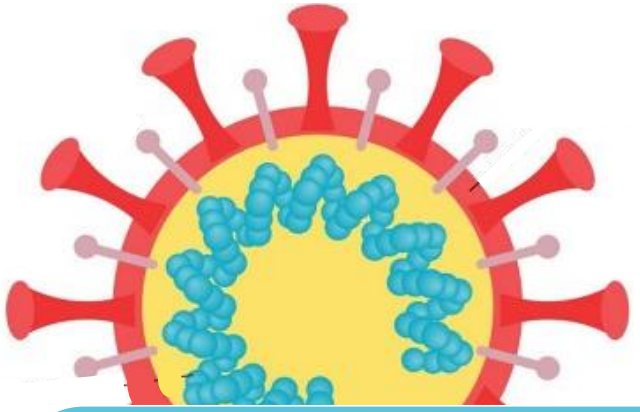
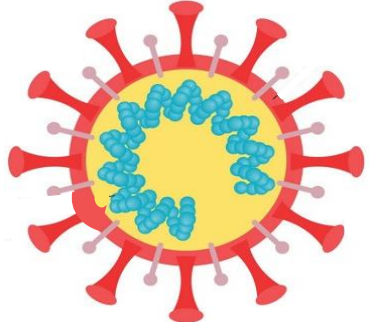




Addendum 4a Viruses

How To Defeat Viruses

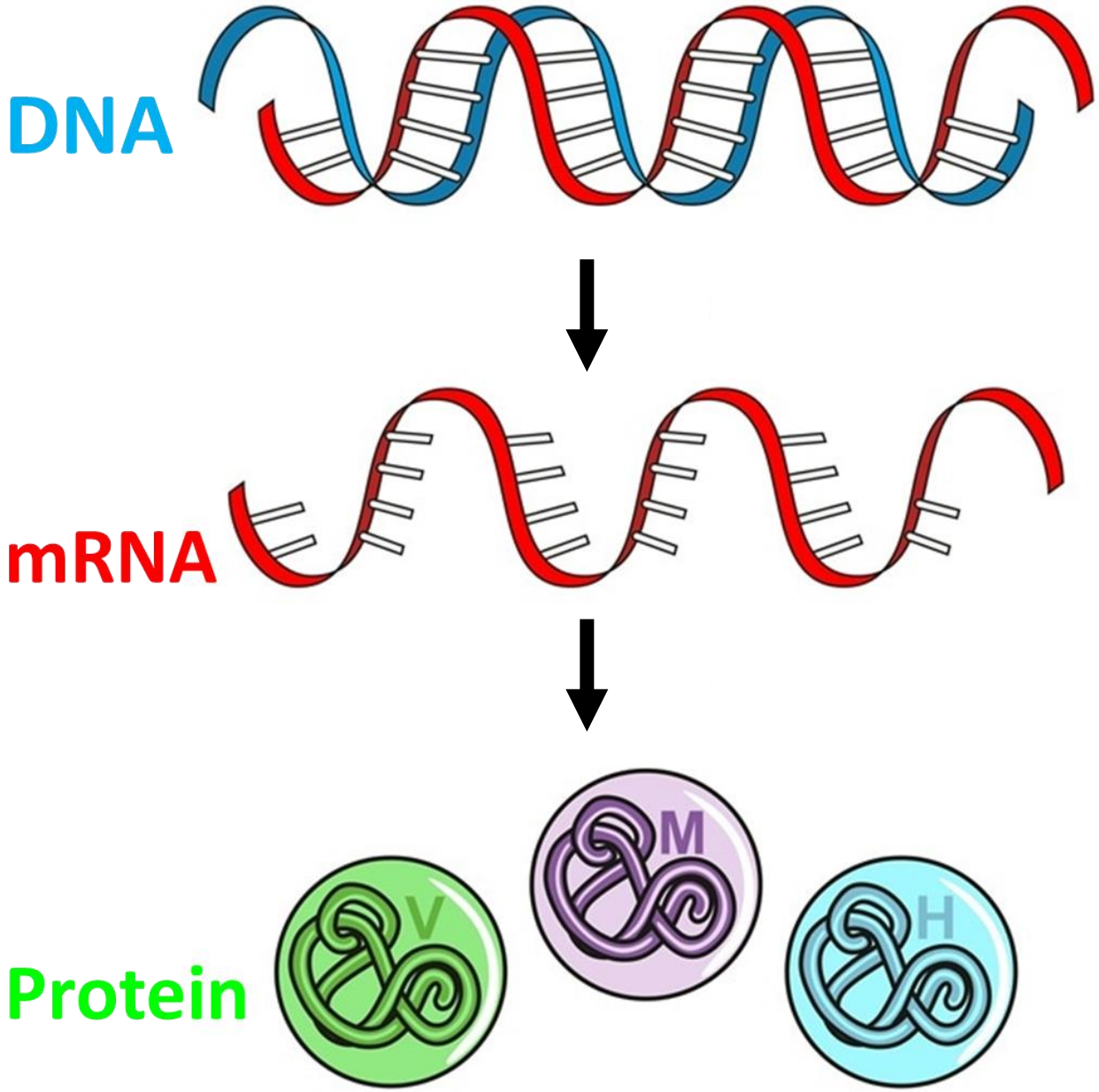
Presenter:

Dr. James Summerton Ph.D.

30 November 2022

This presentation was given to the Academy of Lifelong Learning at Oregon State University. Please see page 36 for the presentation on LK-1

Information flow in Cells

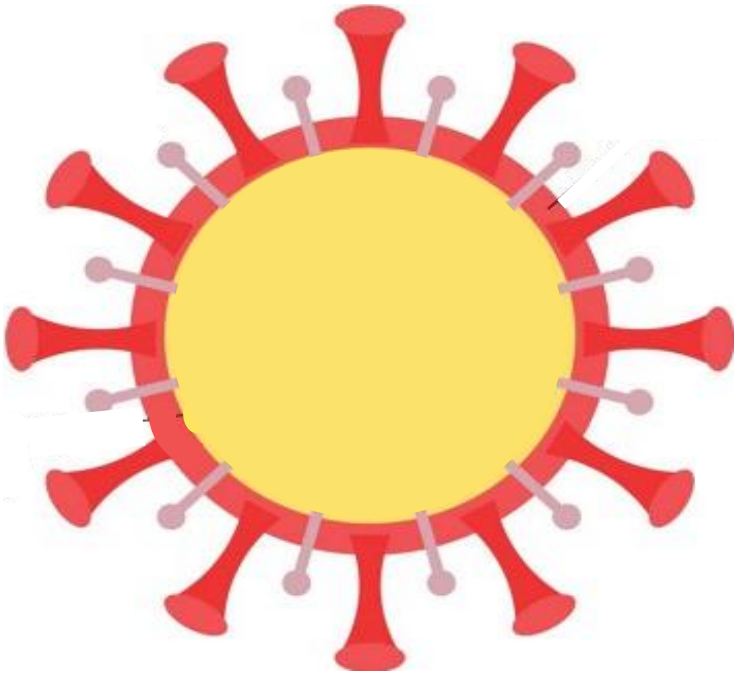


How to defeat COVID-19 and any other viruses

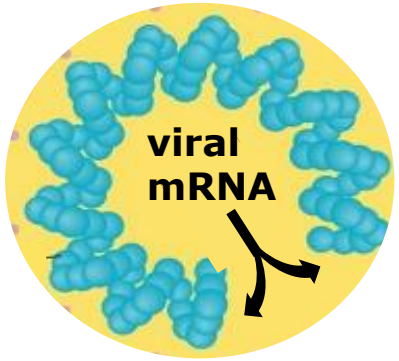
1) Select a suitable viral target

2) Develop a safe, effective and affordable therapeutic to prevent function of that target

The outer structures of COVID-19 serve to carry the viral payload **(**mRNA**)** from an infected cell to a new cell of the host, or to a new host.

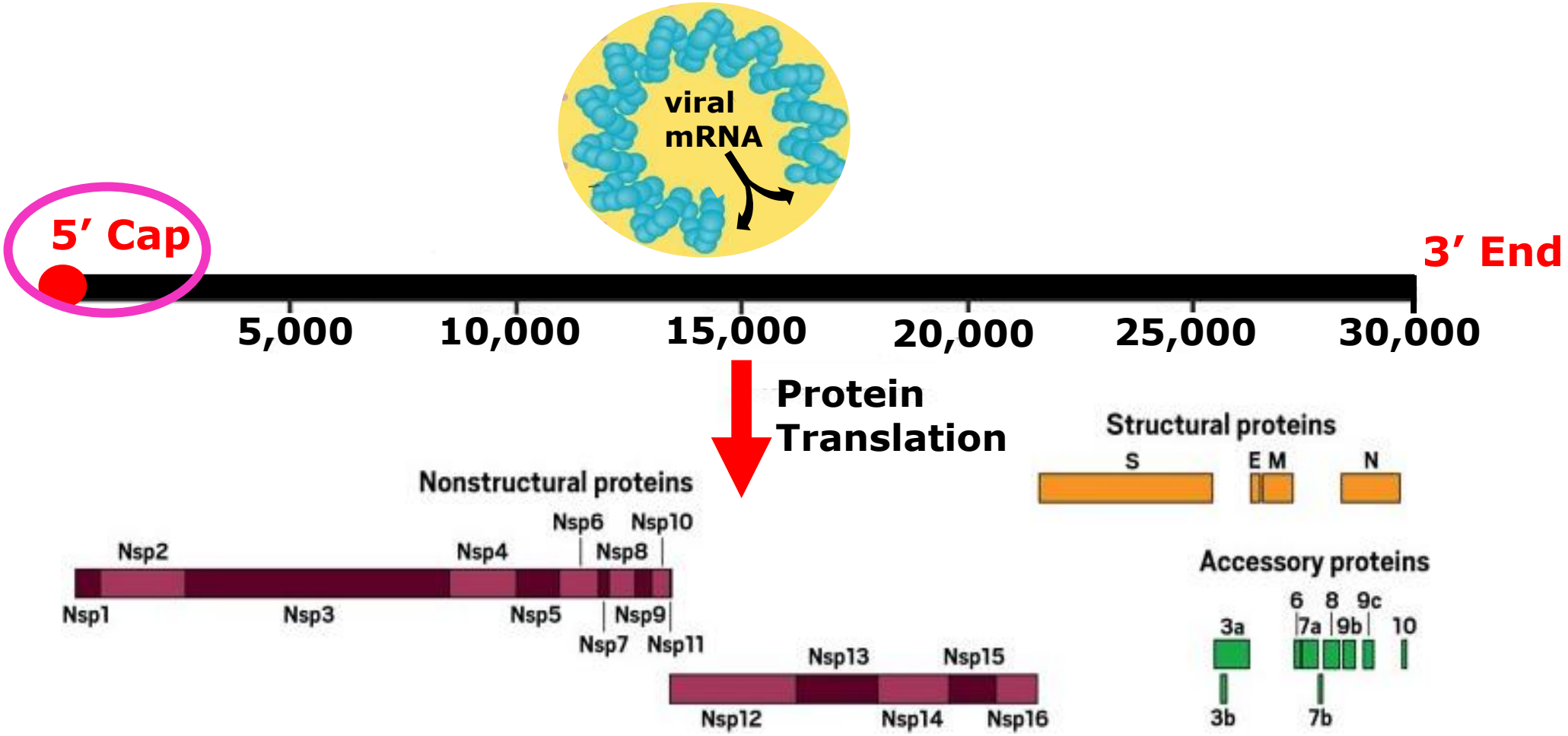


The payload **(**viral mRNA**)** comprises the genetic information for making **all** 29 viral proteins for viral function and reproduction



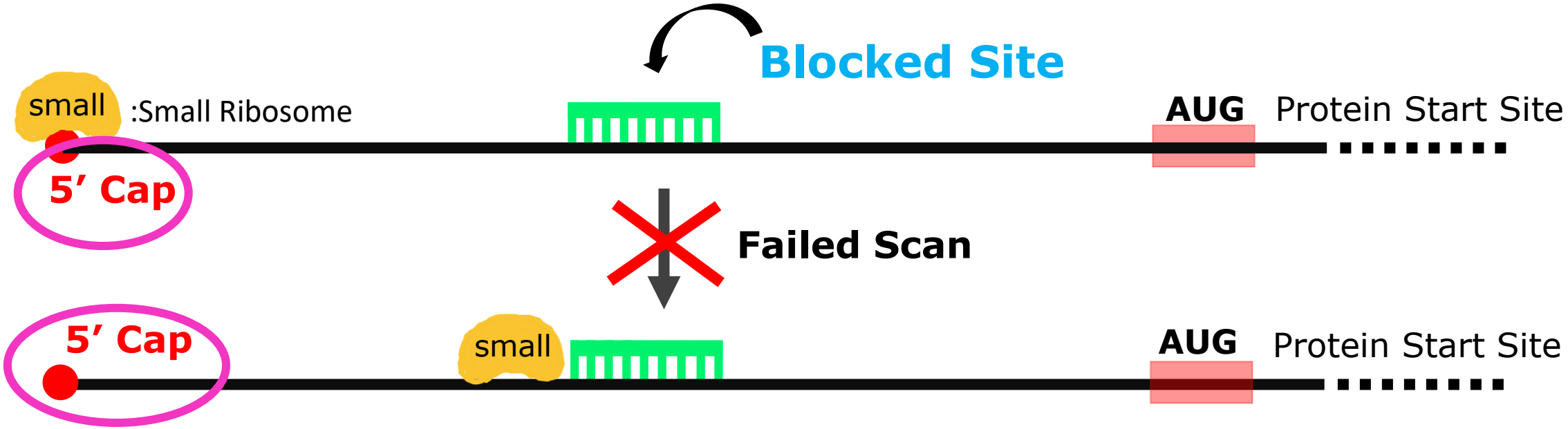
The 29 COVID-19 Proteins

The very-long viral mRNA (30,000 genetic letters) uses the cell's own protein synthesis machinery to make the virus' 29 proteins.



Targeting a Special Region:
5’ cap to AUG start site for protein synthesis

Effective blocking of a site between the 5’ cap site and AUG start site can prevent **ALL** function and reproduction of that COVID-19 mRNA.



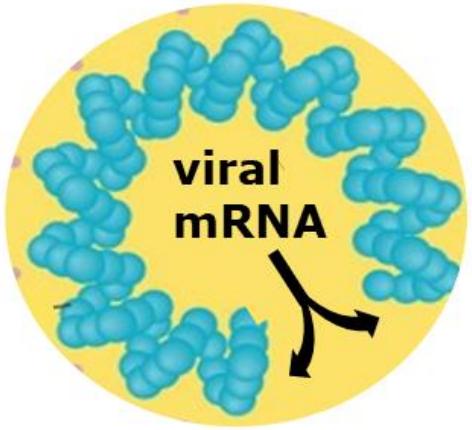
The virus remains **completely** nonfunctional as long as the blockage remains in place

Benefits of Targeting the Special Region

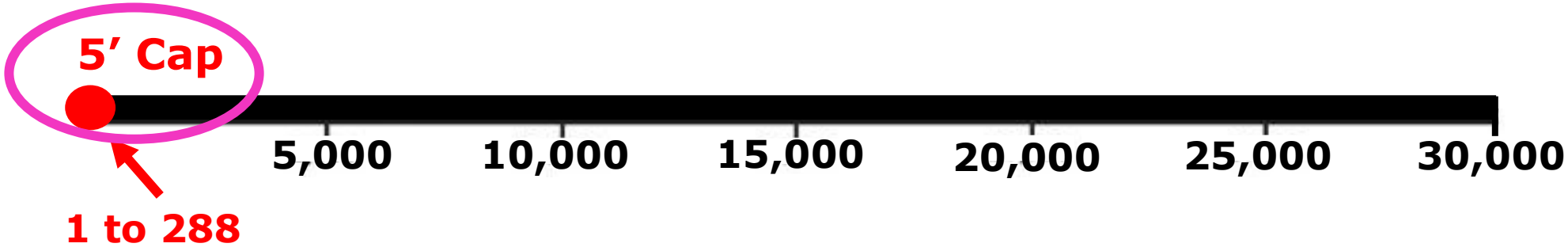
- Prevents synthesis of all 29 covid-19 proteins
- Prevents replication of entire covid-19 virus
- Sequence in special region is well conserved over decades

To Summarize

A) Select a suitable viral target. To date, we have settled on targeting the 30,000-genetic-letter viral messenger RNA (mRNA).



