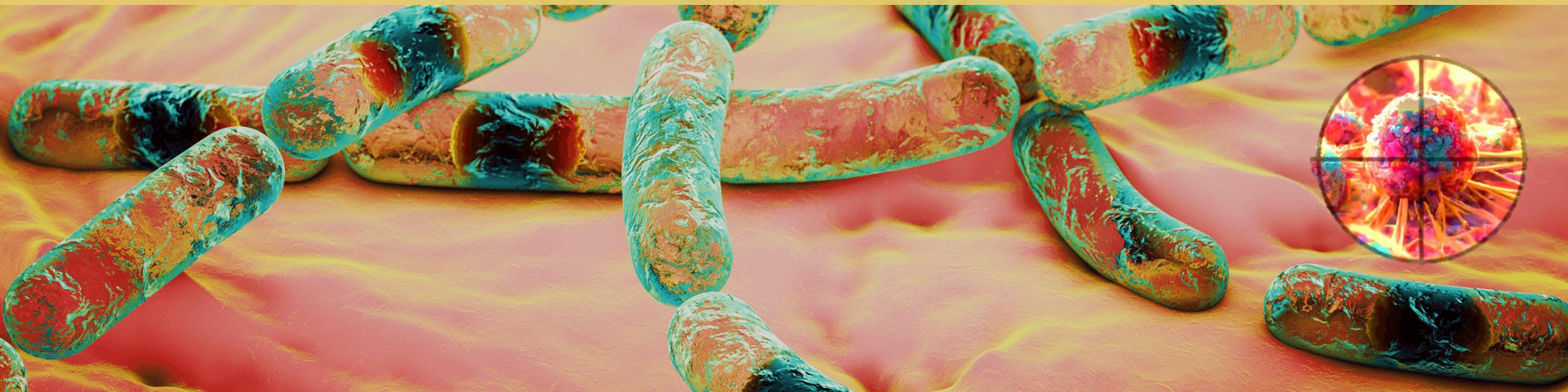


3. Turning anthrax toxin into a cancer killer

4. Studies results & Conclusion

5. References

Introduction



What is cancer?

Cancer is caused by changes (mutation) to the DNA within cells.

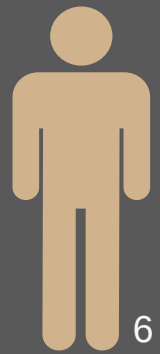
It is disease by the dysregulation of the cell cycle leading to uncontrolled cell division.

Epidemiology of cancer

- ✓ Cancer is a global health problem responsible for one in six deaths worldwide.
- ✓ According to the latest report of the World Health Organization in 2022, there were an estimated 20 million new cancer cases and 9.7 million deaths.

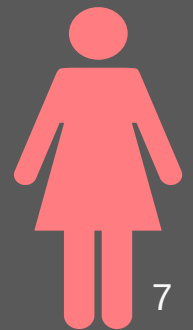
Global cancer incidence in men

Rank	Cancer	%of all cancers
1	Lung	15.4
2	Prostate	15.1
3	Colorectal	11.4
4	Stomach	7.7



Global cancer incidence in women

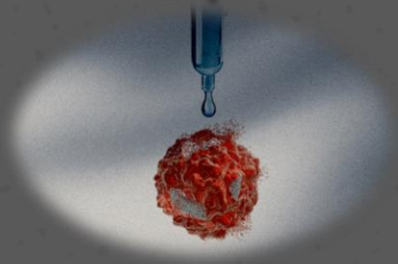
Rank	Cancer	%Of all cancers
1	Breast	25.8
2	Colorectal	9.9
3	Lung	8.8
4	Cervix uteri	6.9

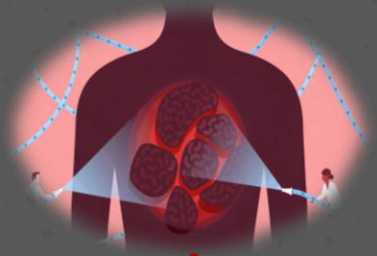


Traditional treatments of cancer

Surgery

Chemotherapy





Radiation therapy

Hormone therapy

Disadvantages of traditional cancer treatment methods

- ✓ Despite great improvements in the treatment of cancer, it is still one of the top diseases that threaten human health. Because we still faces limitations in these treatment such as:

a) Improper specificity → Need for high doses →

Increase in costs

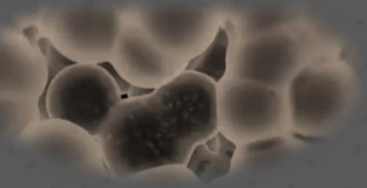
b) Drug resistance

c) Failure in treatment

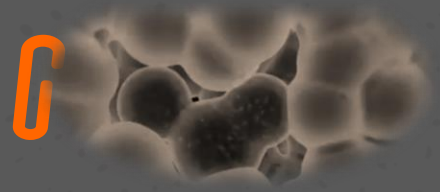
Side effects of traditional cancer treatments on patients

- Fatigue
- Hair loss
- Inflamed skin
- Nausea
- Joint pain
- Peripheral neuropathy

Bacterial toxin therapy



- ✓ Targeted therapeutics have emerged in recent years as an attractive approach to treating various types of cancer.
- ✓ One approach is to modify a cytocidal protein toxin to direct its action to a specific population of cancer cells.



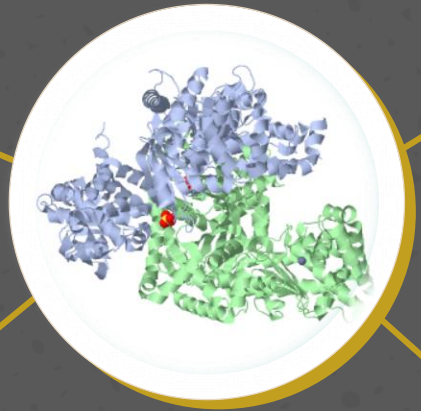
Bacterial toxin therapy

- ✓ Bacteria express and release some toxins that can be cytotoxic.
- ✓ But at lower concentrations, some of these toxins are able to effectively inhibit tumor growth through cell-cycle arrest, tumor cell signal pathway interruption, and other mechanisms.

Bacterial toxin therapy

C. diphtheria
diphtheria toxin

Pseudomonas
exotoxin A (PE)



C. perfringens
type A enterotoxin

B.anthracis
Anthrax toxin

History

- ✓ In 1992, Naveen Arora and Stephen H. Leppla et al. first reported that catalytic domain of *Pseudomonas* exotoxin A and the LF fusion protein could be delivered into the cytosol of mammalian cells by anthrax PA.

- ✓ This discovery opened a new frontier with regard to the use of anthrax toxin PA as a drug delivery system for various non-native cargoes.