B) Target selection was further narrowed to the special region starting at the 5’ cap and ending at genetic letter 288



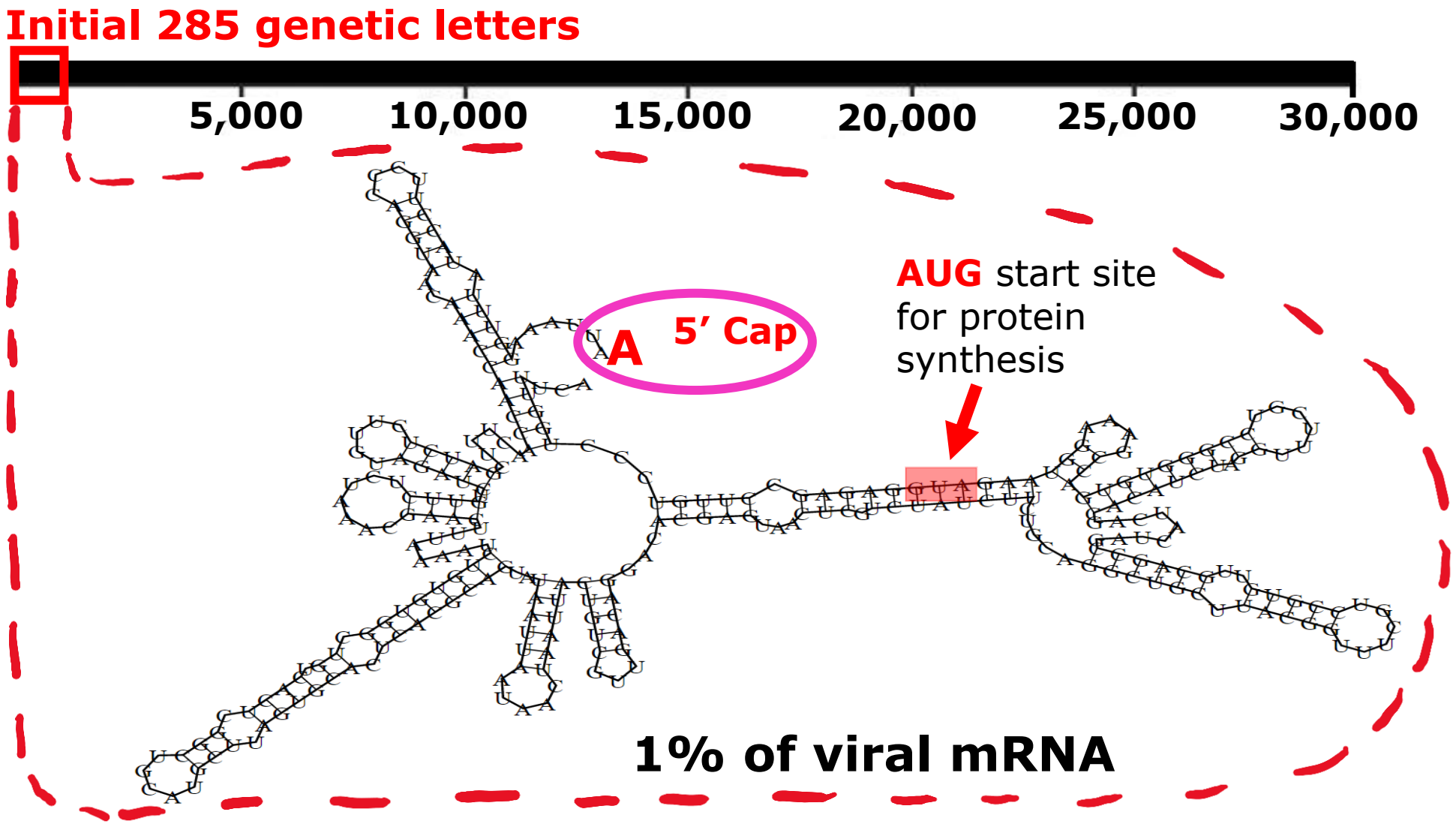
Optimal Target Region:

5’ Cap to AUG start site

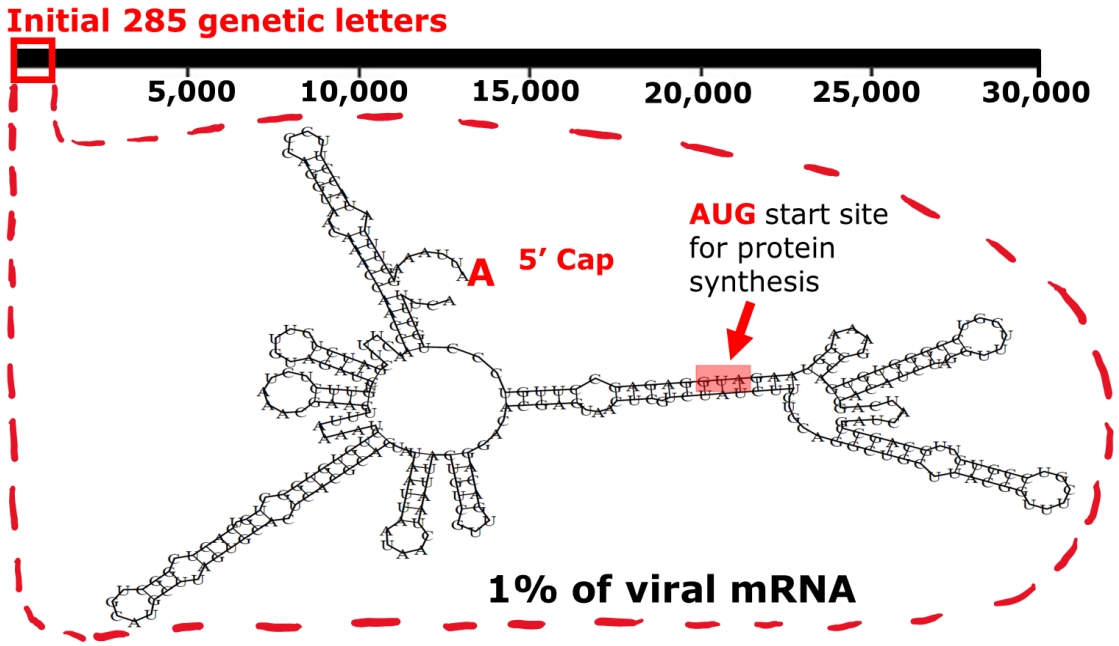
Simplified Optimal Target Region:

~1% of viral mRNA

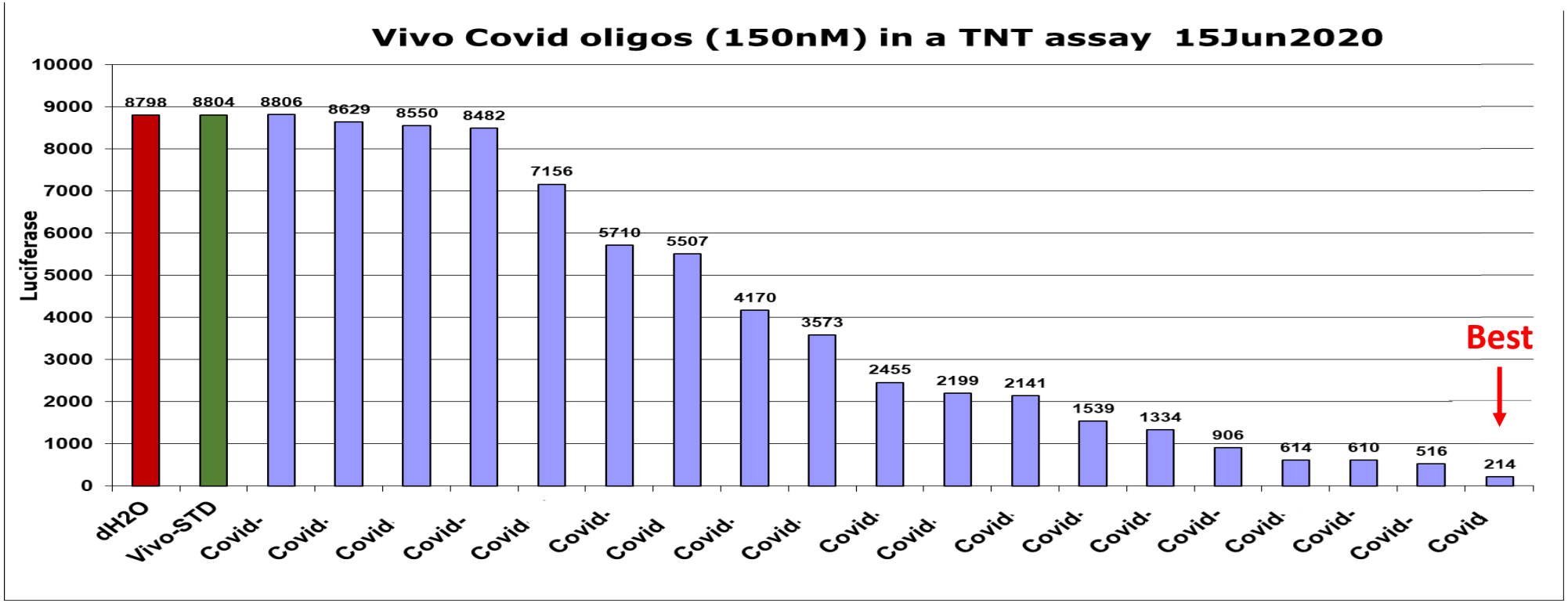
Actual Optimal Target Region



All Targetable Sites Are NOT Equal



Preliminary target blockage results



How To Defeat COVID-19

1. Select a suitable covid-19 target
2. Develop a safe, effective, affordable therapeutic to block the function of that selected covid-19 target

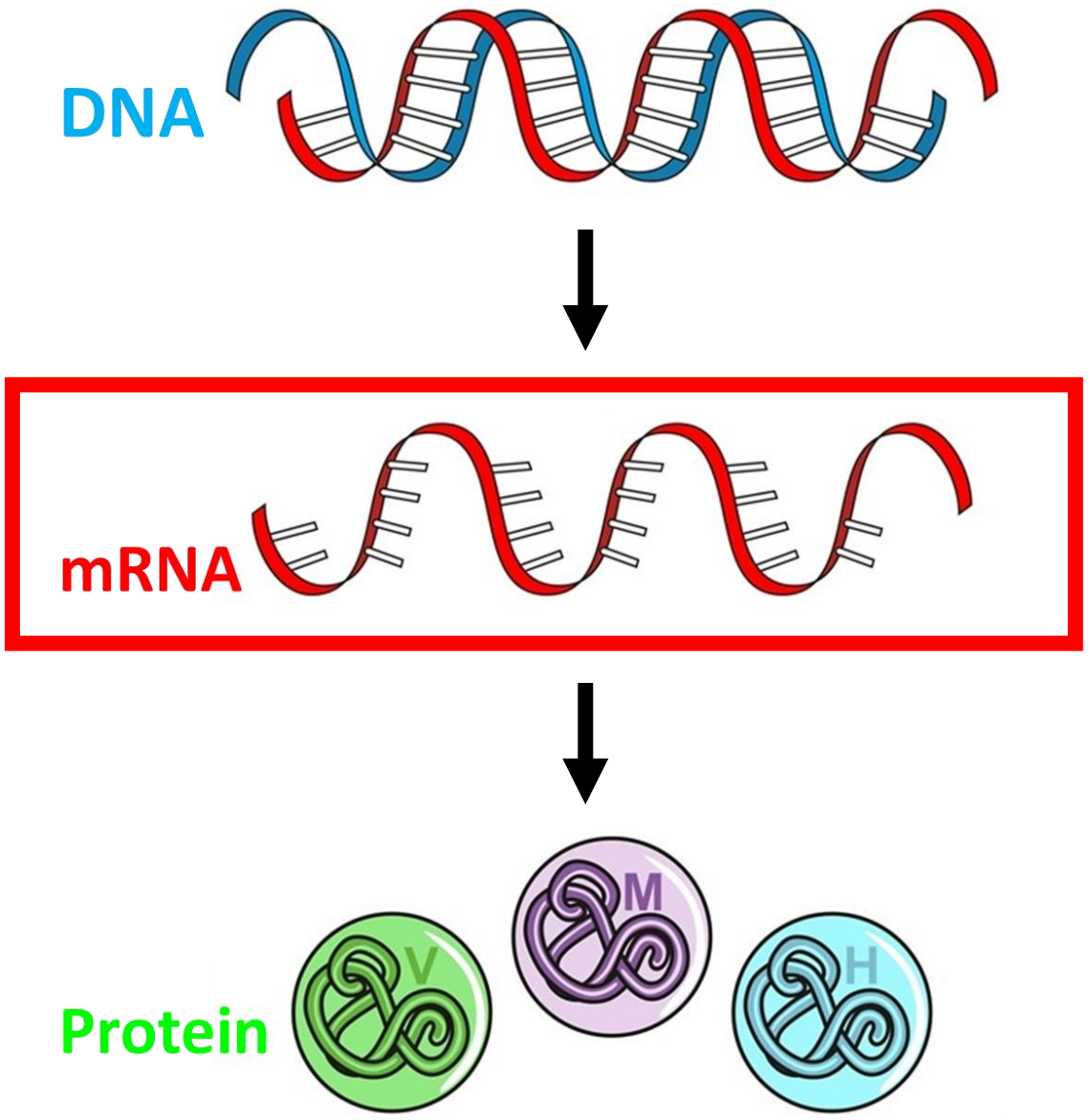
Structural design of covid-19 therapeutic

Morpholino … **Delivery Component**

Precision blocking agent for selected RNA target

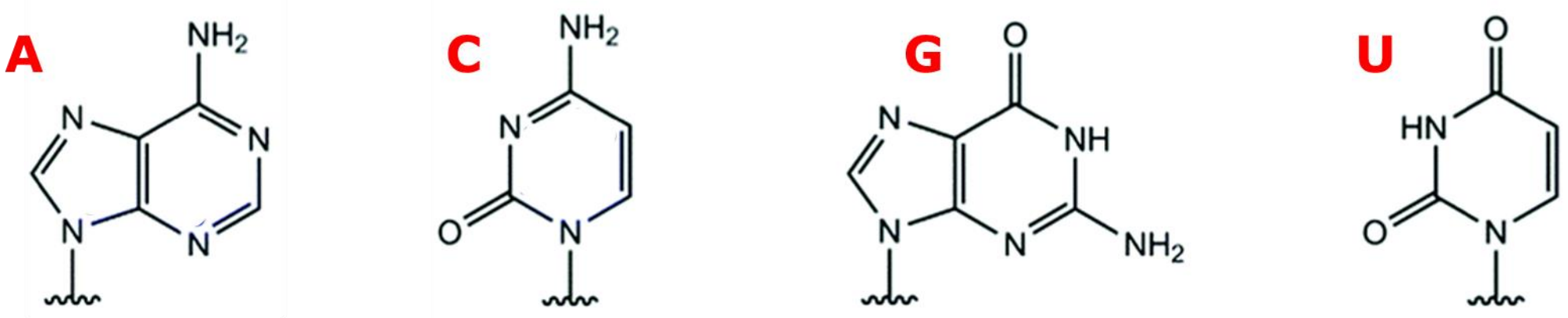
To deliver Morpholino from the blood to the cytosol of cells

Information flow in Cells



Brief introduction to messenger RNA (mRNA)

An mRNA, such as the one in covid-19, is made up of a long string of 4 genetic letters **(A, C, G, U)** in a specific sequence unique to the particular mRNA



The sequence of genetic letters in an mRNA can be read by a ribosome **(the cell's protein synthesis machine)** to assemble a corresponding protein that serves to carry out one or more functions in the body.

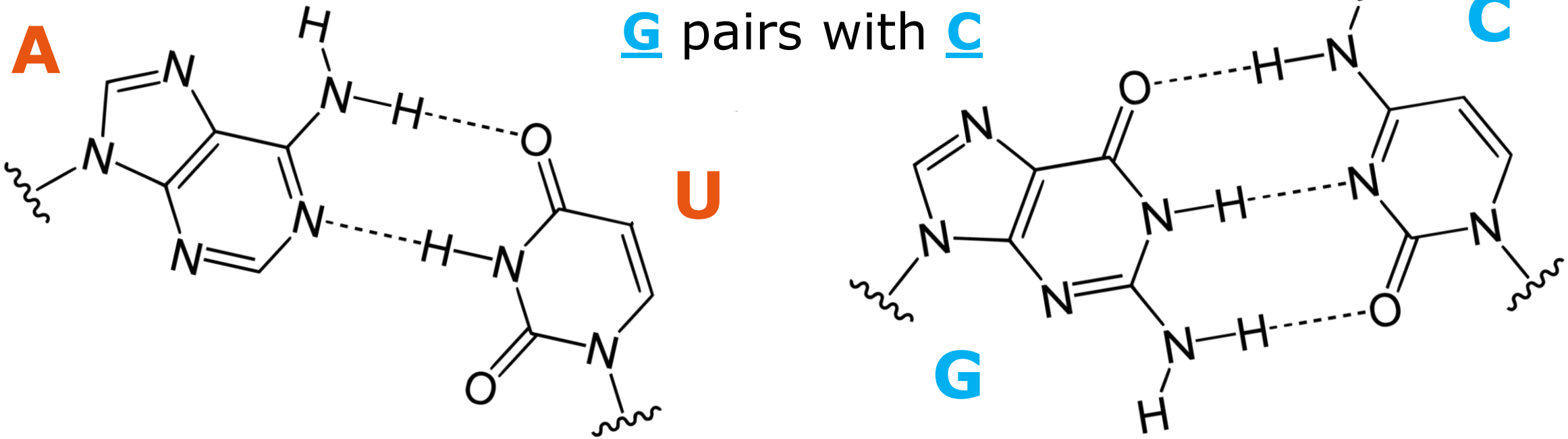
If the mRNA is from a **virus** the resultant protein(s) can instead carry out one or more nefarious functions for the invading virus.

Watson/Crick Pairing:
A key interaction for all life as we know it

As noted earlier, a messenger RNA (mRNA) that codes for a specific protein is composed of a long sequence of 4 genetic letters. Those genetic letters have the special property of complementarity wherein:

A pairs with **U**

G pairs with **C**



Antisense Therapeutics Strategy : Block a selected sequence in RNA

In principle, specific destruction or long-term blocking of a selected mRNA can alter, halt or cure a wide variety of diseases and conditions.

An established technology for very specifically inactivating or altering a selected mRNA is commonly referred to as the **“antisense therapeutics strategy.”**

Antisense Therapeutics

The antisense therapeutics strategy exploits this Watson/Crick pairing property to afford simple and efficient design and production of high-specificity **antisense** blocking agents for selectively blocking any of a wide variety of mRNA sequences (called **sense** strands because they carry the genetic information for making proteins).

Very-long RNA target sequence **(sense strand):**

….GUGACCGAA**AGGUAAGAUGGAGAGC**CUUGUCCCU…

UCCAUUCUACCUCUCG

Short blocking Agent **(antisense strand)**

Evolution of The Antisense Therapeutics Field

1. Initial **conceptions** of antisense strategy: **1967 - 1969**
2. First **papers focused on antisense therapeutics strategy: 1978**

Researchers	Work Done At:
Summerton & Bartlett	Berkeley
Zamecnik & Stephenson	Harvard

3. First **patent** issued in antisense field: **1978**

   (Summerton & Bartlett, US Patent 4,123,610)

4. First **company** founded to pursue development of antisense therapeutics : ANTIVIRALS, Inc., founded by Summerton in **1980** (In 2012 company was renamed Sarepta)
5. By the **mid-1980s** the antisense therapeutics field was becoming wildly popular, with a great many researchers, granting agencies, and pharmaceutical companies becoming heavily involved.
6. In the **late-1980s** venture capitalists became involved by funding start ups of 4 more antisense companies in **1987** to **1989** : Gilead, Genta, Hybridon, and lastly, Isis (recently renamed Ionis)

Many Technical Challenges

In the **1980s** it was becoming clear there were many technical challenges to overcome before the seemingly-simple antisense therapeutics strategy could provide treatments for the diseases and conditions for which that strategy appeared to hold promise

Major problems in the antisense field

While there have been many problems to solve in the antisense field, the most obvious first problems were :

a) How to **avoid rapid degradation** of short DNA (or RNA) antisense oligos.

b) How to achieve **highly-efficient inhibition** of the targeted mRNAs.

c) How to achieve **highly-specific inhibition** of the targeted mRNAs.

d) How to **deliver** the large, polar antisense oligos into the proper subcellular compartment of the patient’s cells.

Problems, problems, and more problems

By the **mid–1980s**, the **antisense therapeutics field** somewhat resembled the arcade game : **“Whack-a-mole”**

In Whack-a-mole the problem is a toy mole that pops up. Your solution to that problem is to whack that toy mole with a mallet, causing it to disappear. But when that mole disappears one or more other moles pop up. When you whack those moles other moles pop up.

It seemed in the **1980s** antisense therapeutics followed a similar path – when one problem was fixed, that fix generated new problems.

Progress at Last ?

During the **1980s** dozens of antisense structural types were made and tested – but it proved very difficult to devise a single structural type that solved most or all the most obvious problems deterring development of antisense agents suitable for use in patients.

However, in the **mid–1980s** a research group led by Cohen at the US National Institutes of Health **(NIH)**, in collaboration with a research group led by Zon at the US Food and Drug Admin. **(FDA)**, developed **Phosphorothioate–linked DNA oligos (S–DNA)** which showed substantial promise for antisense applications.

Solutions – but with problems

The new S-DNA structural type was quickly and widely adopted by much of the antisense research community. This was because **S-DNAs** offer **:**

A) **Moderate resistance to degradation –** because their pendant sulfur on each intersubunit link slows attack by degradative enzymes;

B) **Excellent efficiency** in inhibiting their targeted mRNA – because the paired mRNA**/**S-DNA duplex is rapidly cleaved by the cell's RNase H;

C) a rather **limited level of specificity** for their targeted mRNA – limited because only a low amount of sequence information is recognized by the RNase H (only 6 or 7 base**–**pairs of mRNA**/**S-DNA duplex); and,

D) A **ready–made delivery** capability **–** because delivery systems developed for transfecting DNA into cells can be used to deliver S-DNAs.

Fundamental flaws in S–DNA antisense oligos

1. Why S-DNAs are plagued with so many off–target effects (causing undesired biological effects)

The pendant sulfurs on the intersubunit linkages of **S-DNAs** strongly interact with a wide variety of proteins – which leads to a host of serious off–target effects that have been extensively documented starting in the **1990s**

The Solution : Morpholinos

Not just a simple substitution of a sulfur for an oxygen

But instead

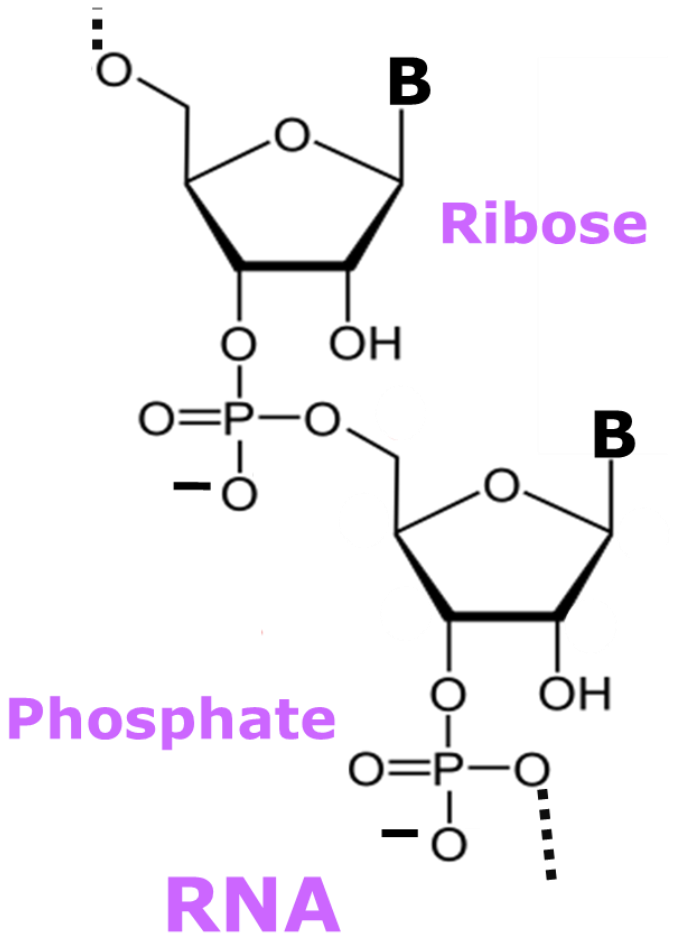
A radical re-design of genetic material

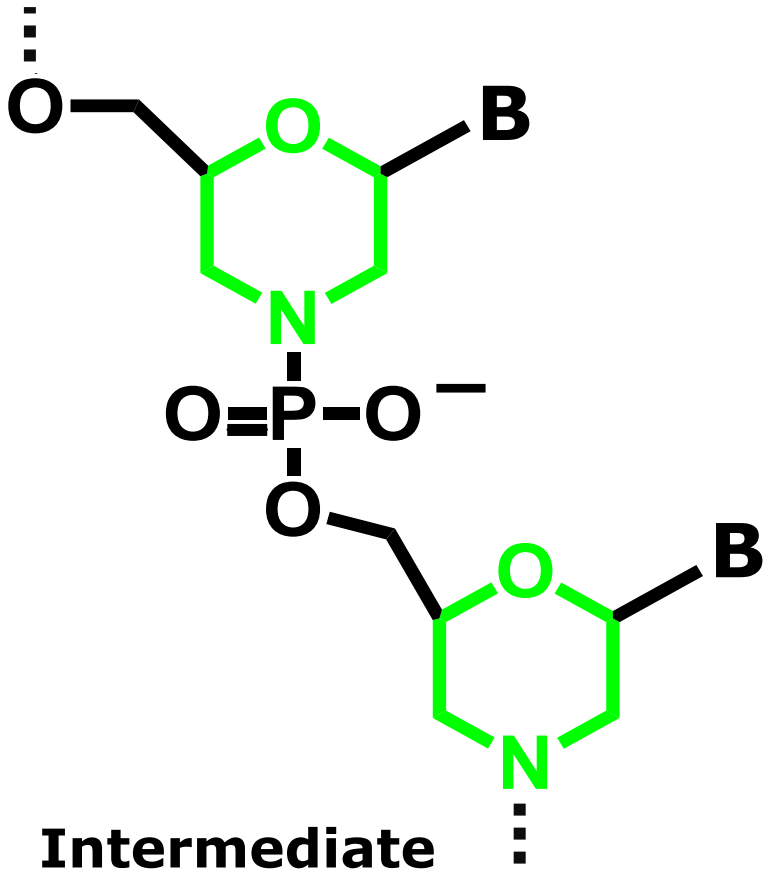
Radical Redesign of Genetic Material

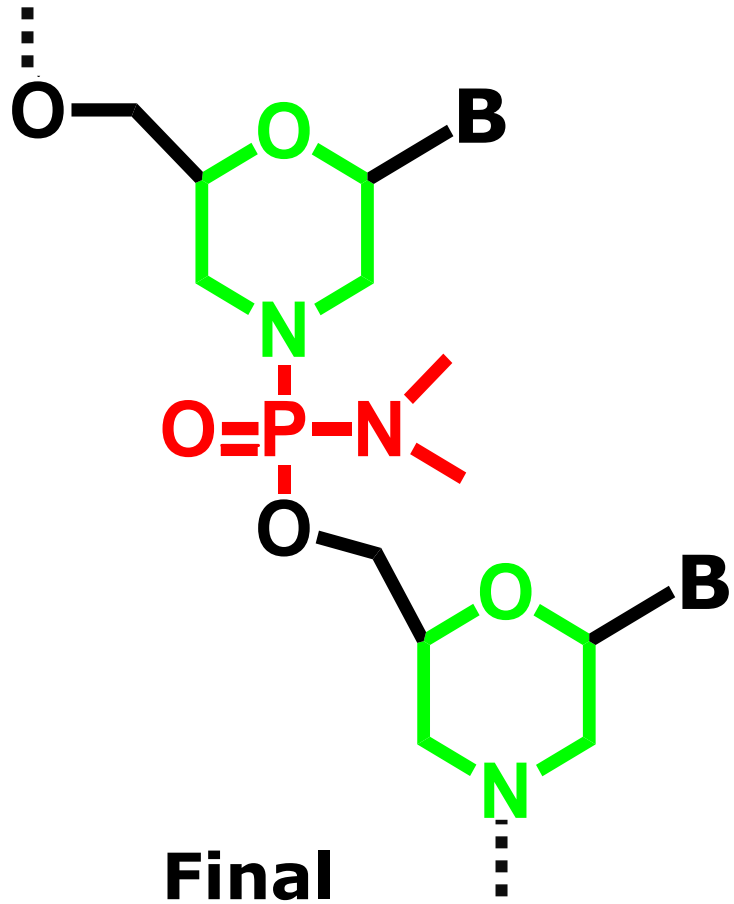
Start: 1985

B (Base): A, C, G, U

Finished: 1989





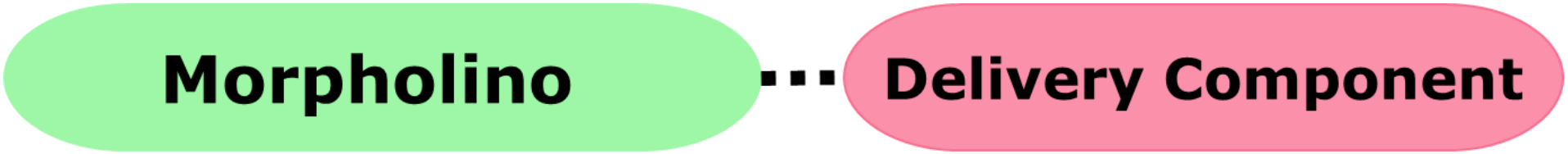


Functional Properties of "Magic Morpholinos"

1. **Complete resistance to degradation** in biological systems
2. **Greatest sequence specificity** of all antisense structural types
3. **Highly effective** without need for RNase H or RISC
4. **Little or no non-antisense activity**
5. **Predictable targeting**
6. Freely passes between cytosol and nucleus, and functions in both
7. Versatile : a) alter splicing in the nucleus
   b) block translation in cytosol
   c) and much more
8. Good aqueous solubility
9. Affordable : a) cheap starting materials
   b) efficient assembly
   c) easy workup

Morpholinos achieve these outstanding properties by virtue of their novel 6-membered morpholine backbone moieties joined by uncharged intersubunit linkages. These provide major advantages over the 5-membered ribose/deoxyribose moieties and negatively-charged intersubunit linkages characteristic of natural nucleic acids.

Our Therapeutic Designed For Preventing and Curing COVID-19



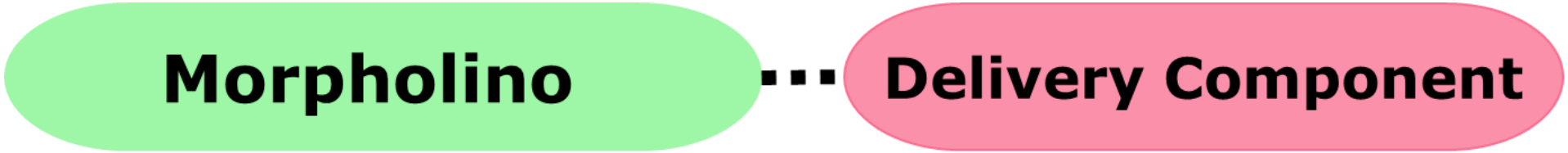
Precision blocking agent for selected RNA target

To deliver Morpholino from the blood to the cytosol of cells

1985 – 1993

1993 – 2022

Therapeutic Applications

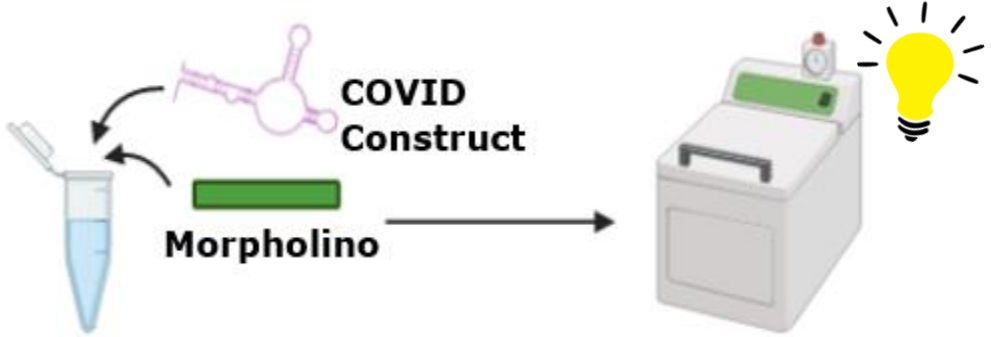


By the year 2022

- Over **200,000 custom–sequence** **Morpholinos** have been designed and produced for researchers around the world

- Over **11,000 scientific publications** have described research with **Morpholinos** (Tabulated at : gene-tools.com)

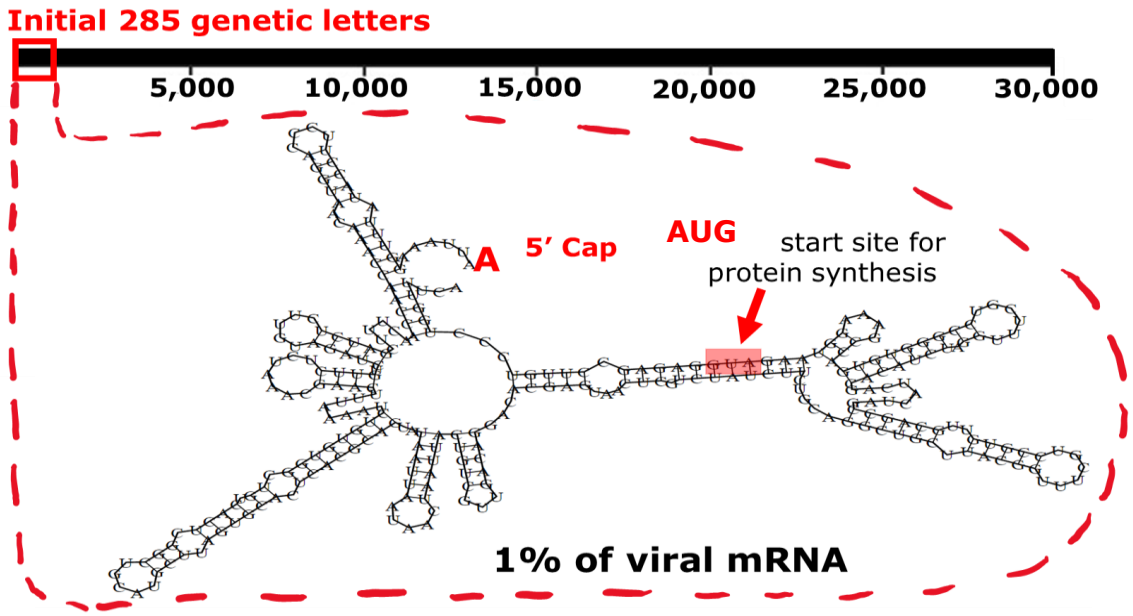
- **3 FDA – approved** **Morpholino** therapeutics are being used to treat muscular dystrophy patients – with more nearing FDA approval.



Cell Free TNT Luciferase Assay
To Determine Best Target Sequence

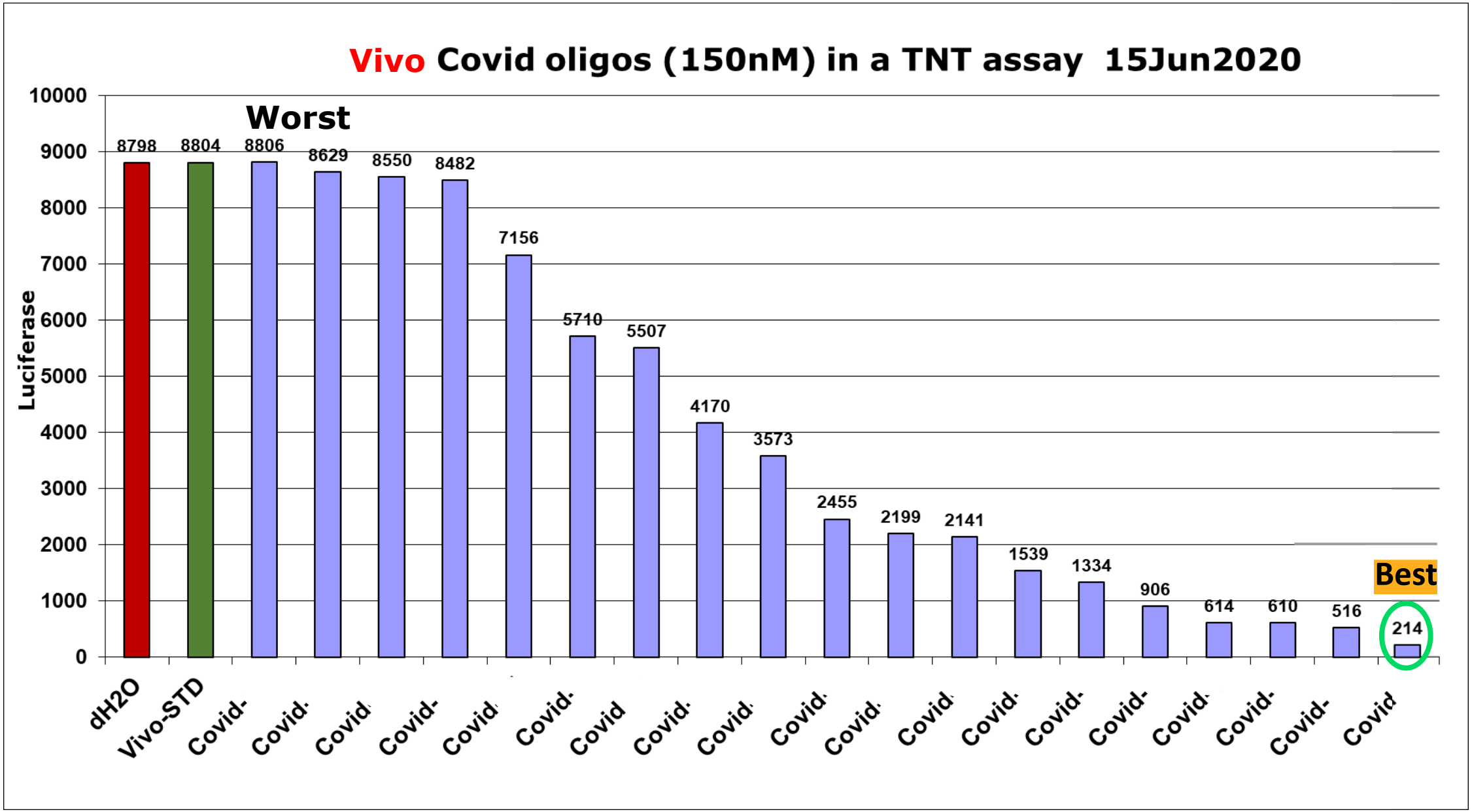
Cell Free Assay

Purpose: To determine the **BEST** targeted sequence for effective translating and blocking of Covid-19 virus.