Bacillus anthracis

Characteristics of *Bacillus anthracis*

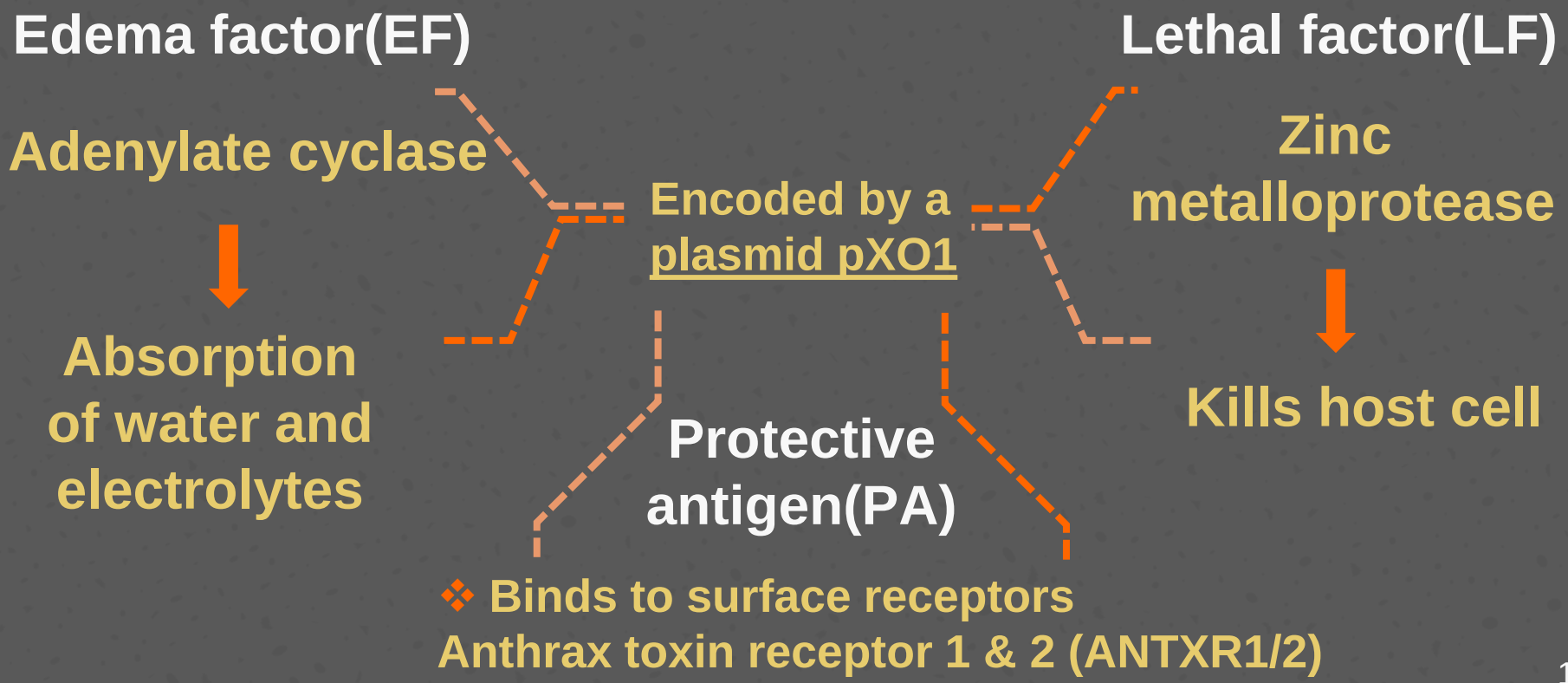
01 Very large, Rod shape

02 Aerobic

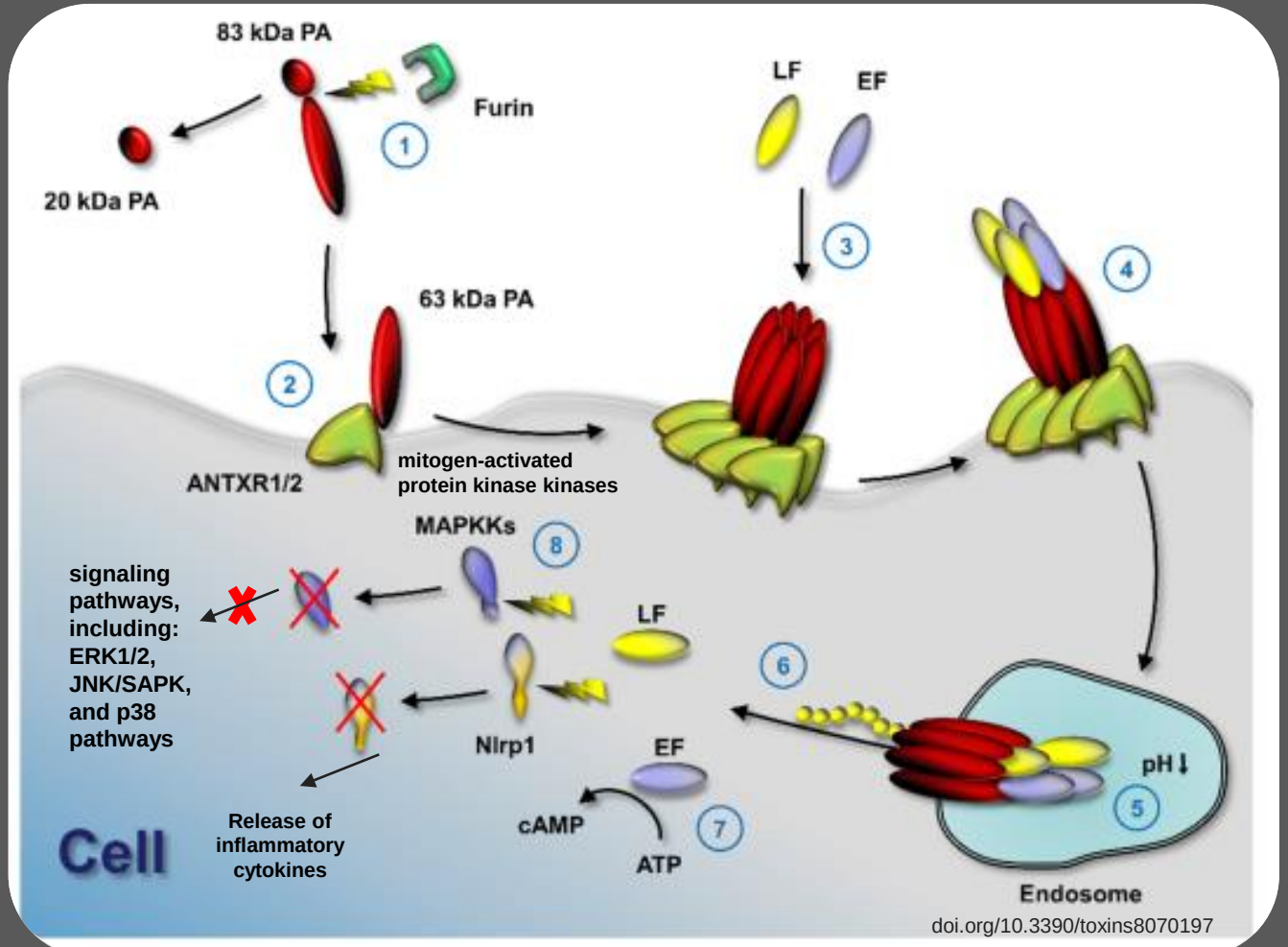
03 Central spores that develop under all condition except in the living body

04 Non-motile
Non- hemolytic

Anthrax toxin



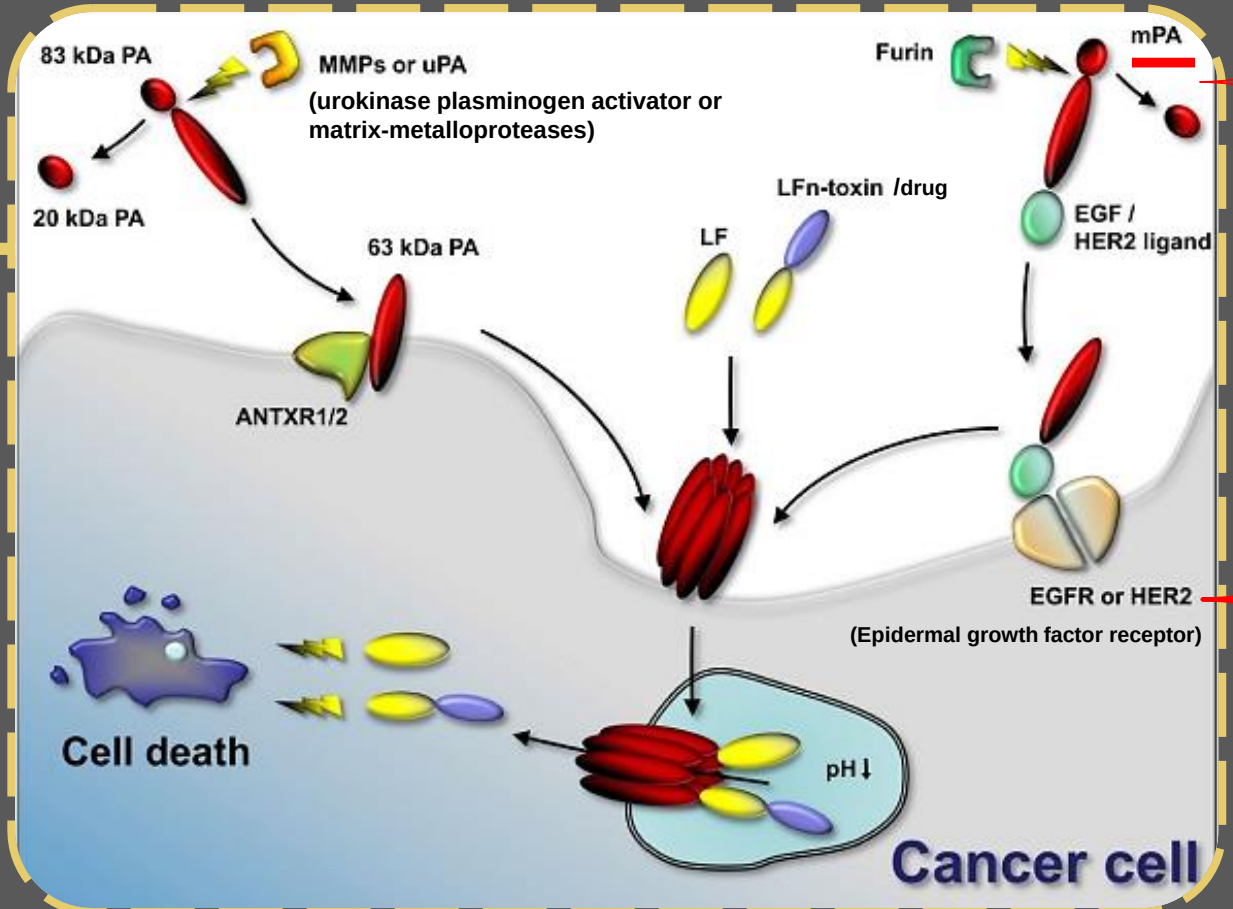
Mechanism of anthrax toxin



doi.org/10.3390/toxins8070197

Turning anthrax toxin into a cancer killer

Tumor-selective activation of protective antigen and Tumor-selective formation of PA octamer