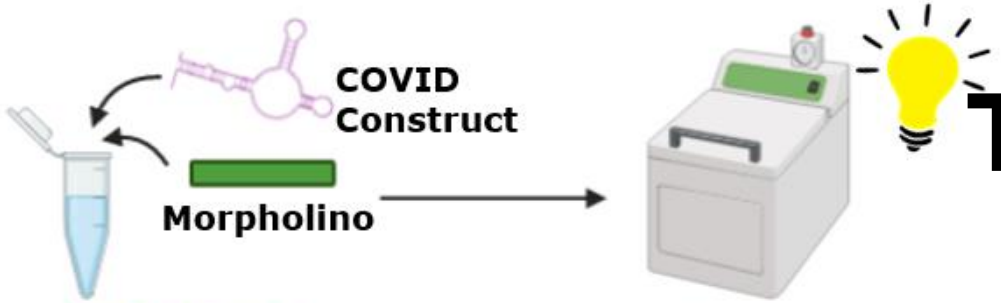
Genetically Modified with Luciferase Gene; a gene found in fireflies!

Preliminary Data From Cell Free Assay

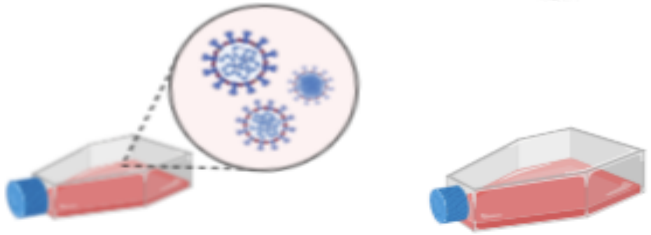


Unpublished

The Steps to Biological Testing



1. Cell Free TNT Luciferase Assay
To Determine Best Target Sequence



2. Cell Culture to study the efficiency of the COVID-19 target
To study to efficiency of the delivery component

3.

Animal Studies

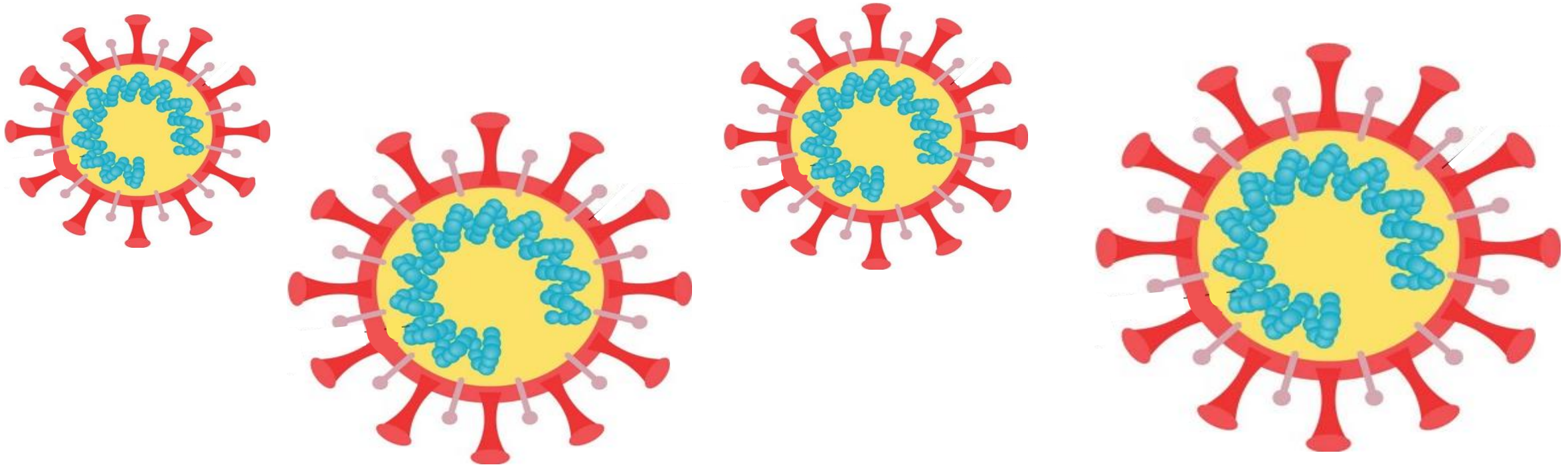
1. Cell Free Assay
2. Cell Studies
3. Animal Studies

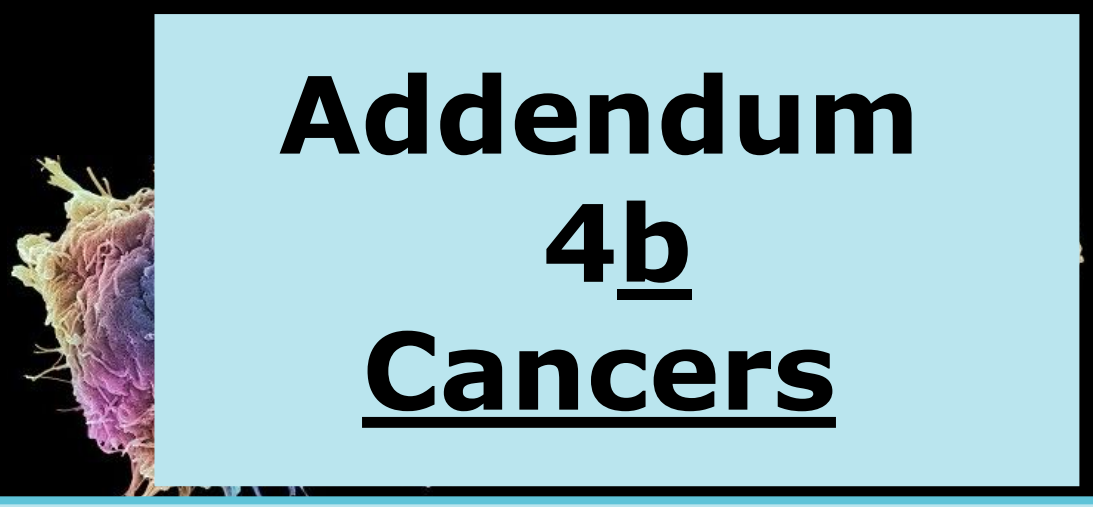
Compassionate Use

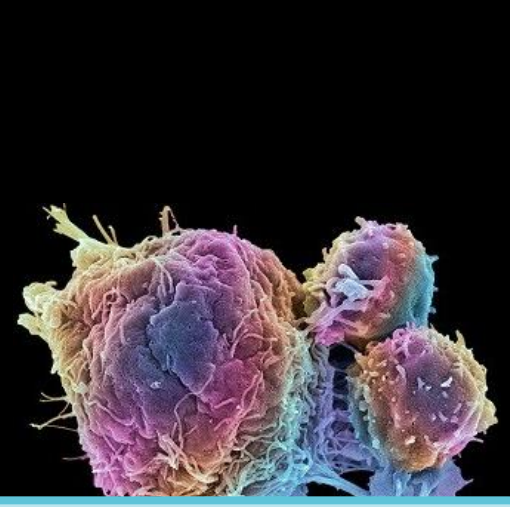
An alternative route to humans

Gilead’s Remdesivir Precedent

Questions ?







Addendum 4b Cancers

How To Defeat Cancers

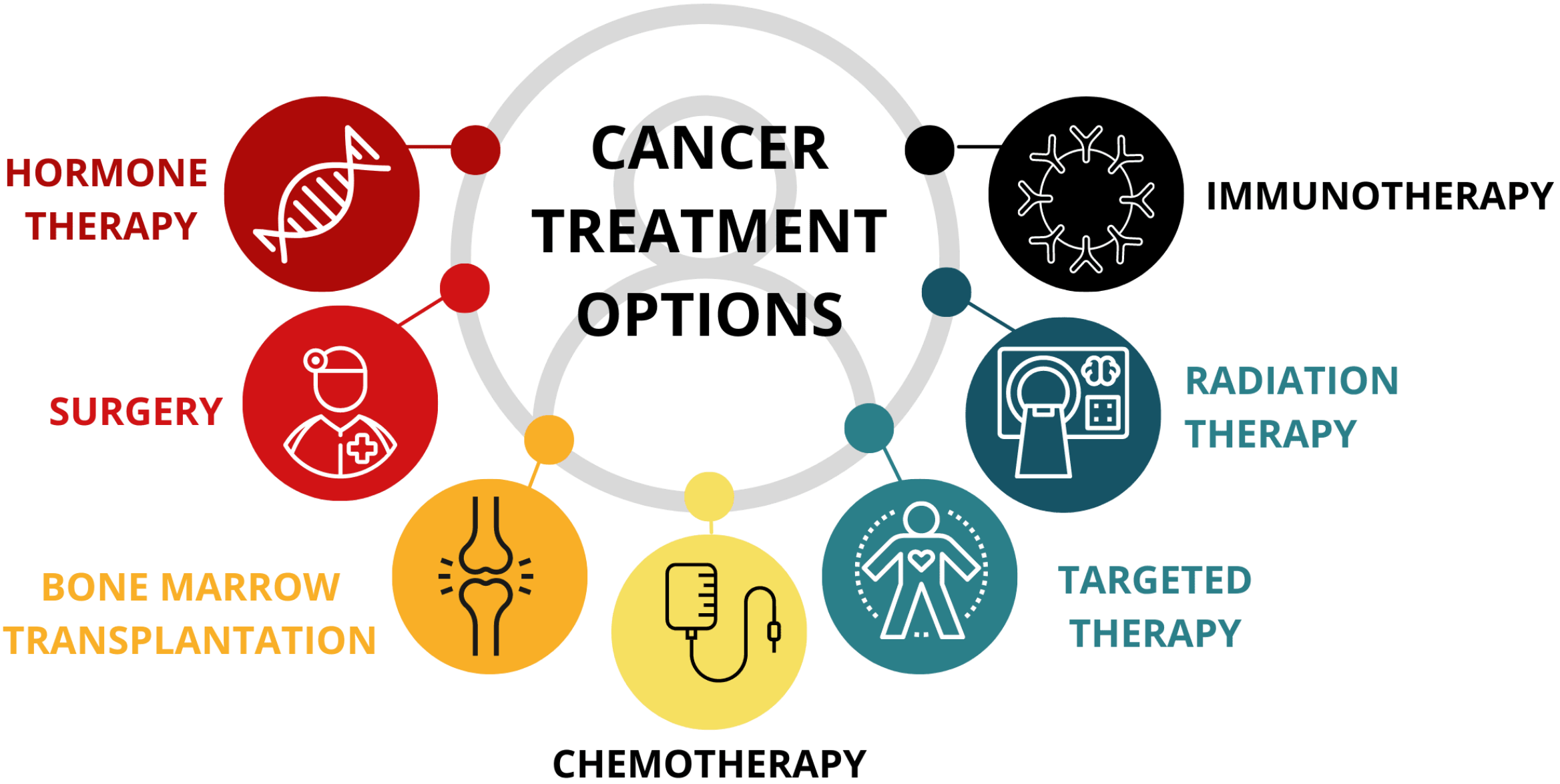
Presenter:

Lena Kinion (Ph.D. Candidate)

30 November 2022

This presentation was given to the Academy of Lifelong Learning at Oregon State University. Please see page 36 for the presentation on LK-1

Currently There Are No Reliable Cures For Cancer

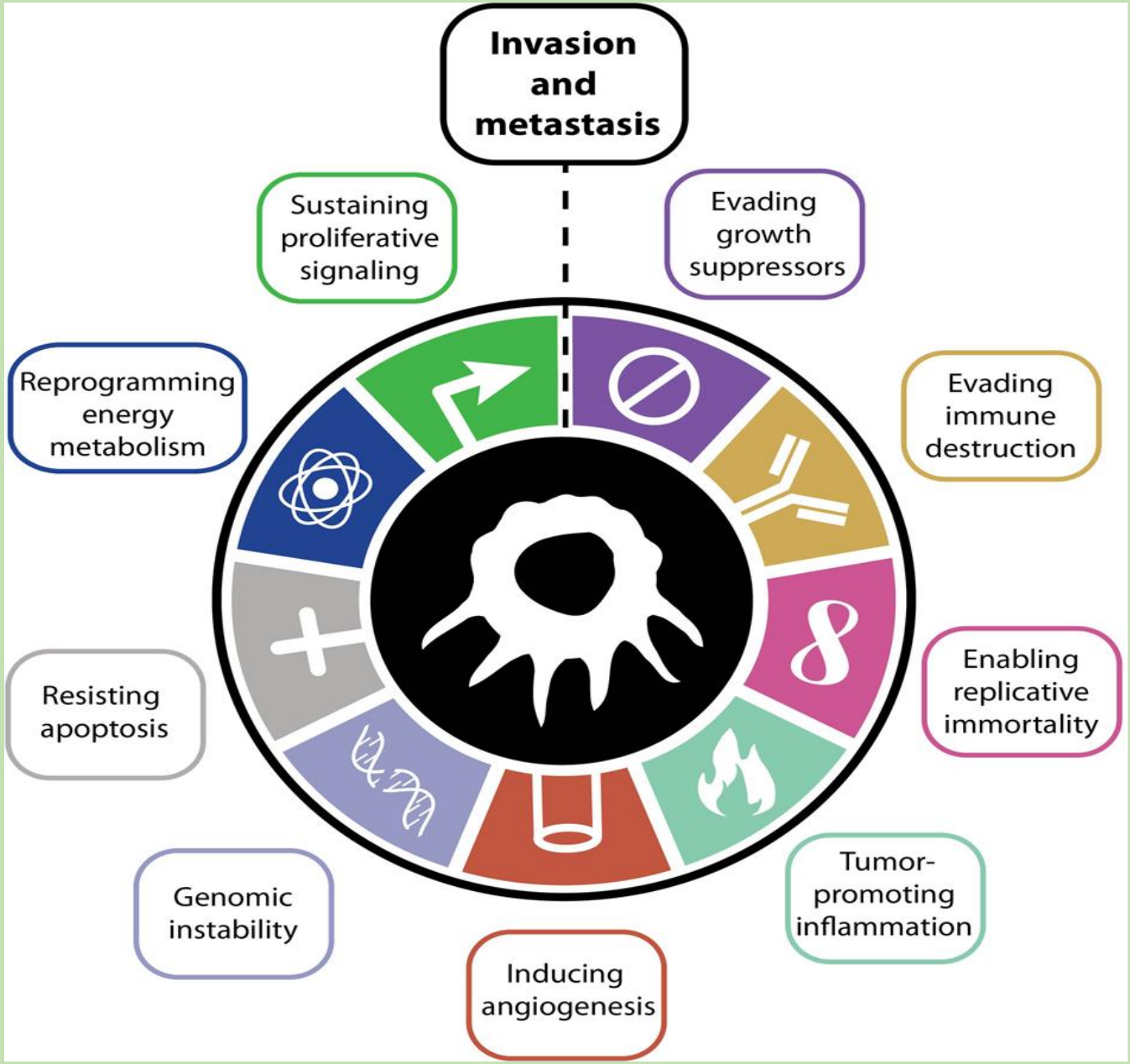


How To Safely Defeat Cancer

1. Select a suitable target that will destroy the cancer

2. Avoid harm to the patient

How To Find An Ideal Target That Will Destroy Cancer

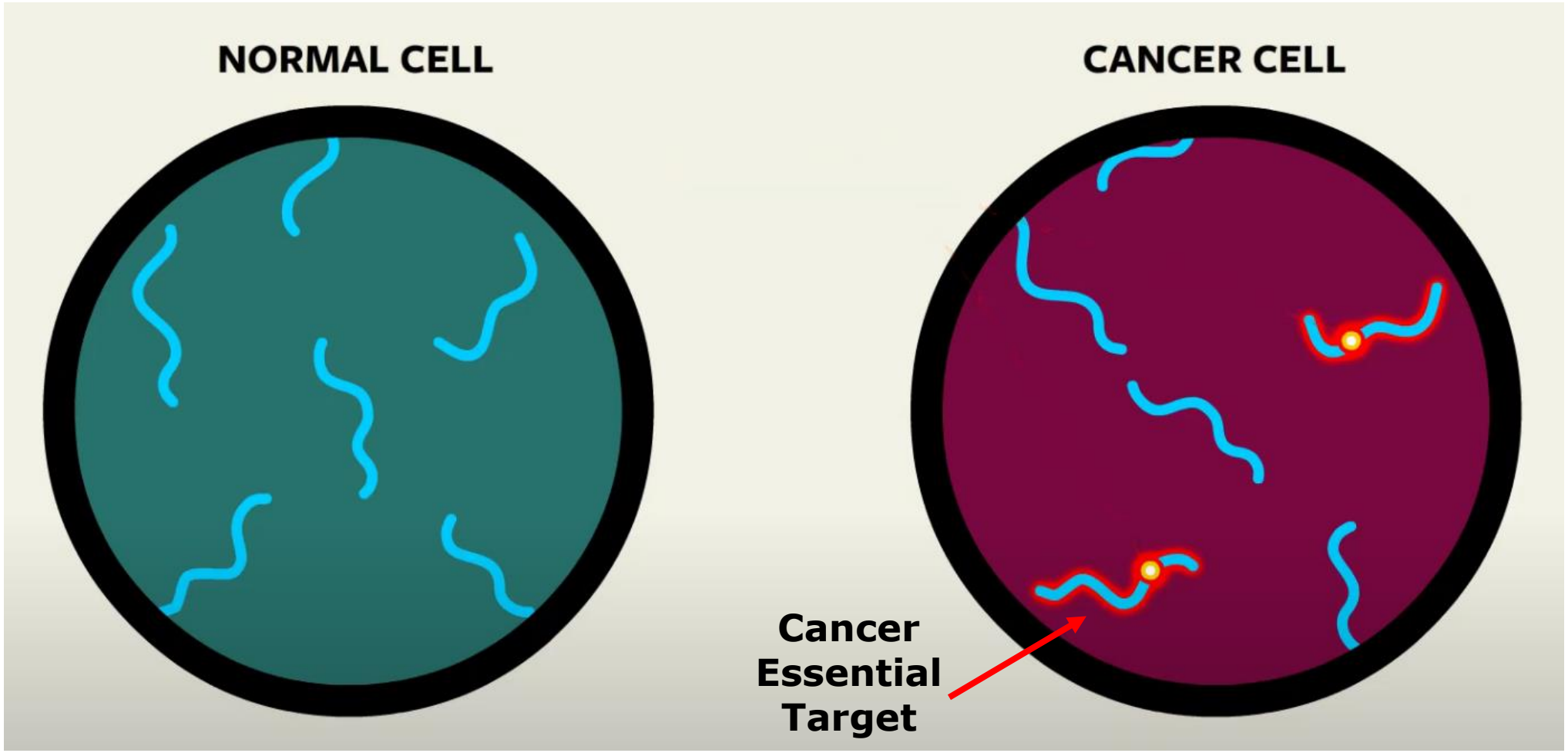


Meirson et al, 2020

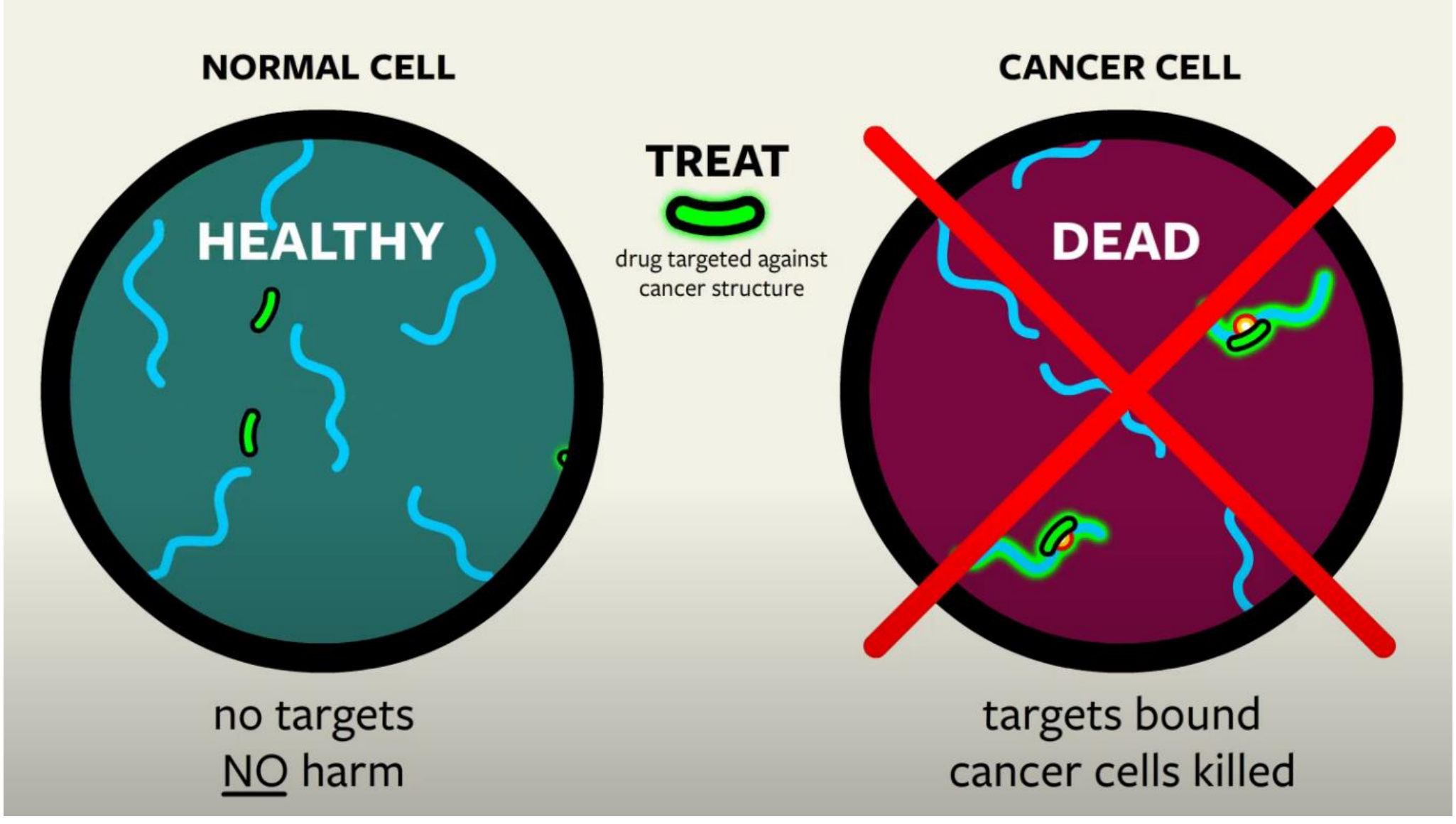
Weinberg identified multiple properties that are essential to cancers survival

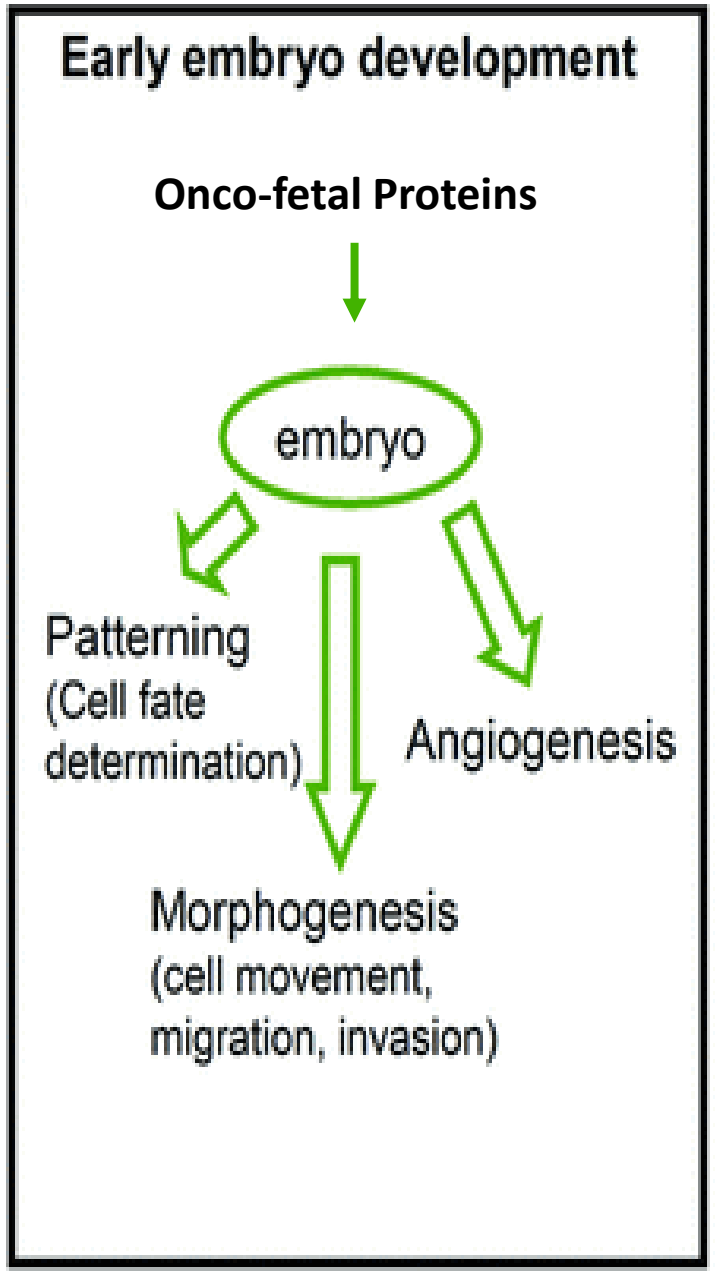
Onco-Tools
Cure cancer without harming patients

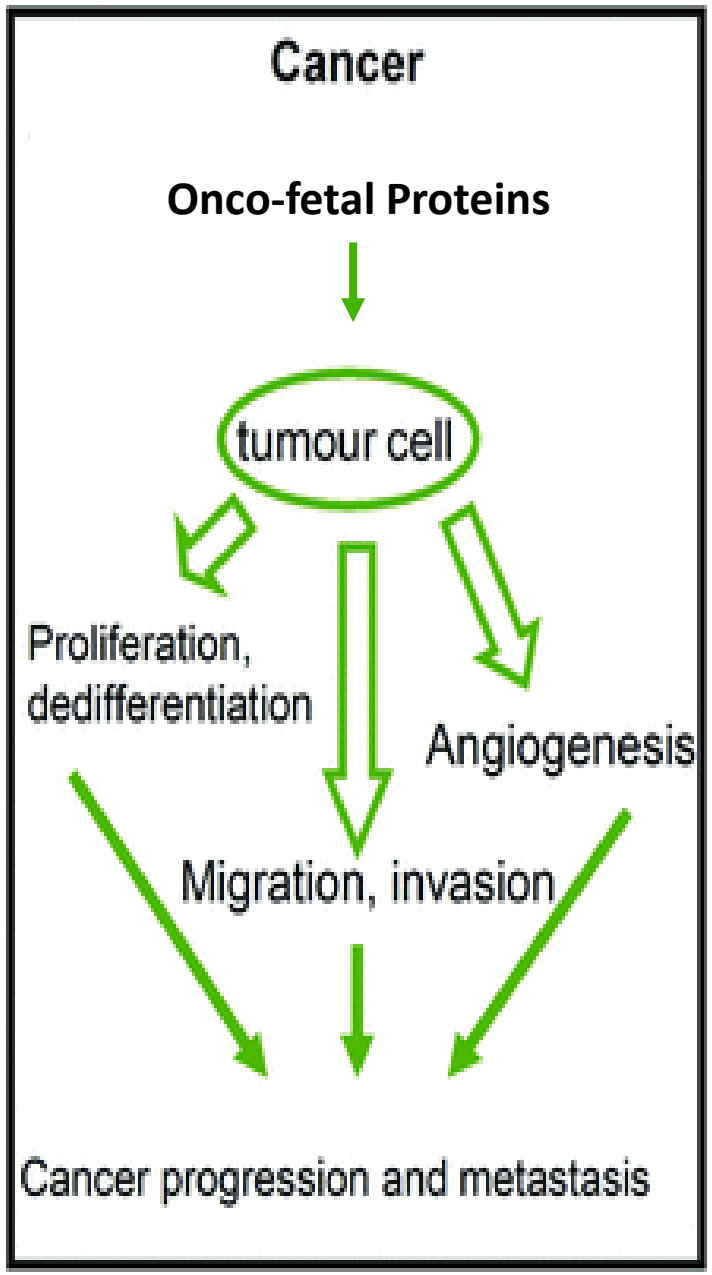
Strategy For Developing A Cure For Cancer Without Harming The Patient



Strategy For Developing A Cure For Cancer Without Harming The Patient





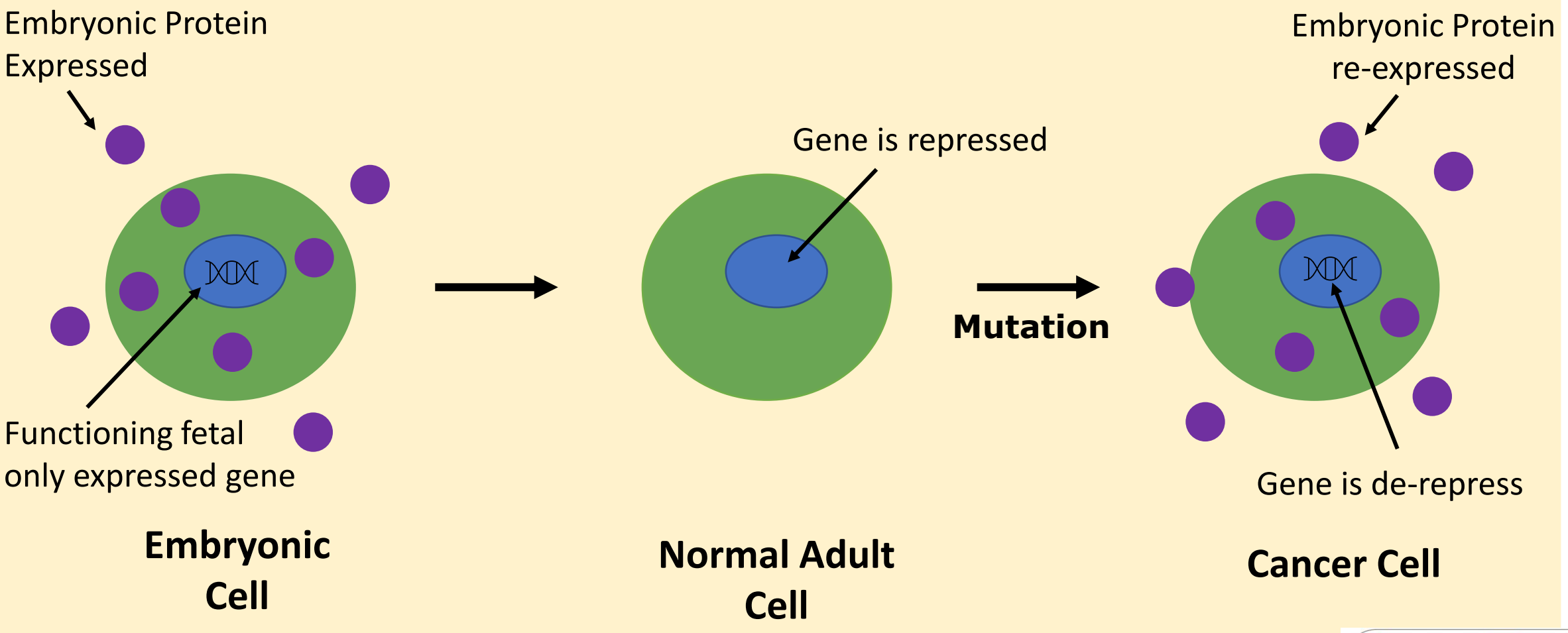


The relationship between early embryo development and tumorigenesis

- Similar signaling pathways in early embryo development and tumorigenesis.
- During tumorigenesis, these pathways are reactivated and contribute to cancer progression and metastasis.

Ma et al, 2010

Why Target Onco-Fetal Proteins?

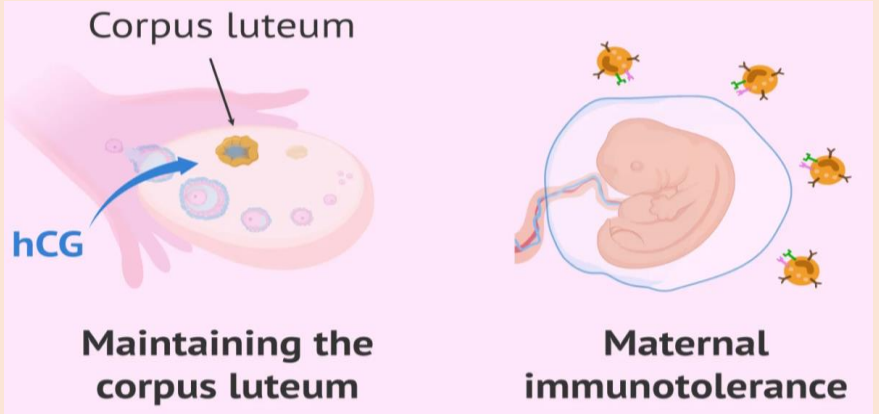


Onco-Fetal Proteins

- These are proteins produced during fetal life
- They are present in high concentrations in fetuses & are disposed of after birth
- They reappear in individuals with cancer
- This demonstrates that certain genes are reactivated by mutation
- There are several oncofetal genes one being **human chorionic gonadotropin-beta** (hCG-b)

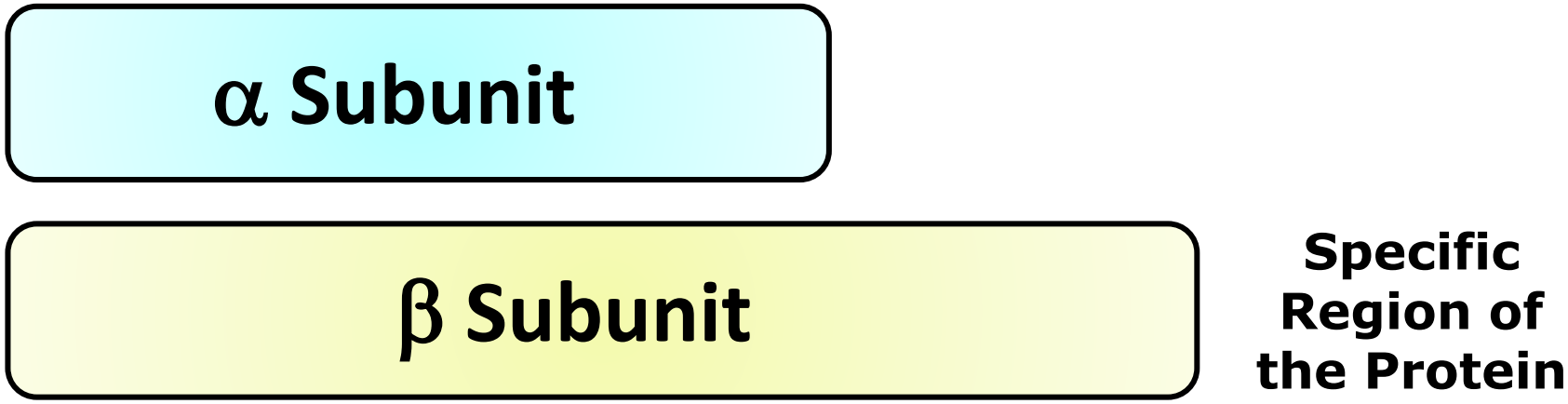
What is Human Chorionic Gonadotropin (hCG)