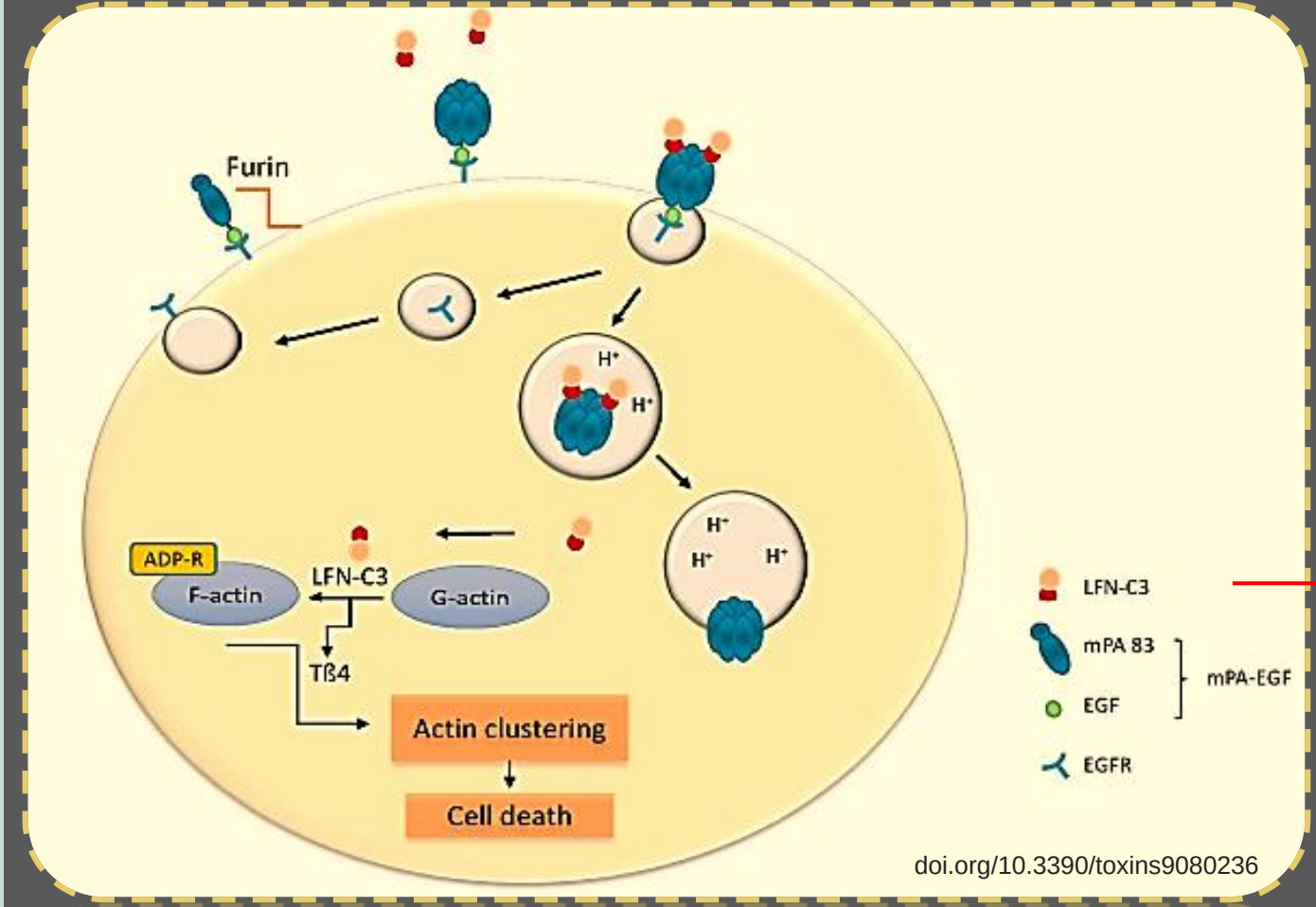
PA as a double mutant (N682A, D683A)

LFn-toxin:
Is a fusion between LFN, the N-terminal PA63-binding domain of LF, and the catalytic domain of toxin.

Bladder/Breast/ Gastric/Prostate and ovarian cancer

doi.org/10.3390/toxins8070197

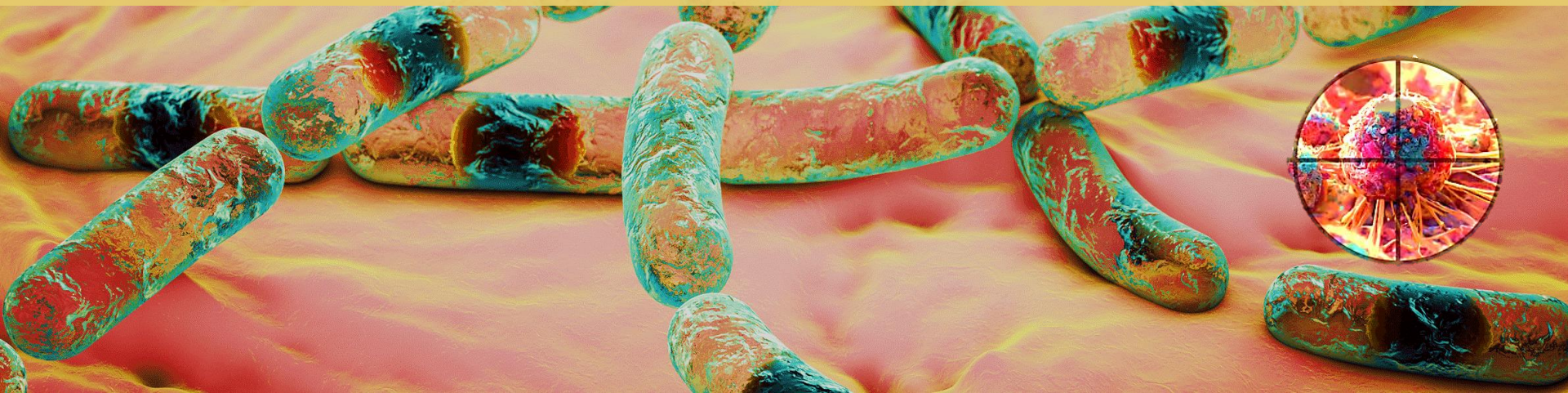
Anthrax toxin as a transporter



Anthrax lethal factor-N-terminus fused to the catalytic domain of *Photorhabdus luminescence* TccC3 toxin

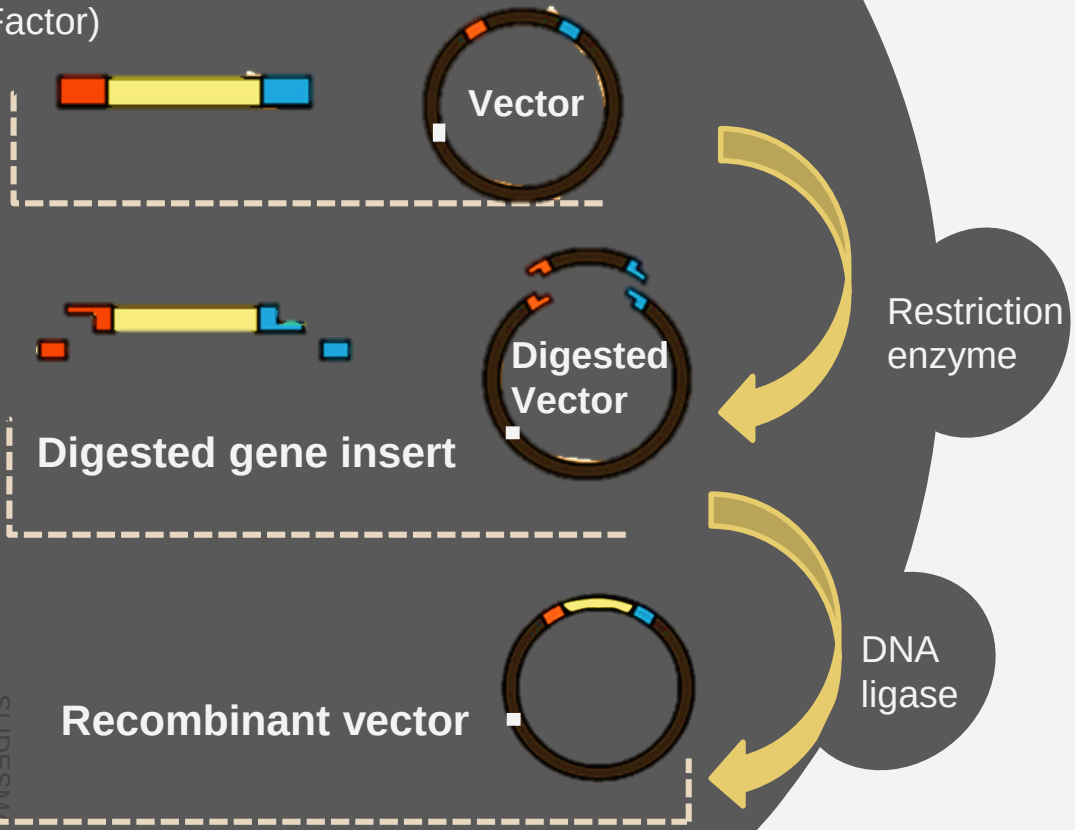
doi.org/10.3390/toxins9080236

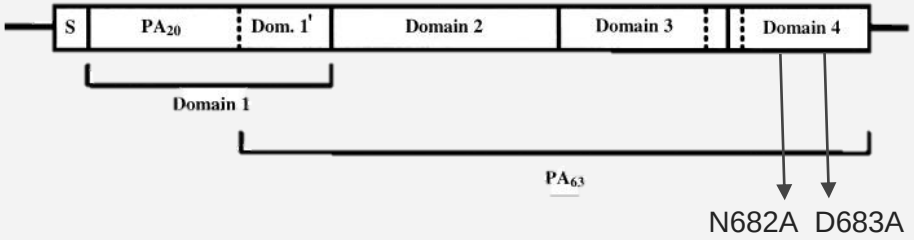
Mechanism of toxin synthesis



1. Produce a vector consist of mPA –EGF

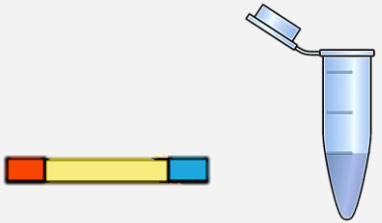
(mutated protective antigen- Epidermal Growth Factor)