- hCG is used as a marker for at home pregnancy tests (known as pregnancy hormone)
- hCG is produced by the placenta during pregnancy
- Maintains other essential functions during pregnancy and protects the fetus from the mother's immune system



hCG Protein Structure

Identical to other Reproductive Proteins

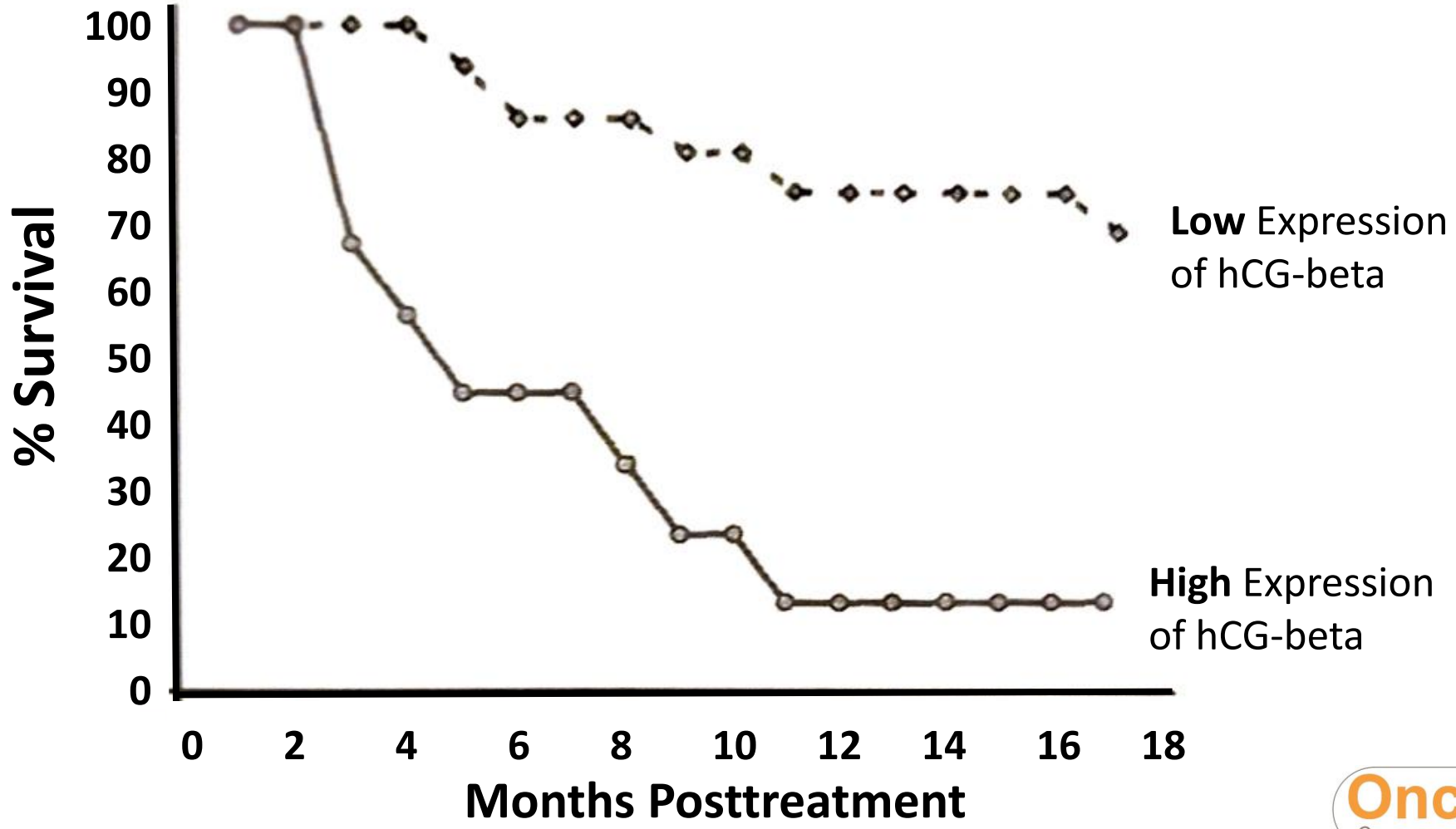


Specific Region of the Protein

hCG-βeta has been linked to many cancers

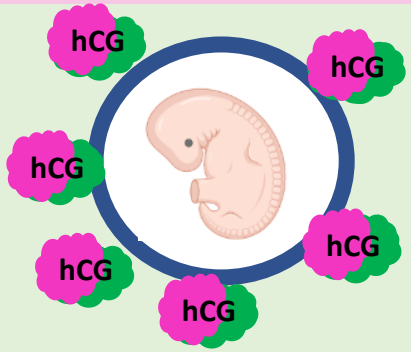
- When at home pregnancy tests were being developed, men were used as controls.
- Surprisingly, some of the controls came up positive!
- Researchers then, started looking at hCG and its influence in cancer

Low Prognosis For Patient's Who Express hCG-β In Cancers



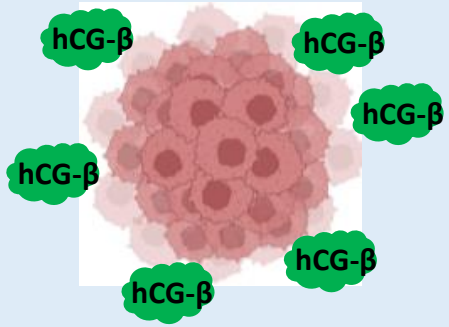
Butler & Cole, 2015

hCG-β Activity in Humans



Embryos

- Blocks programmed cell death
- Promotes implantation into uterus
- Hides the embryo from the mother's immune system
- Induces new blood vessels

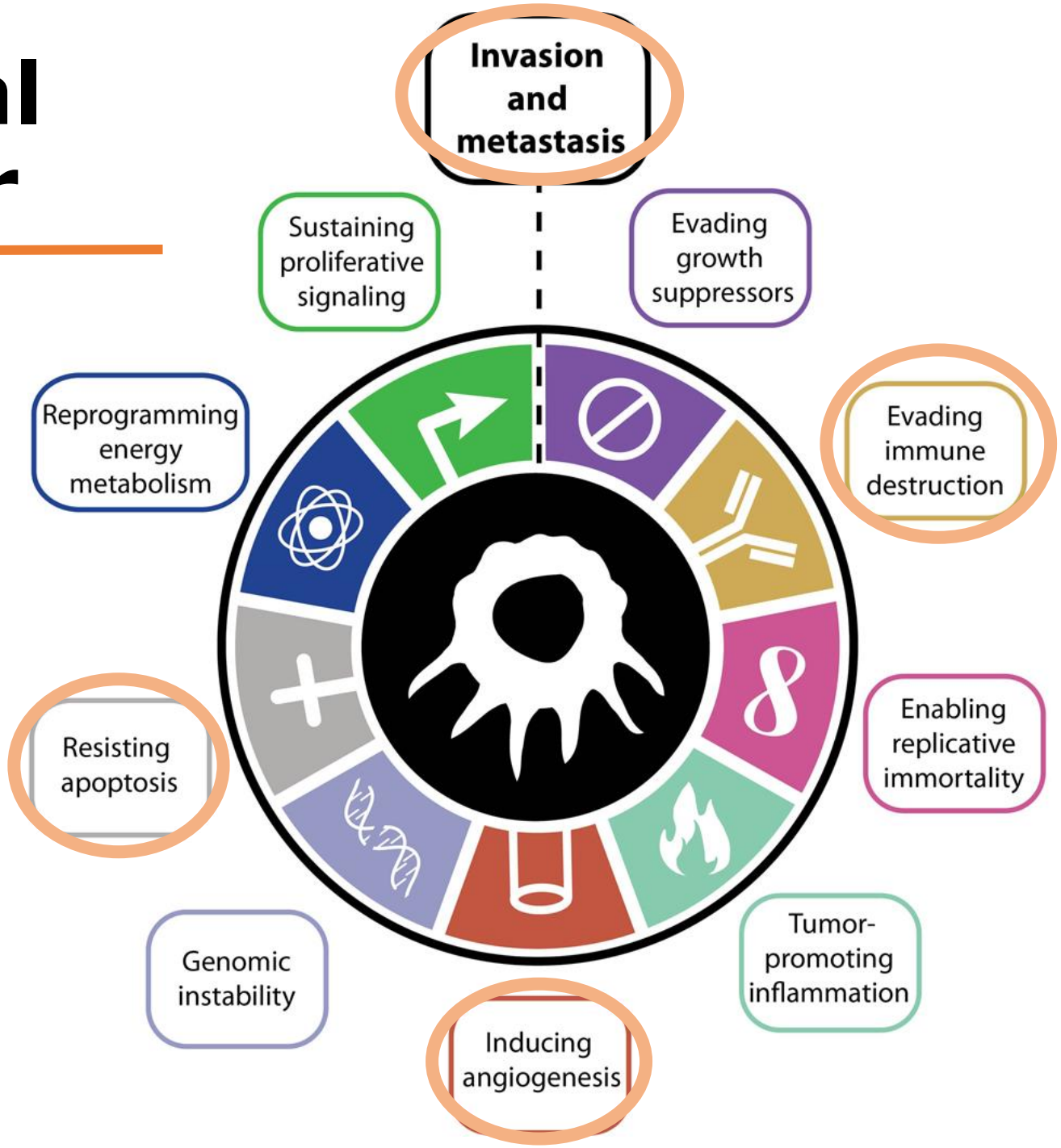


Cancers

- Blocks programmed cell death
- Promotes invasion into normal tissues
- Hides the cancer from the patient's immune system
- Induces new blood vessels

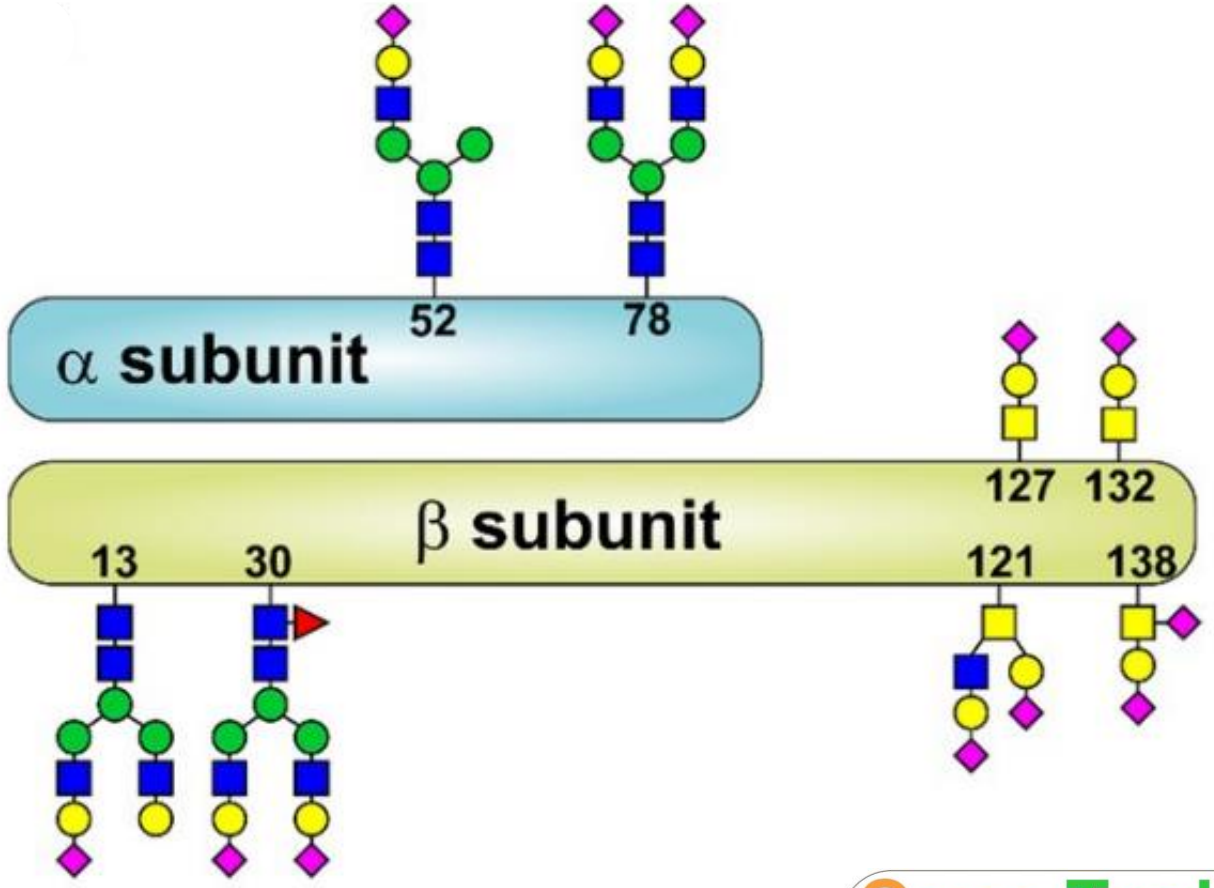
hCG-β Is The Ideal Target For Cancer

- hCG-β plays a major role in many different cancers
- Prevalent in many different cancers:
  - Prostate
  - Ovarian
  - Triple negative breast
  - Breast
  - Bladder
  - Skin, etc
- Essential to cancer but **NOT** essential in normal cell



Why haven't Scientists been successful targeting hCG-β?

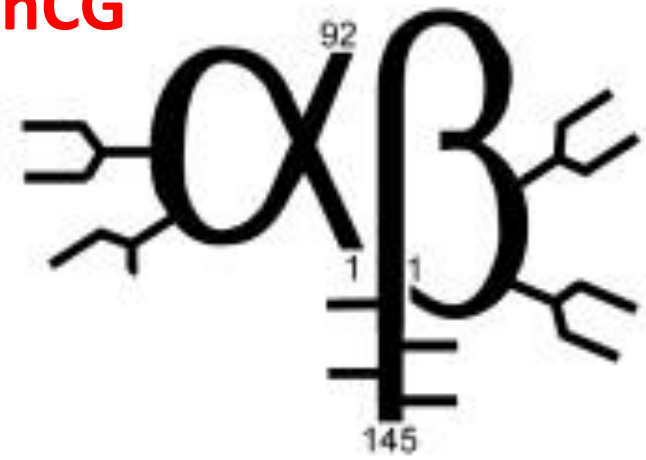
- hCG-β is a very complicated protein to target
- 3,000 out of 25,000 protein coding genes are druggable
- ~10% of the druggable proteins are FDA-approved drugs
- What does druggable mean to NIH?
  - Proteins with ability to bind to drug-like small molecules



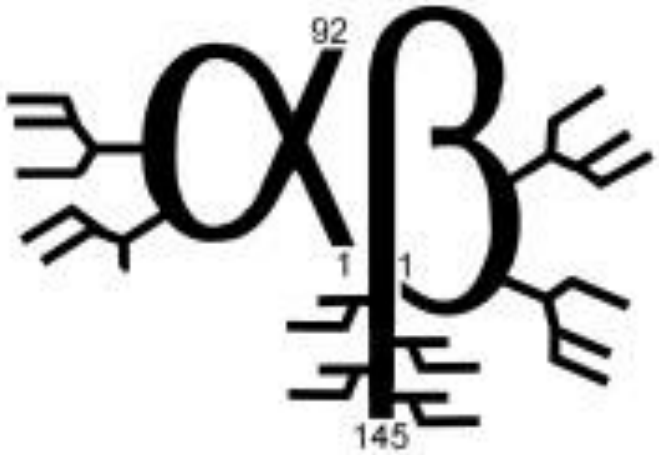
Hires et al, 2019

Human Chorionic Gonadotropin Comes in Many Forms

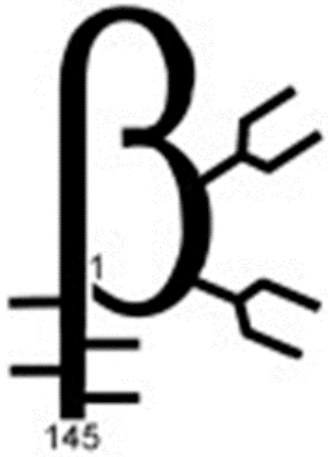
hCG



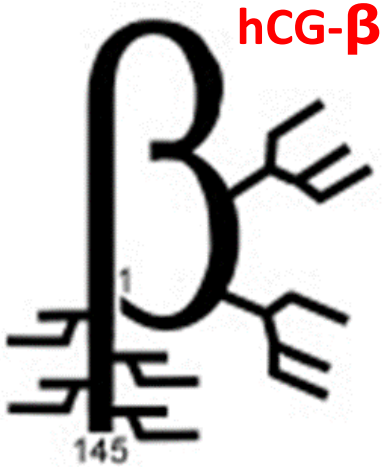
Hyperglycosylated hCG



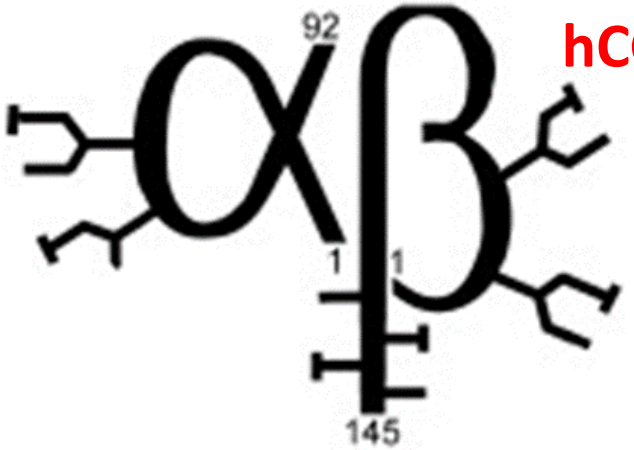
hCG-β



Hyperglycosylated hCG-β

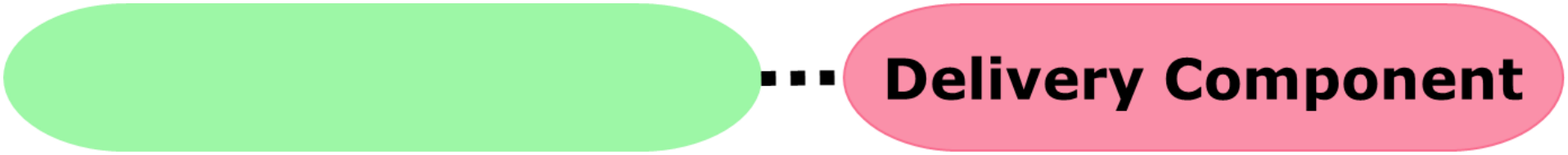


Sulfated hCG



L. Cole, 2012

Our Therapeutic Designed For Blocking Function of hCG-β



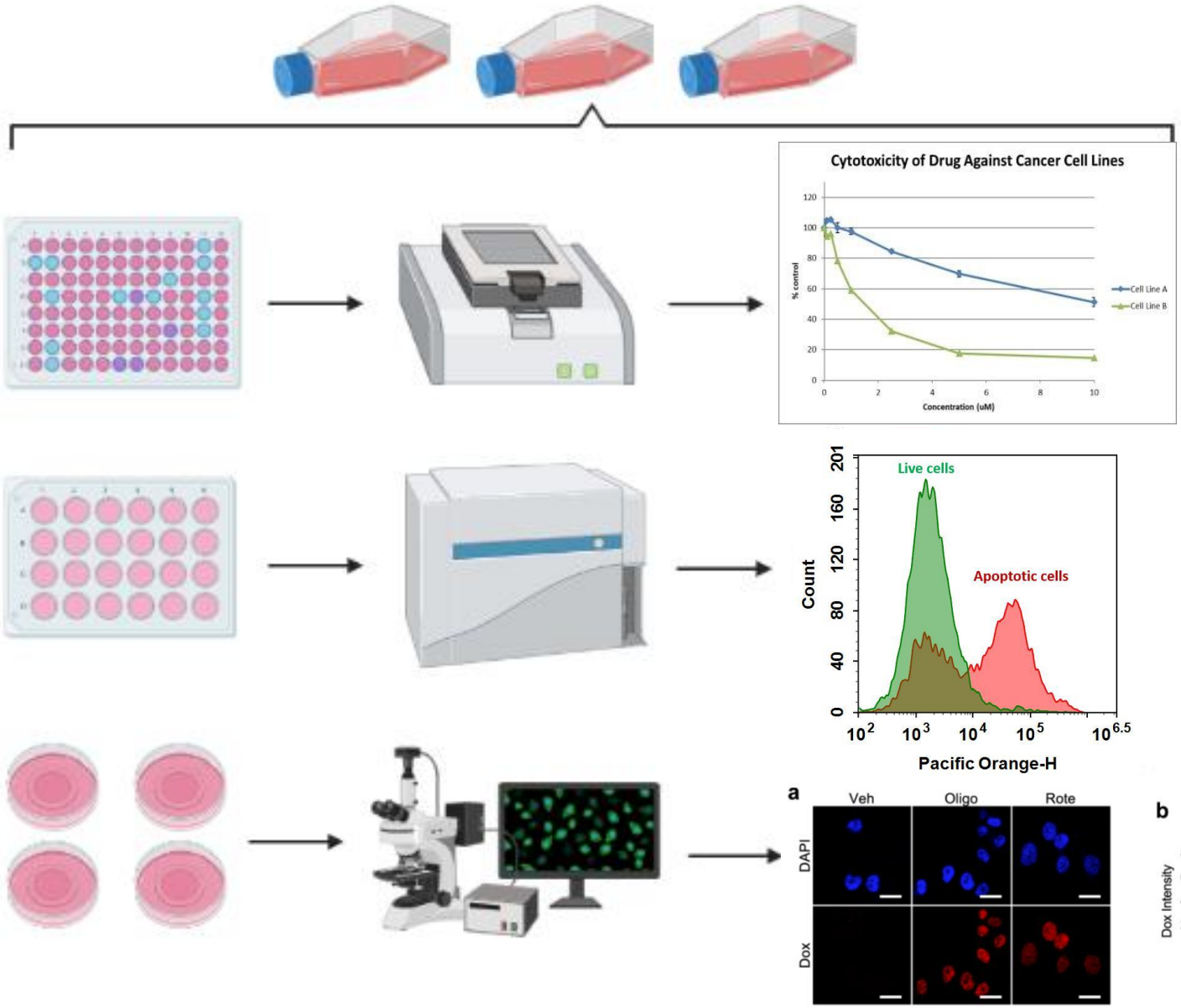
Precision blocking agent for selected target