Introduced 2 mutations within domain 4

mPA gene were amplified



Vector

Epidermal Growth Factor gene

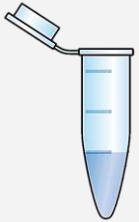
Digested Vector

Restriction enzyme

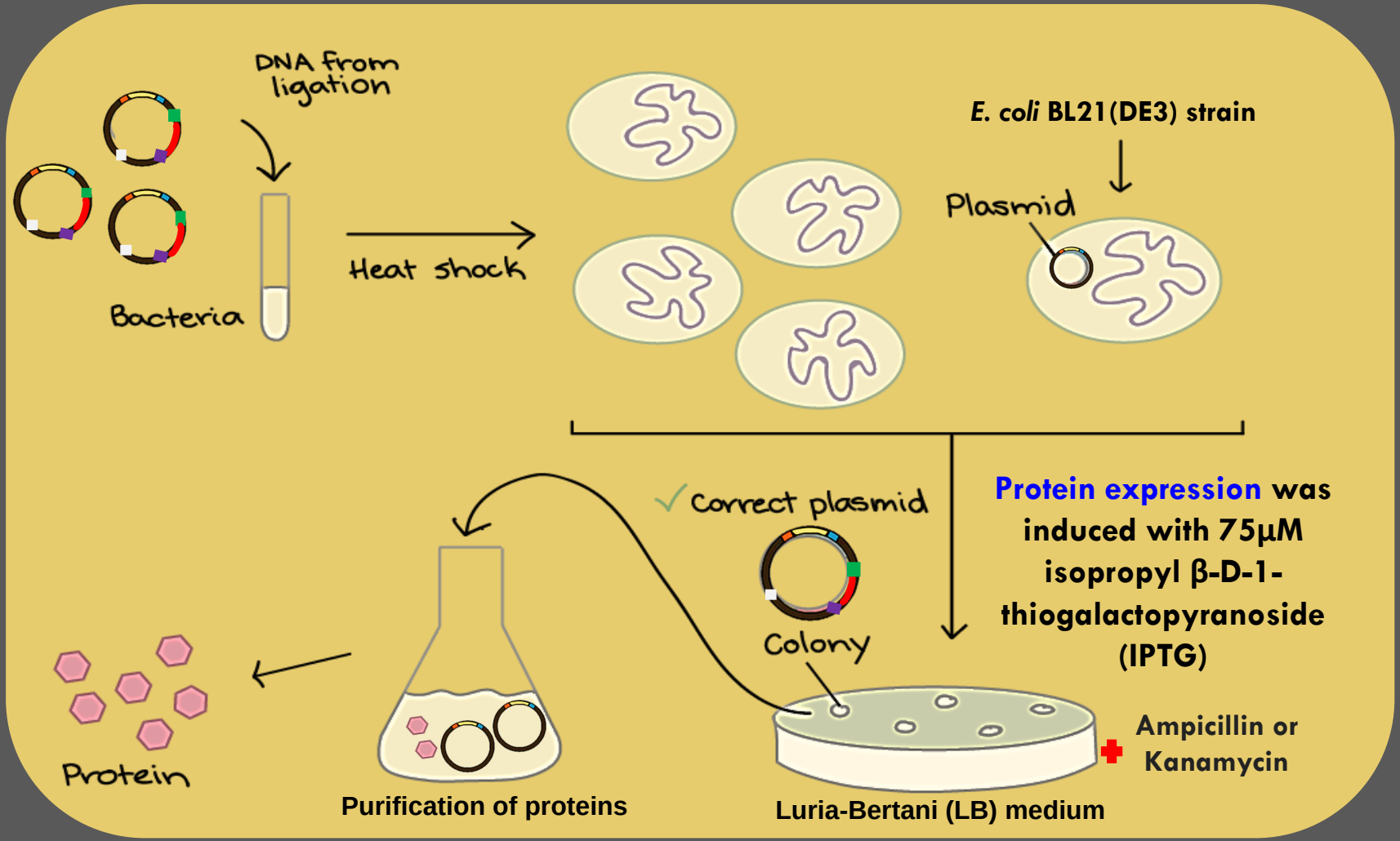
Digested gene insert

Recombinant vector include **EGF & mPA**

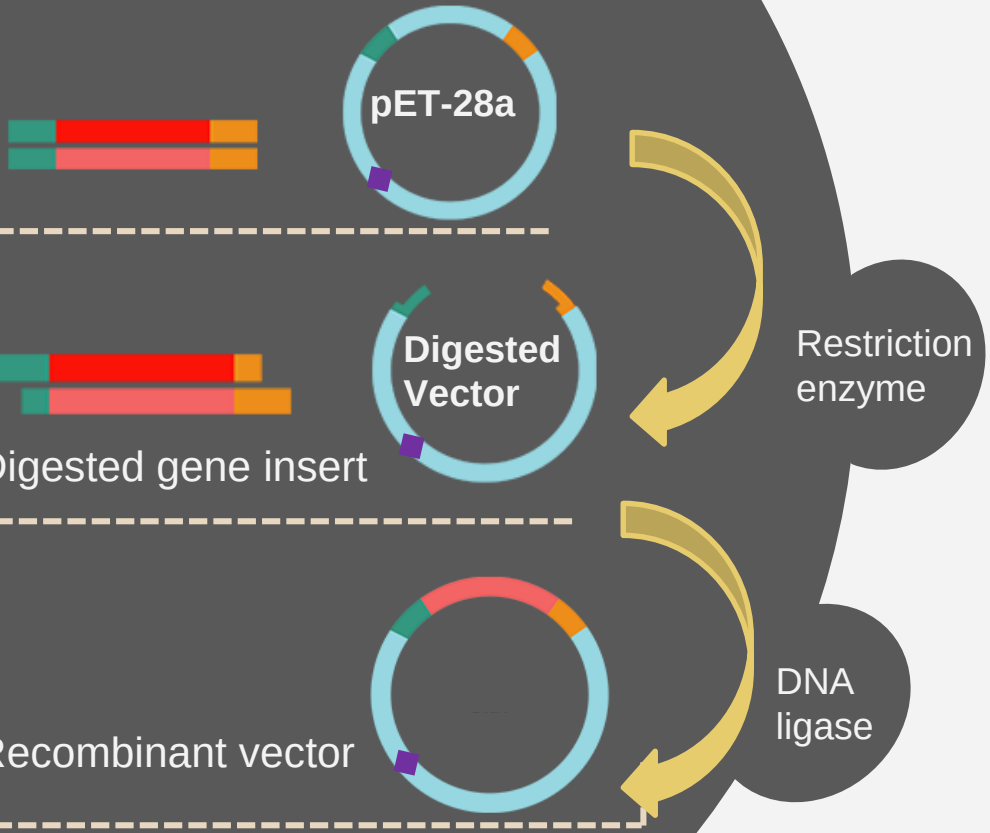
DNA ligase



2&3: Protein expression & Purification

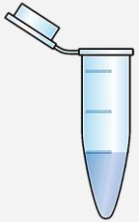


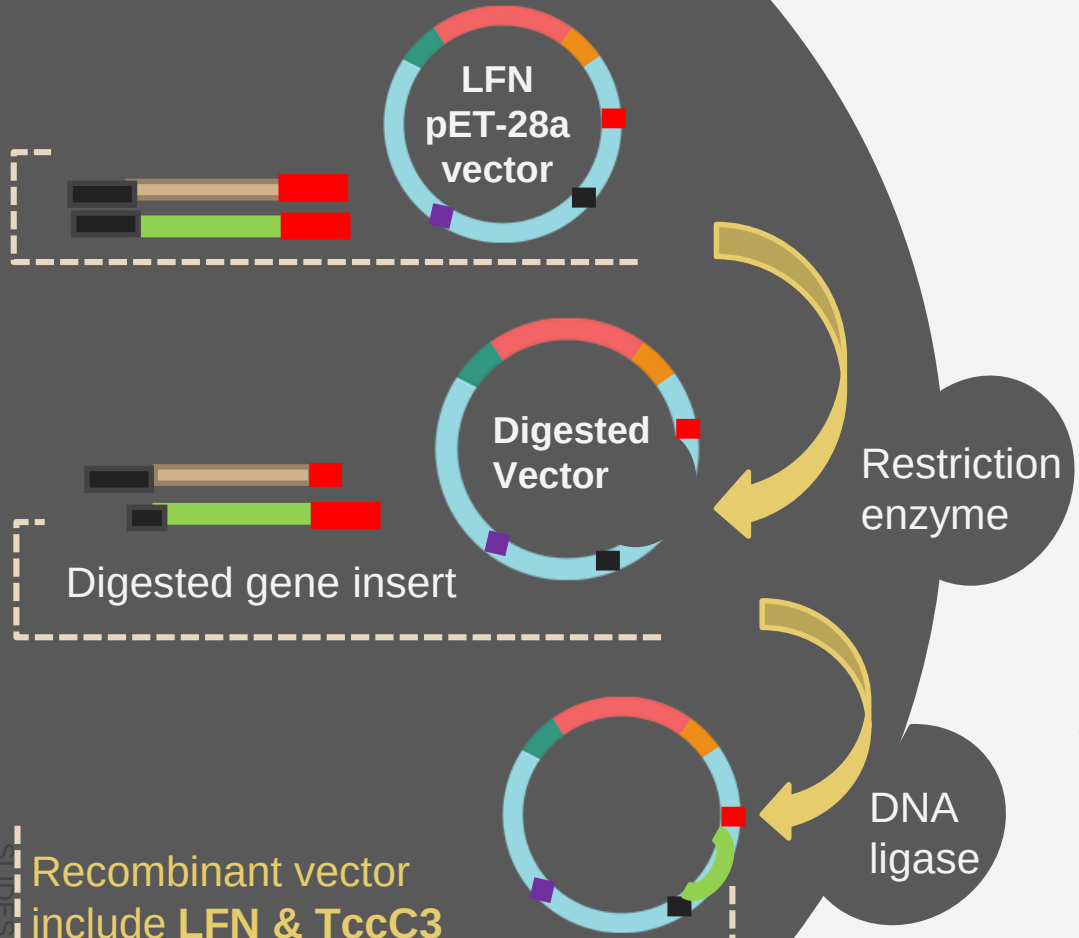
4. Produce a vector consist of LFN-C3



Lethal factor gene

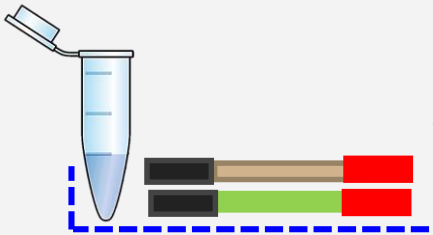
The LFn was amplified





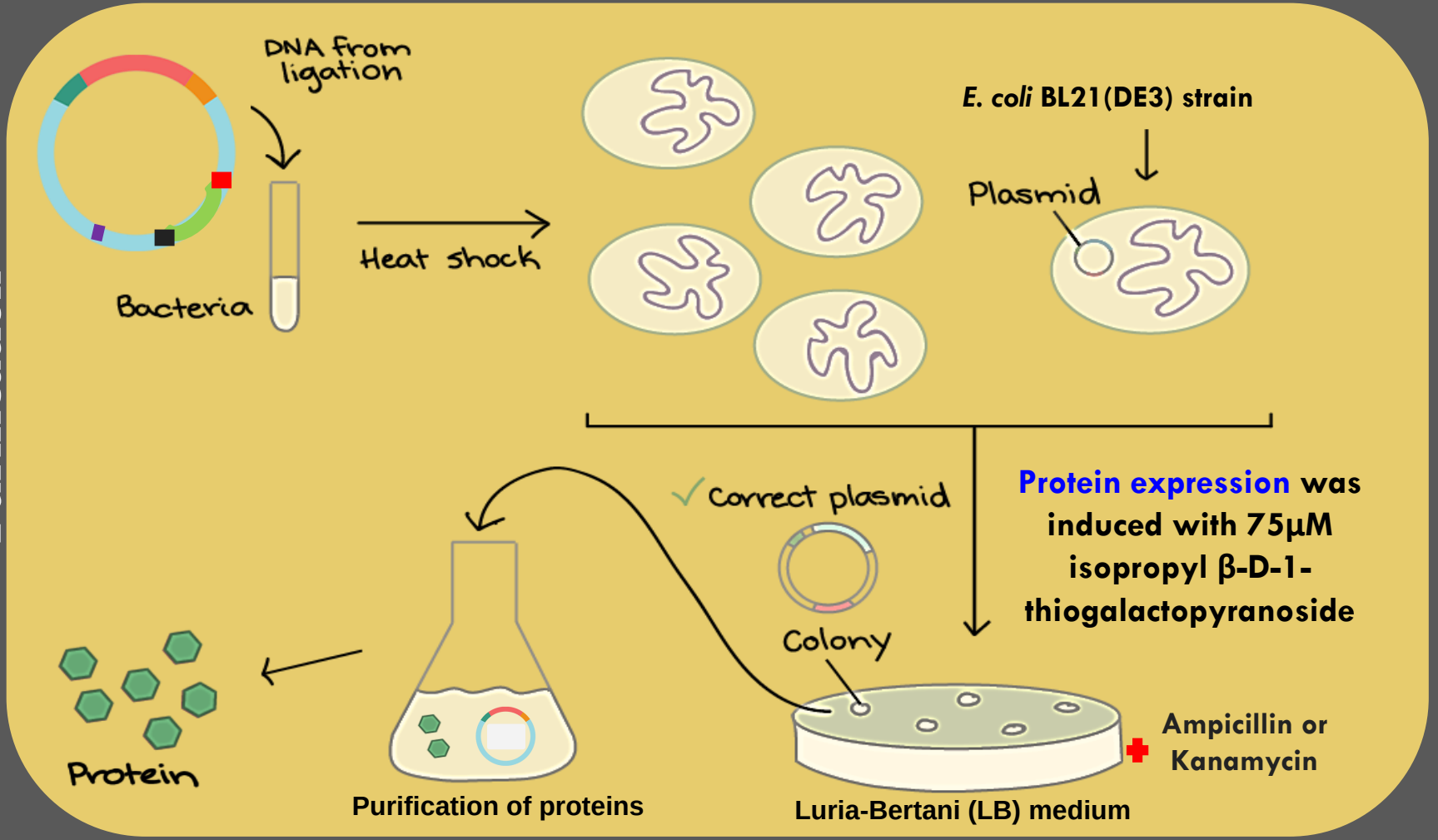
Photorhabdus luminescence
TccC3 gene

Amplification of the
ADP-ribosyltransferase
domain of TccC3

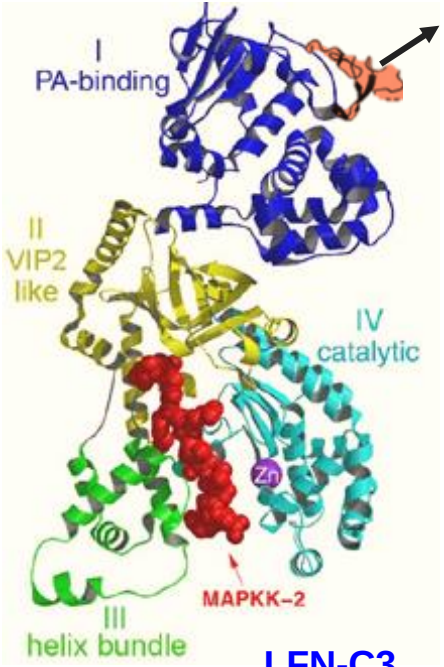


The PCR product
of TccC3

5&6: Protein expression & Purification



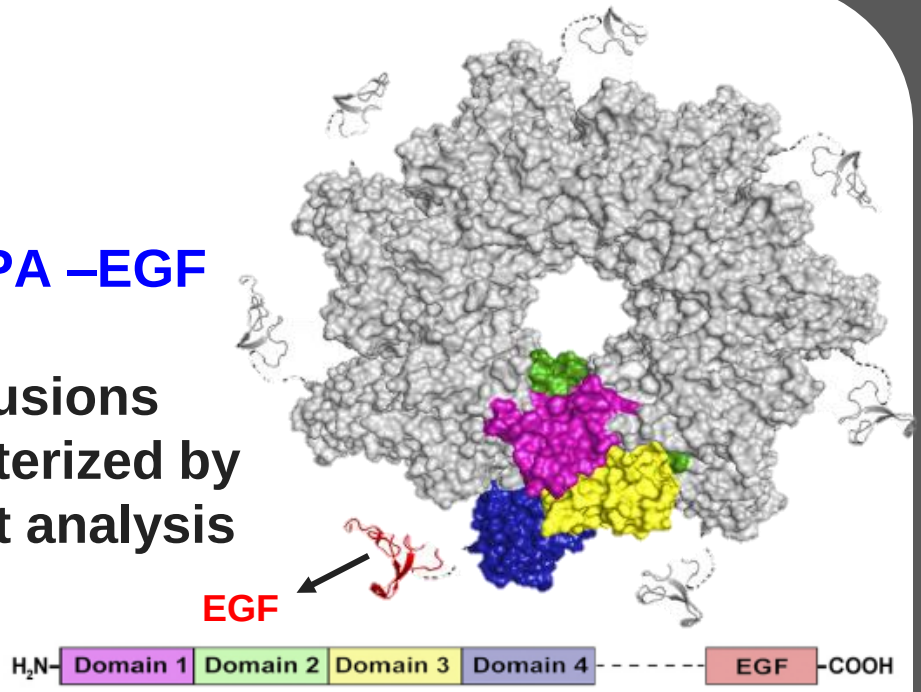
7.Western blot technique



LFN-C3

(Is a fusion between LFN, the N-terminal PA63-binding domain of LF, and C3, the catalytic domain of toxin TccC3)

Purified mPA –EGF
&
LFN-C3 fusions were characterized by Western blot analysis



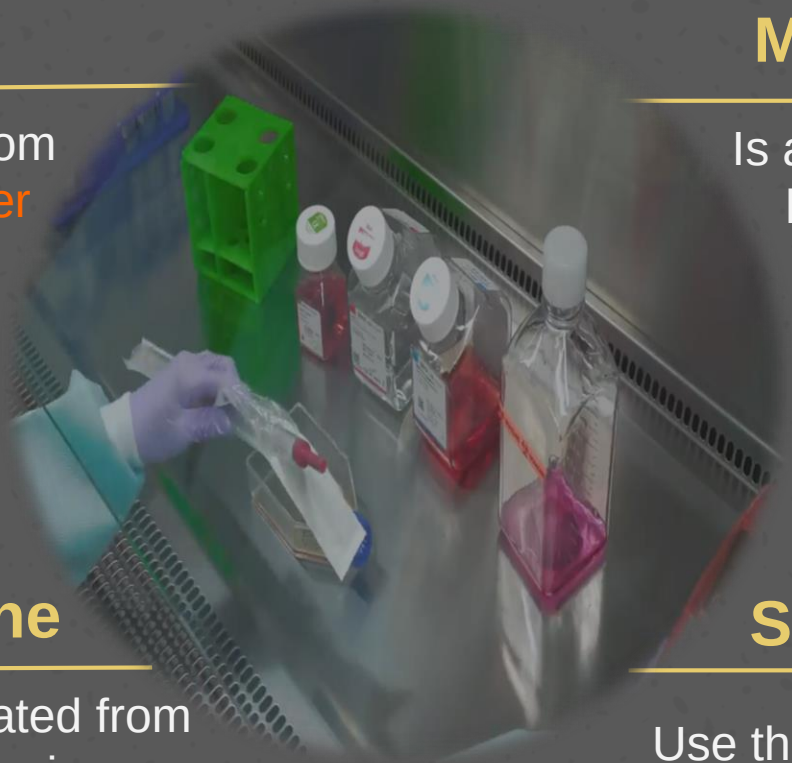
mPA –EGF

(mutated protective antigen- Epidermal Growth Factor)

8. Cell culture

T24 Cell Line

A cell line established from a human urinary bladder cancer patient.