To deliver Morpholino from the blood to the cytosol of cells

1985 – 1993

1993 – 2022

Various Cancer Types Tested

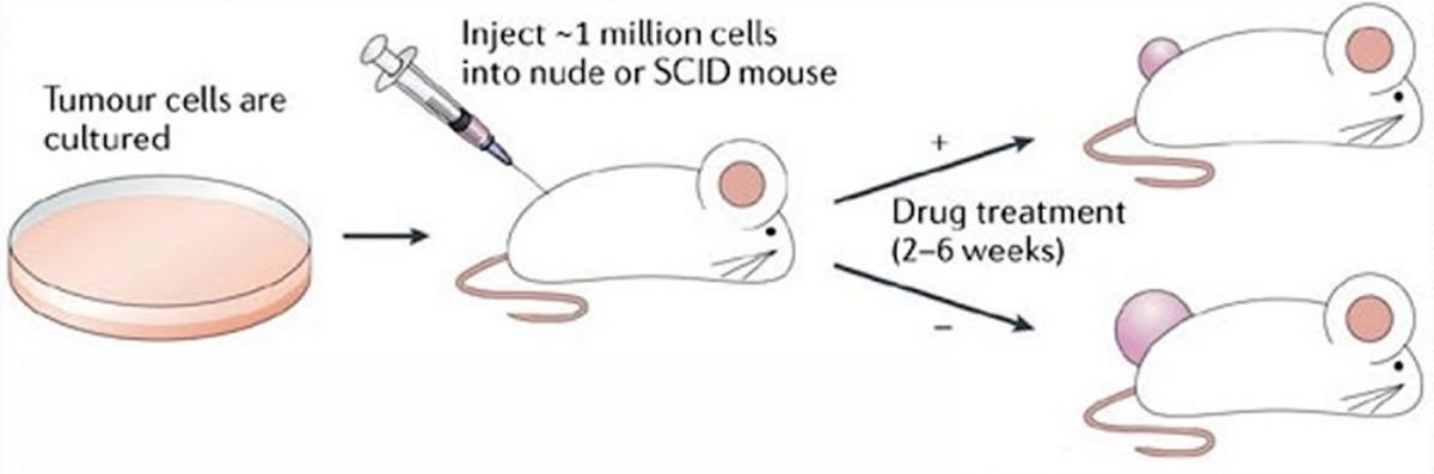


Steps to Develop a Cancer Therapeutic

1. Screen different treatment combinations
2. Identify dead cells
3. Validate best drug combination in several biological assays
4. Animal Studies

Onco-Tools
Cure cancer without harming patients

Steps to Develop a Cancer Therapeutic

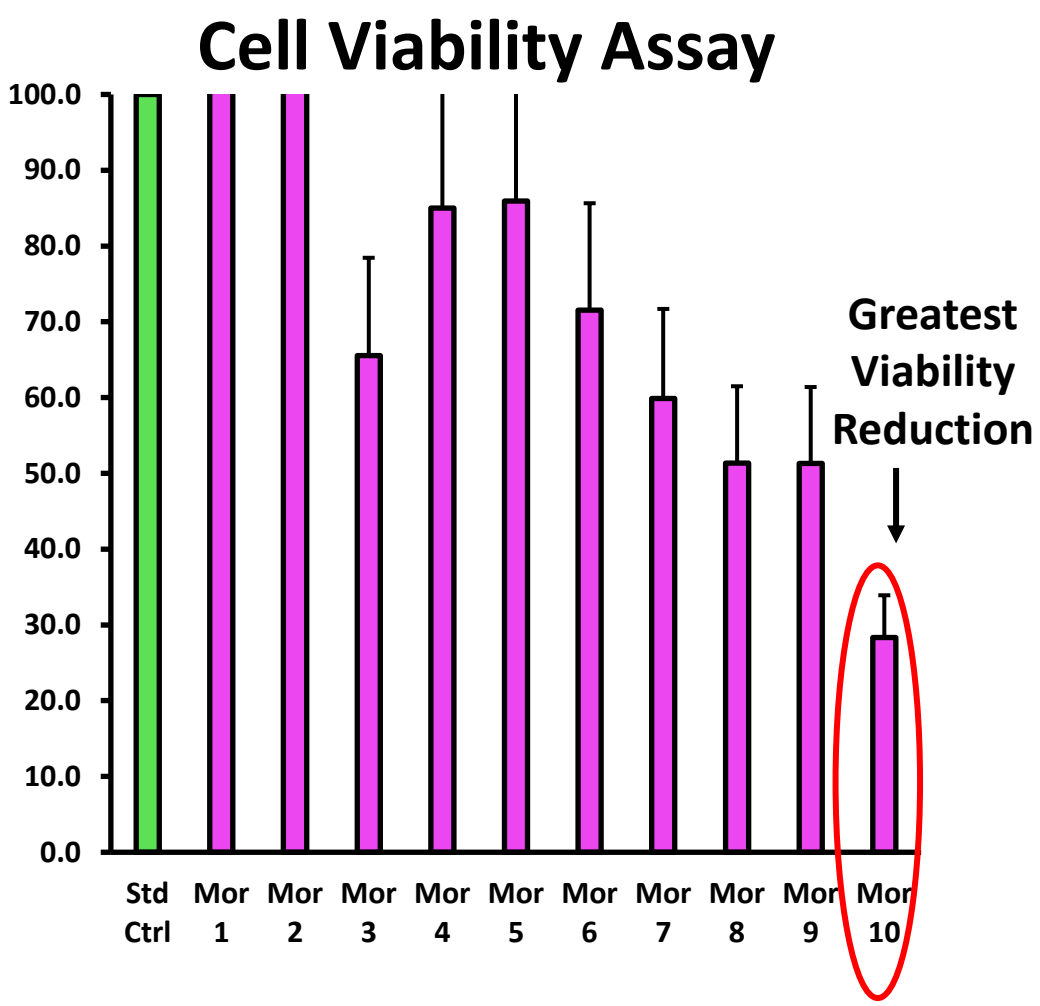


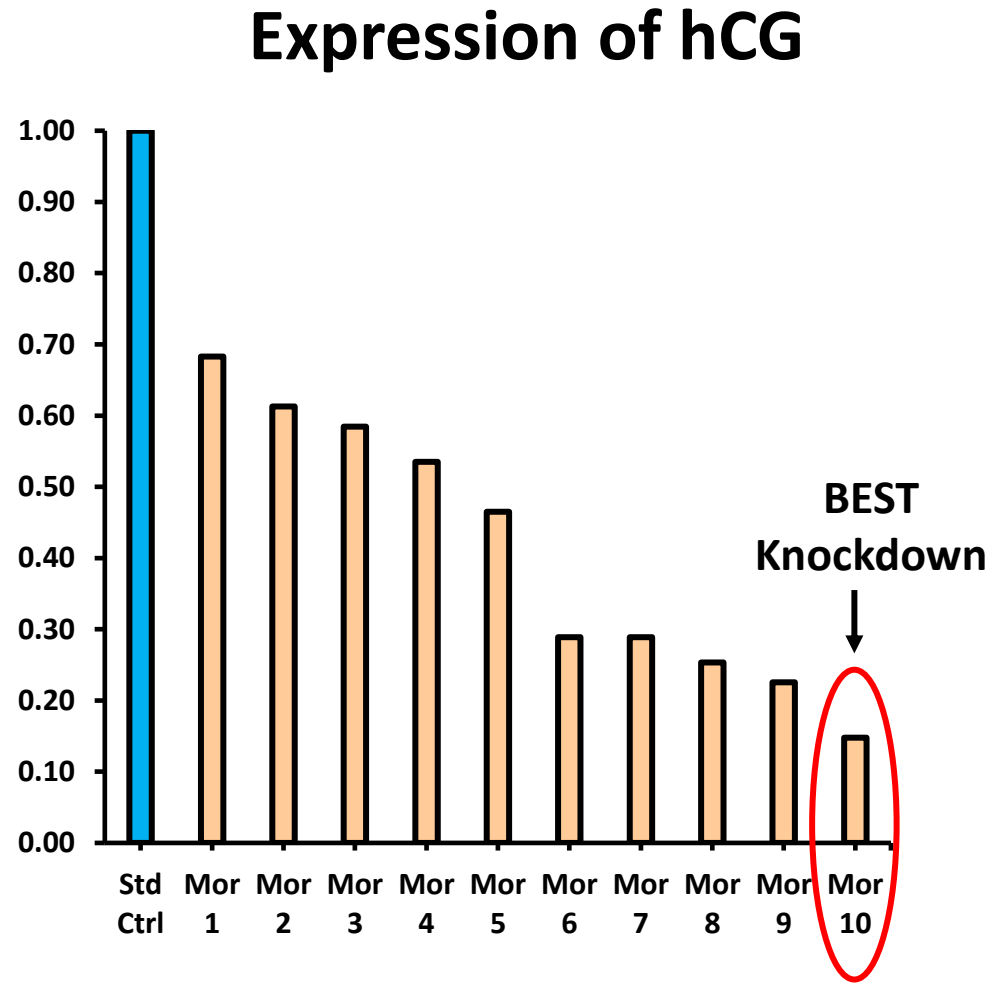
Xenograft studies can be designed to:

- **Identify lead compounds**
- **Optimize dose schedules**
- **Identify combination strategies**

1. Screen different treatment combinations
2. Identify dead cells
3. Validate best drug combination in several biological assays
4. Animal Studies