MB49 Cell Line

Is a urothelial carcinoma cell line derived from a male mouse

BT- 474 Cell Line

Is a cell line that was isolated from a solid, invasive ductal carcinoma from a breast cancer patient

SK-BR-3 Cell Line

Use these cells in breast cancer research.

8. Cell culture

CHO-K1 Cell Line

Derived from epithelial cells of the ovary of the Chinese hamster, often used in medical research and in the production of recombinant therapeutic proteins

Canine primary bladder cancer cells

A431 Cell Line

Were established from an epidermoid carcinoma in the skin (epidermis) of an 85- year-old female patient.
(express abnormally high levels of the EGFR)

8. Cell culture

OE33 Human Cell Line

Was established from the adenocarcinoma of the lower esophagus of a 73 year old female patient.

HeLa cells

They were isolated in 1951 from a cervical carcinoma derived from a 31-year-old patient.

OE21 Human Cell Line

Was established in 1993 from a squamous carcinoma of mid esophagus of a 74 year-old male patient.

8. Cell culture

MDA-MB-468 Human Cell Line

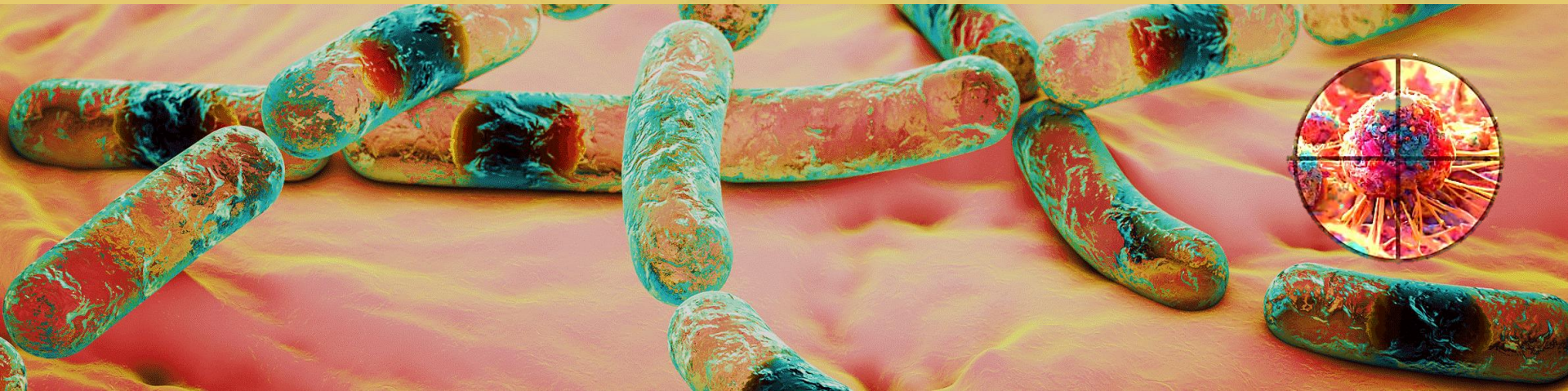
It was isolated from a pleural effusion of a 51-year-old Black female patient with metastatic adenocarcinoma of the breast.

MDA-MB-231 Human Cell Line

It is commonly used to model late-stage breast cancer

Anti-tumor activity *In-vivo* in dogs with bladder cancer

Studies results



Studies results

- They designed EGF-mPA/LFN-DTA toxin system.
- Specifically, human T24, mouse MB49 and cells isolated from several spontaneous canine tumors showed similar sensitivity to the LFN-DTA with a LC50 ranging from 0.21-0.54nM
- Their datas showed that exposures as short as ≈3min were enough to commit human (T24), mouse (MB49) and canine (primary) bladder cancer cells to apoptosis.

Int J Cancer. 2020 Jan 15;146(2):449-460. doi: 10.1002/ijc.32719. Epub 2019 Nov 1.

A novel, safe, fast and efficient treatment for Her2-positive and negative bladder cancer utilizing an EGF–anthrax toxin chimera

Sherwin Jack [1 2], Kayalvizhi Madhivanan [1 2], Swetha Ramadesikan [1 2], Sneha Subramanian [1 2], Daniel F Edwards 2nd [1 2], Bennett D Elzey [1 3 4], Deepika Dhawan [5], Andrew McCluskey [6], Erin M Kischuk [1 3], Alexander R Loftis [7], Nicholas Truex [7], Michael Santos [7], Mike Lu [7], Amy Rabideau [7], Bradley Pentelute [7 8 9 10], John Collier [6], Hristos Kaimakliotis [4], Michael Koch [4], Timothy L Ratliff [1 3], Deborah W Knapp [1 5], Ruben C Aguilar [1 2]

Affiliations + expand

PMID: 31584195 PMCID: PMC10303116 DOI: 10.1002/ijc.32719

IJC INTERNATIONAL JOURNAL of CANCER | UICC

in the United States

- ✓ Indeed, 6 dogs with terminal, treatment resistant bladder cancer exhibited consistent tumor mass reduction as a result of exposure to this EGF-toxin.

- ✓ As first step towards *In-vivo* studies, we tested the EGF-toxin for potential adverse effects in tumor-free animals. Specifically, the toxin was instilled into the bladder of control animals:

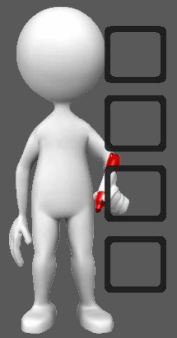
6 mice and 4 dogs. No toxicity was detected in the animals by any analysis made.

- ✓ The EGF-toxin was then instilled intravesically via the urinary catheter into the bladder at a concentration of 200nM EGF-mPA/400nM LFN-DTA (in 25-75 ml total volume depending on the size of the dog) and left for 1h.
- ✓ In all cases, dogs treated with the EGF-toxin had some reduction in tumor volume (even when multiple tumors were present) with an average of ~30% decrease in size after a single treatment cycle.

✓ Because of its *In-vitro* and *In-vivo* high efficiency, fast action (reducing treatment time from hours to minutes) and safety, They propose that this EGF-anthrax toxin conjugate provides the basis for new, transformative approaches against bladder cancer.

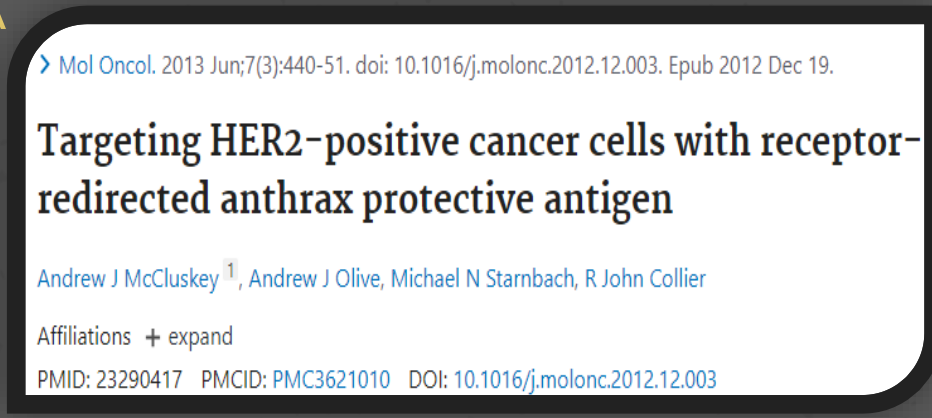
Conclusion:

- EGF-mPA/LFN-DTA induces apoptosis in these cell lines by inhibiting protein synthesis.
- Also, the reduction of bladder tumor growth in dogs with resistant bladder cancer was induced by using this protein fusion by inhibiting protein synthesis.



Studies results

- ✓ They designed mPA-ZHER2/LFN-DTA toxin system.
  - ✓ They tested the fusion protein on:
    1. BT-474 Cell Line
    2. SKBR3 and A431
    3. MDA-MB-231
    4. CHO-K1, MDA-MB-468



01

BT-474 Cell Line

Highest level of HER2r

most sensitive to mPA-ZHER2/LFN-DTA

required the lowest concentration (EC50) of LFN-DTA for 50% inhibition of protein synthesis.

02

SKBR3 & A431

Moderate levels of HER2r

showed intermediate levels of sensitivity

03

MDA-MB-231 Cell Line

low level of HER2r

low sensitivity to mPA-ZHER2/LFN-DTA

04

CHO-K1 & MDA-MB-468

No detectable HER2r

were unaffected, even at the highest concentrations of LFN-DTA tested.

❖ **Thus, EC50 was inversely related to the level of HER2r on the cell surface**

- ✓ Also, the resulting fusion protein (mPA-ZHER2) delivered cytocidal effectors specifically into HER2r-positive tumor cells, including a Trastuzumab-resistant line, causing death of the cells.
- ✓ No off-target killing of HER2r-negative cells was observed, either with homogeneous populations or with mixtures of HER2-positive and HER2-negative cells.

Conclusion:

- The most effective induction of apoptosis by mPA-ZHER2/LFN-DTA is through inhibition of protein synthesis in BT-474 cell line.
- We can use mPA-ZHER2/LFN-DTA against Trastuzumab-resistant cell line that express HER2r

Studies results

- ✓ They designed EGF-mPA/LFN-DTA toxin system.
  - ✓ They tested the fusion protein on:
    1. A431 cells
    2. CHO-K1 cells

in the United States

- ✓ A431 cells, which express high levels of the EGF receptor (EGFR), were killed by LFN-DTA (50% effective concentration [EC50] of ~10 pM) in the presence of mPA-EGF due to inhibition of protein synthesis, whereas CHO-K1 cells, which do not express the EGF receptor, were not killed

Conclusion:

EGF-mPA/LFN-DTA can induce apoptosis in A431 cell line.

Studies results

- ✓ They designed cell specific cell rounding induced by LFN-C3 mediated by different PA transporters

- ✓ They tested the fusion protein on:
1. HeLa cells
2. OE21 cells
(Express high amounts of EGF receptors & little HER2)
3. OE33 cells
(Express high amounts of HER2 & a small amount of EGFR)

Targeted delivery of an ADP-ribosylating bacterial toxin into cancer cells

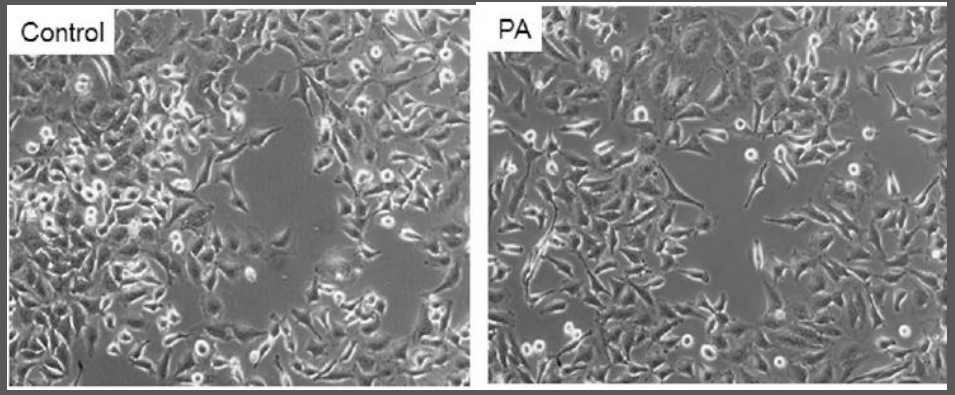
N.-I. Zahaf, A. E. Lang, L. Kaiser, C. D. Fichter, S. Lassmann, A. McCluskey, A. Augspach, K. Aktories & G. Schmidt

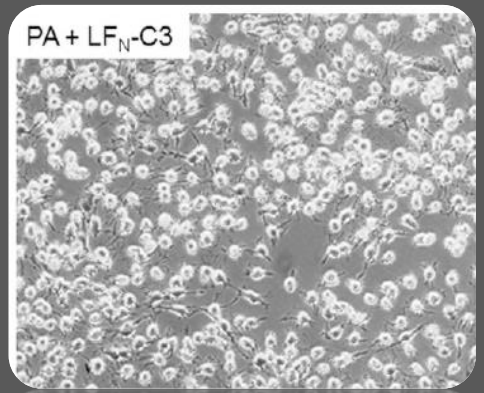
Scientific Reports **7**, Article number: 41252 (2017) | Cite this article

3280 Accesses | **19** Citations | **7** Altmetric | Metrics

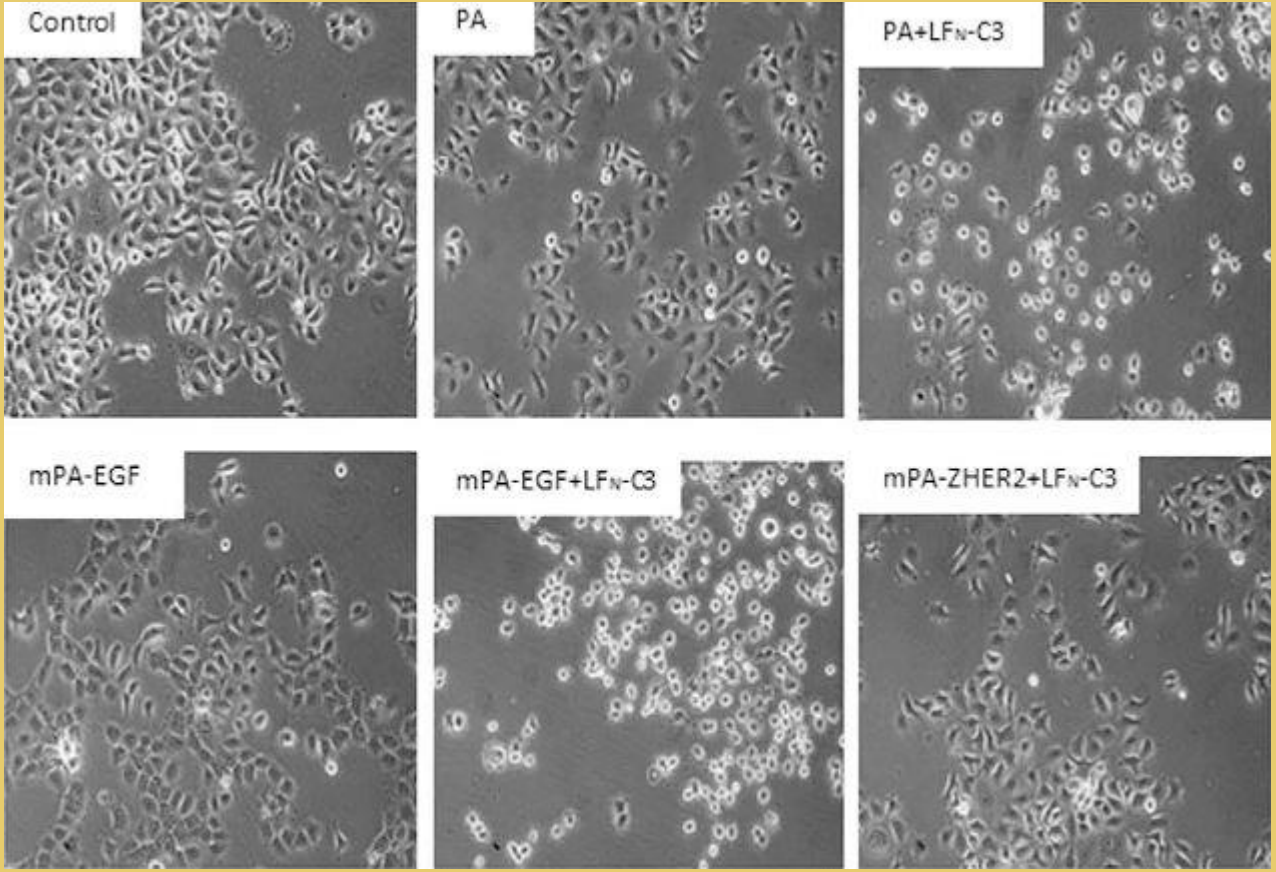
nature SCIENTIFIC REPORTS

in the Germany



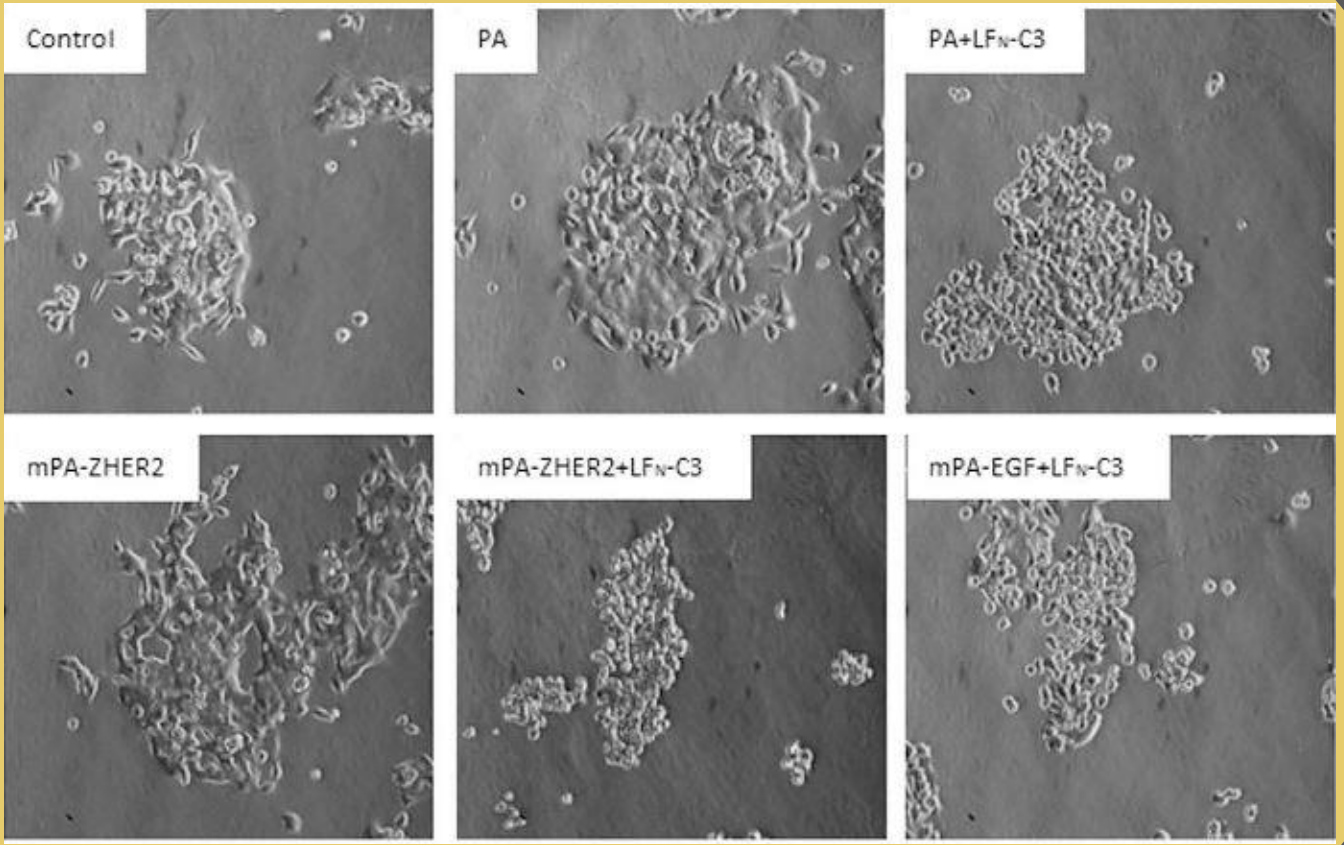


- ✓ HeLa cells treated with PA together with the fusion toxin rounded up, showing that the fusion toxin is transported through the PA pore and is active in the cells.
- ✓ In contrast, the pore-forming PA alone alone does change cellular morphology.



OE21 Cell Line

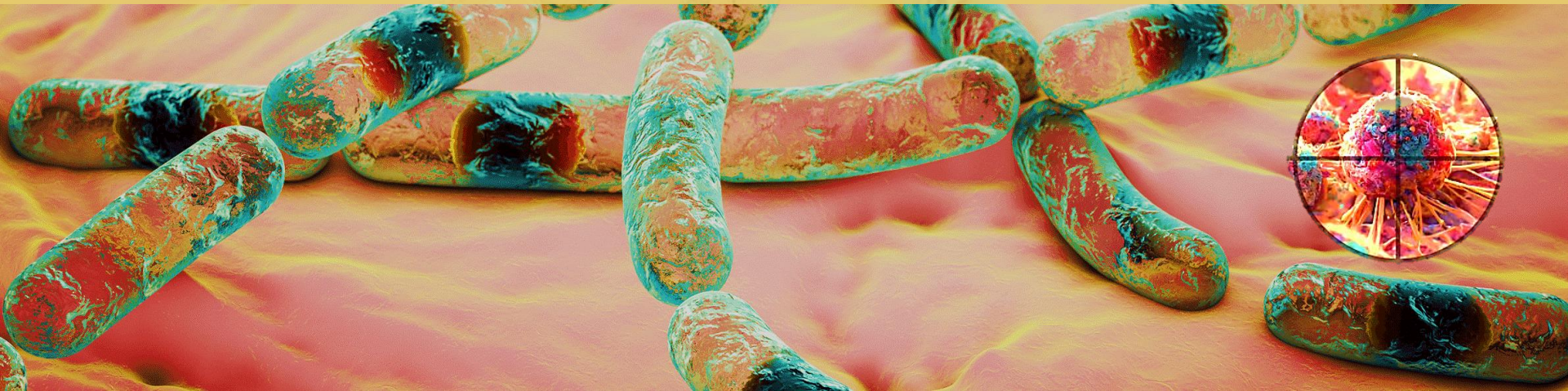
Conclusion:
The best protein fusion for transfecting LFn-c3 into OE21 cells and observing apoptosis is mPA-EGF.



OE33 Cell Line

Conclusion:
The best protein fusion for transfecting LFn-c3 into OE33 cells and observing apoptosis is mPA-ZHER2.

Advantages & Limitations



Advantages

1. In some studies, it has been mentioned that cancer cells resistant to common treatments can be overcome by this fusion of proteins.
   (e.g. bladder & breast cancer)

2. Specify of therapy

Limitations

01

AT is a bacterial toxin

↓

Contains many immunogenic epitopes on its surface

↓

An investigation to determine and mutate these antigens needs to be done in order to limit the immunogenicity of AT

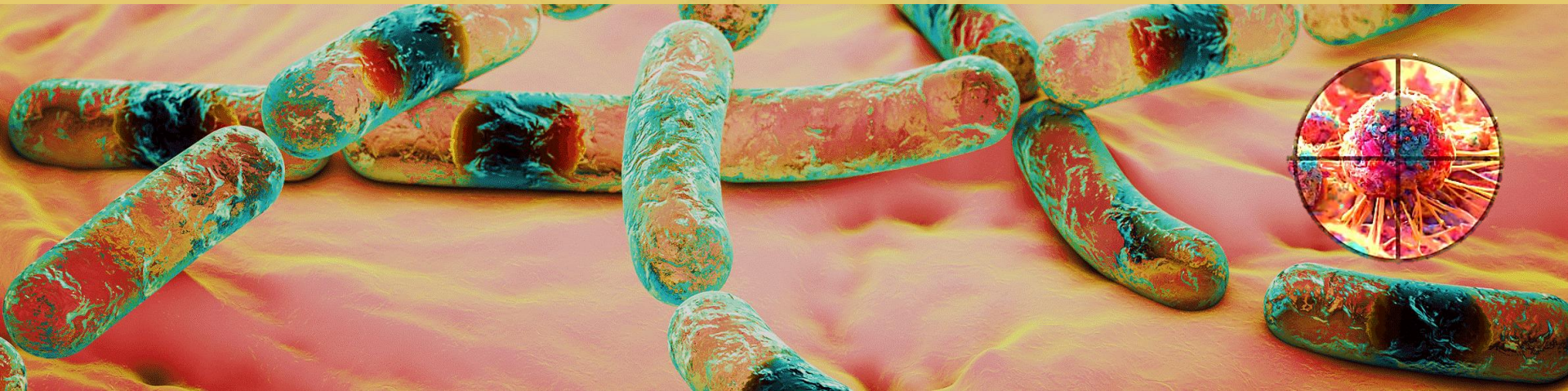
Limitations

02

The size of delivered fusion proteins varies from a few amino acids to proteins of 100 kDa

Bulky effectors and highly stable proteins will not be delivered effectively

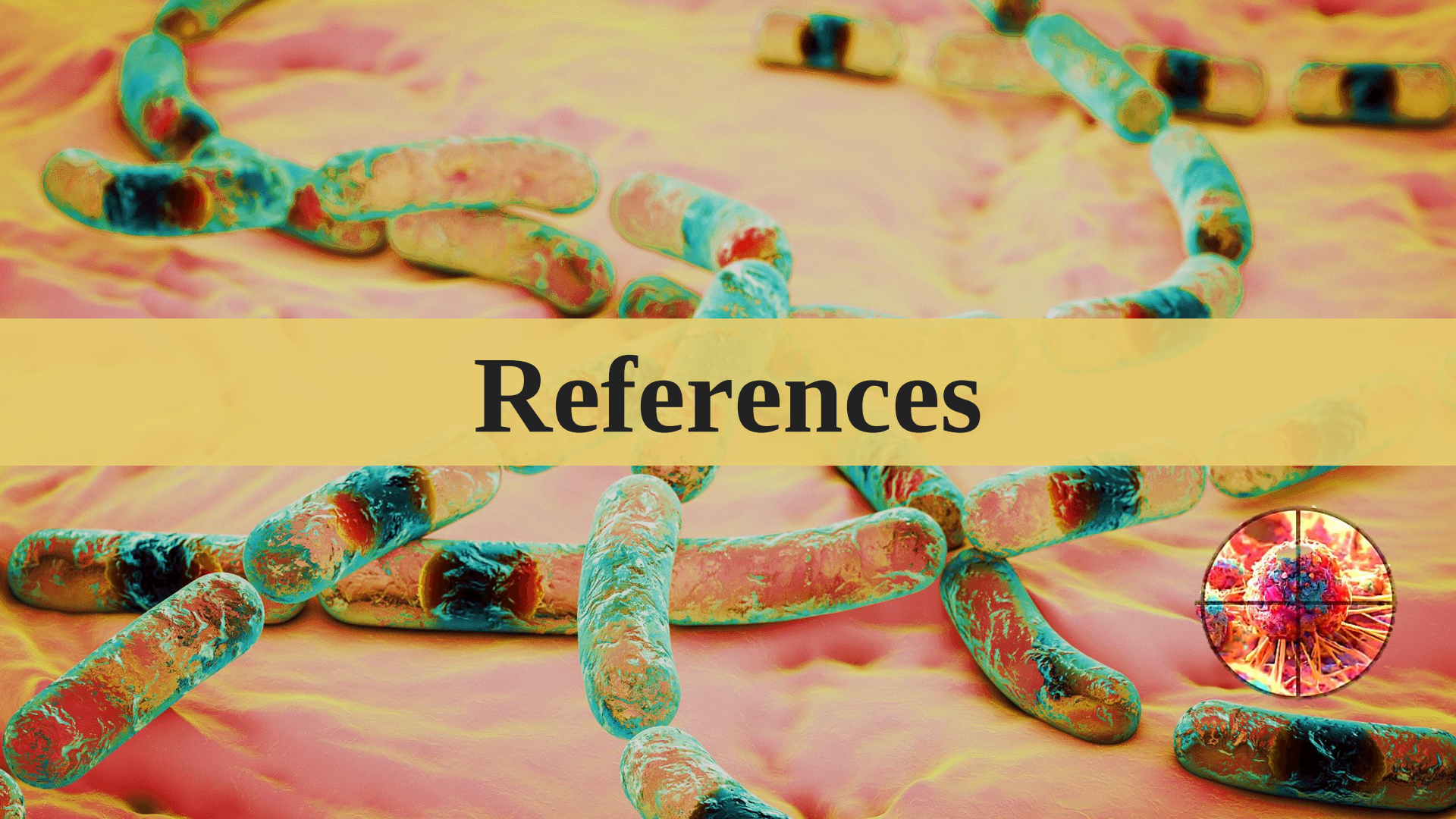
Conclusion



Conclusion

- Altogether, the idea of using bacterial toxins for tumor therapy is still attractive but it needs further creative work for better success.

- According to the results of research conducted in the laboratory, on different types of cancer cells, which indicate the effectiveness of the death of these cells by anthrax poison, as well as the challenges mentioned in the treatment of cancer by traditional methods, we hope that in the not so distant future far from using this technique to treat human cancer.



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I wish you life without any pathogens…

Thanks for your attention…