Various Morpholinos Designed to Block hCG-β Function



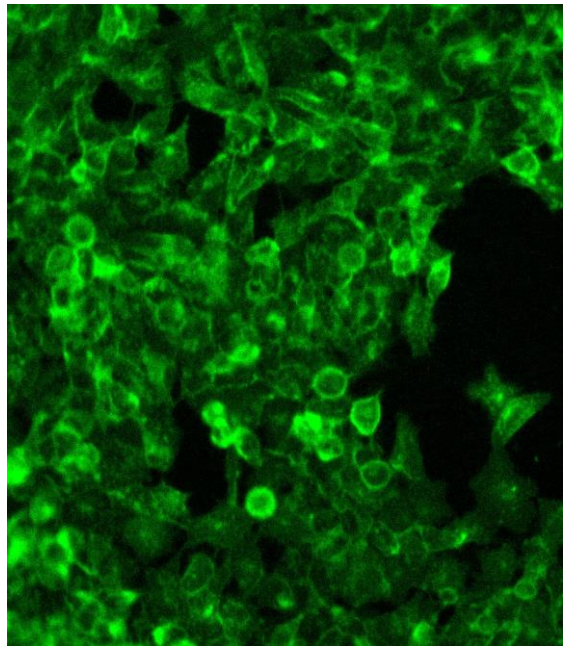


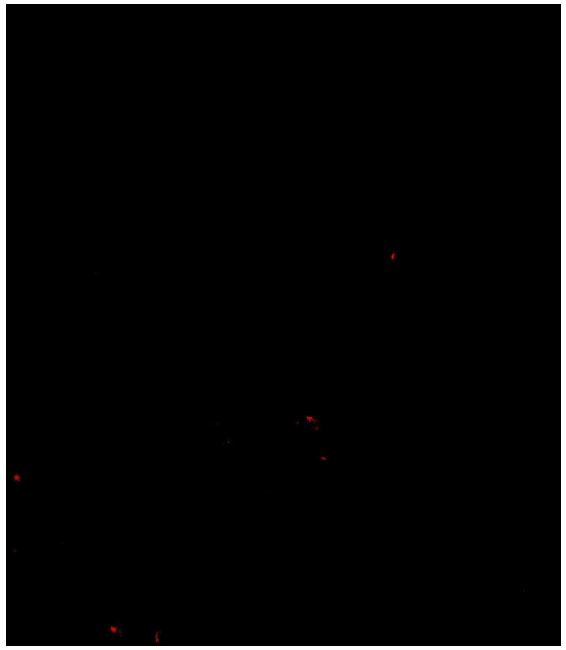
unpublished

Membrane Stain

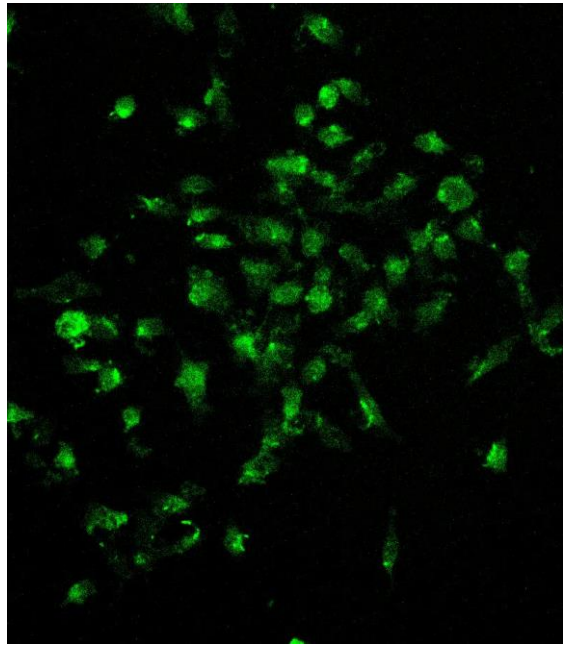
Dead Cells

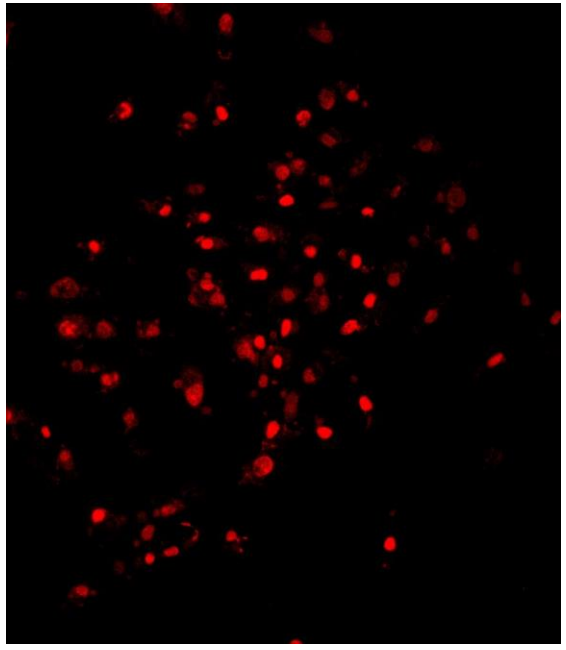
Untreated





Treated Cells





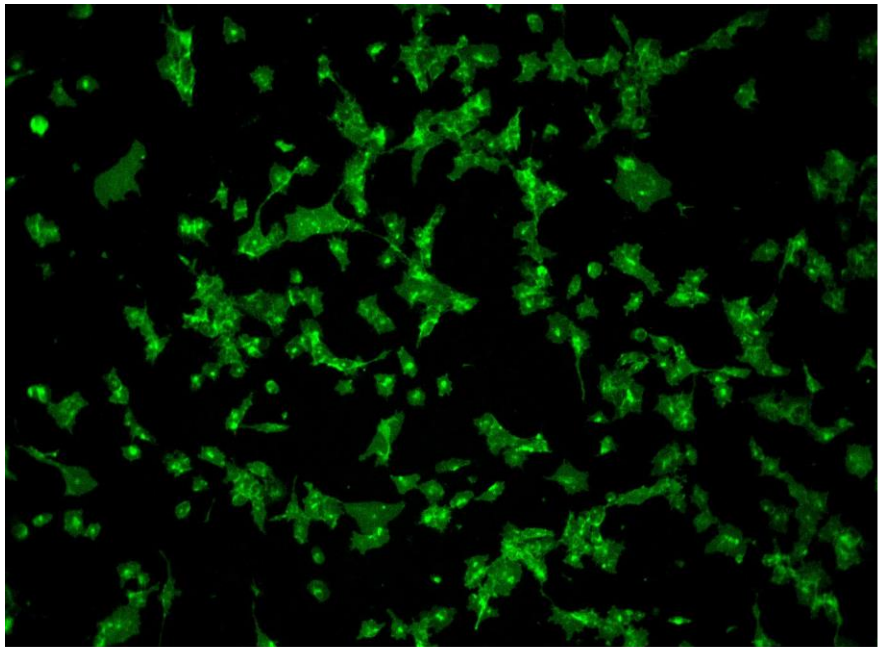
Triple Negative Breast Cancer Cell Line

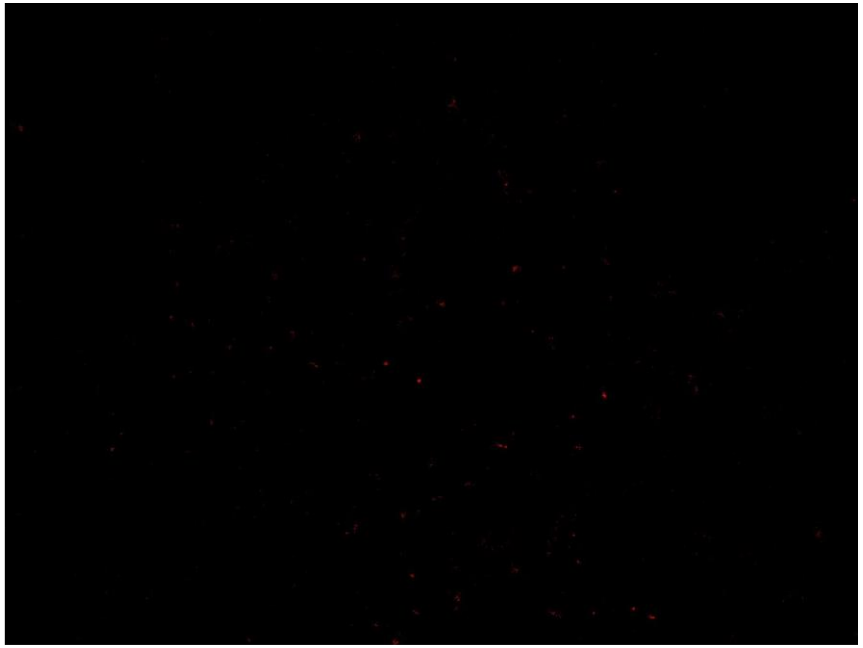
unpublished

Membrane Stain

Dead Cells

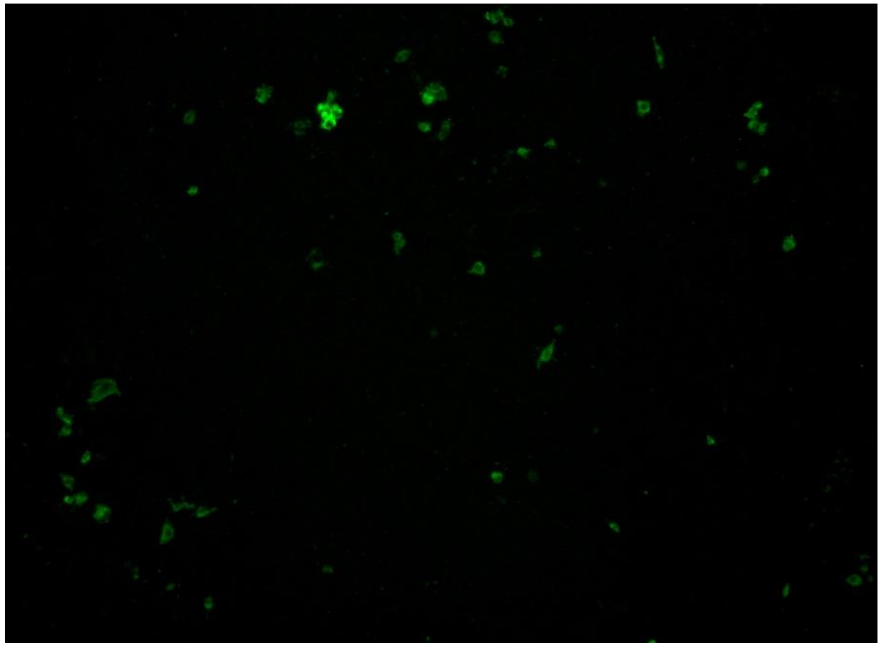
Untreated

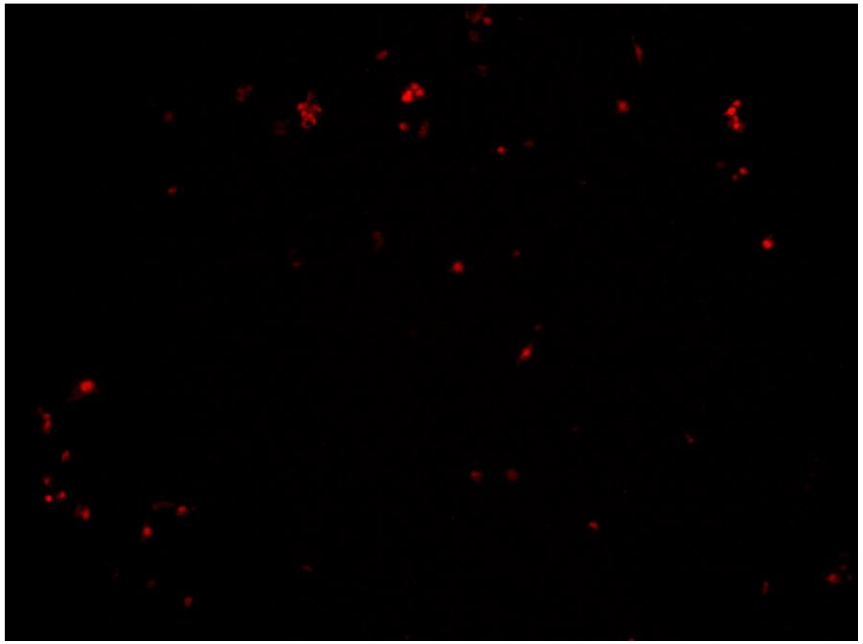




Triple Negative Breast Cancer with BRCA1 mutation

Treated Cells





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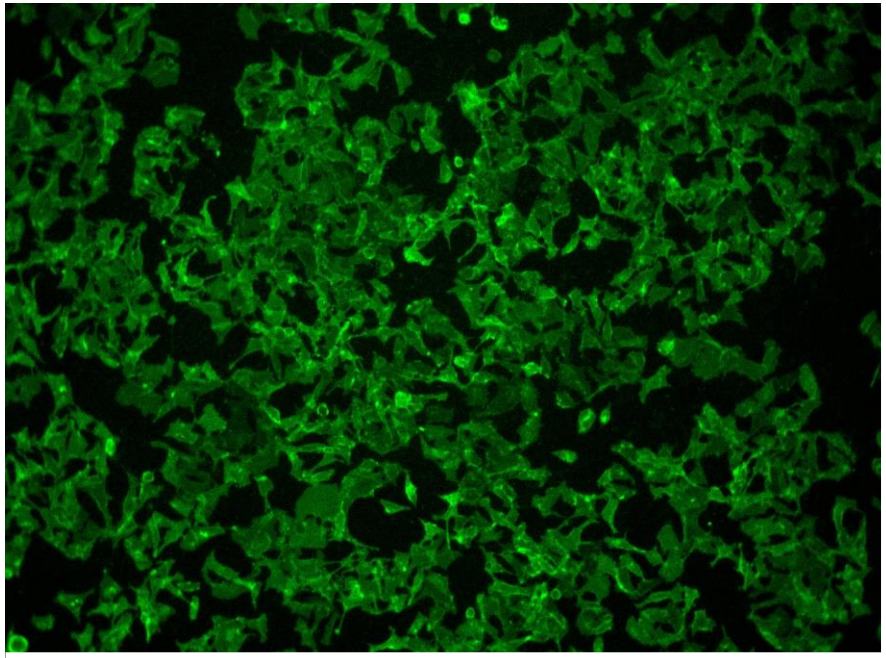
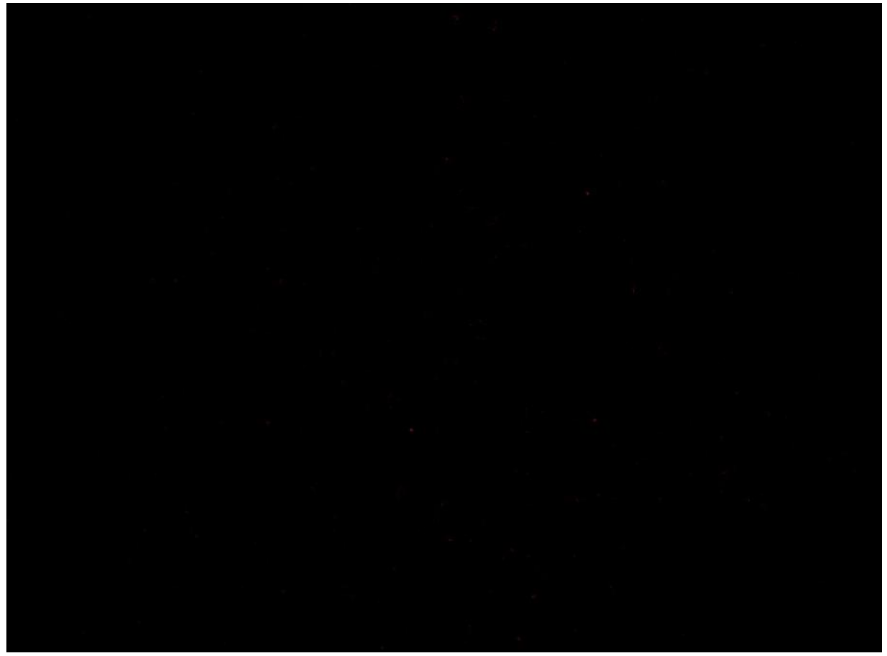
Membrane Stain

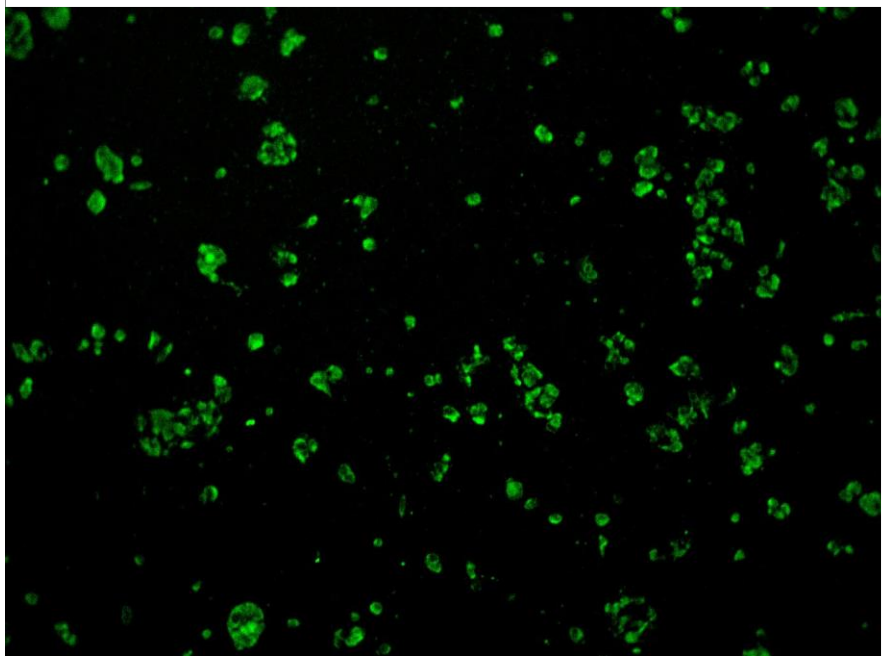
Dead Cells

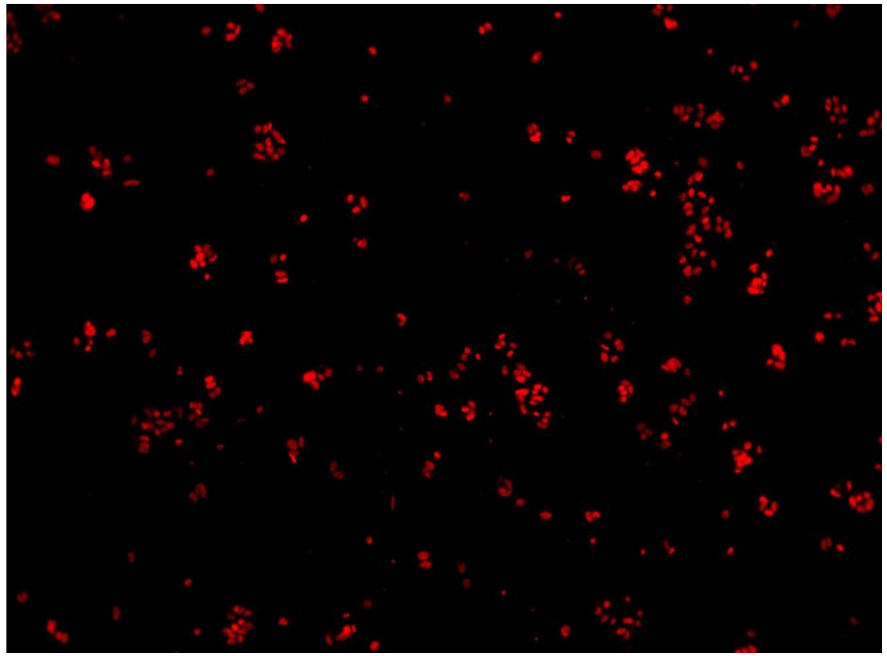
Untreated





Treated Cells





Breast Cancer – Hormone Positive Cell Line

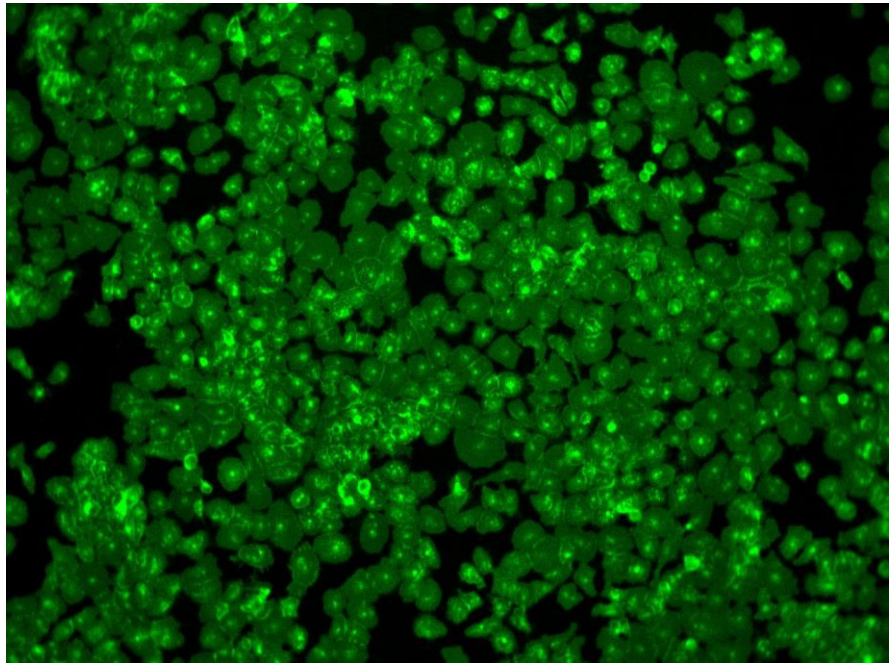
unpublished

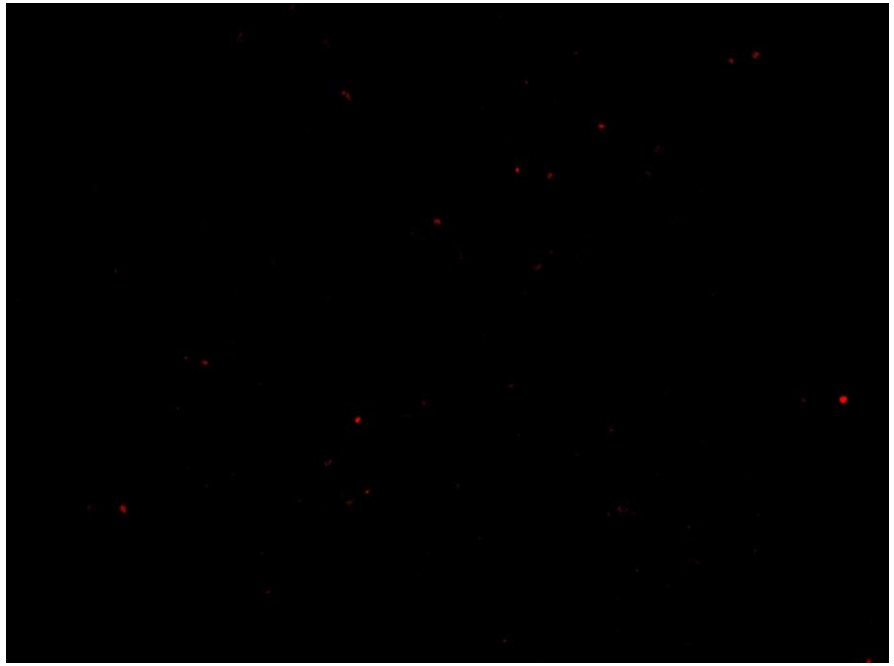
Onco-Tools
Cure cancer without harming patients

Membrane Stain

Dead Cells

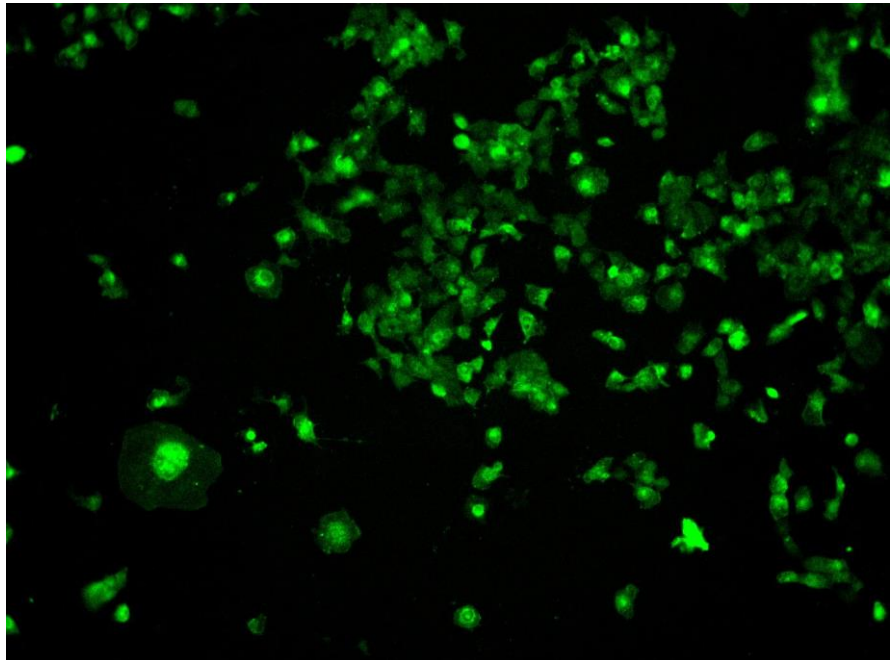
Untreated

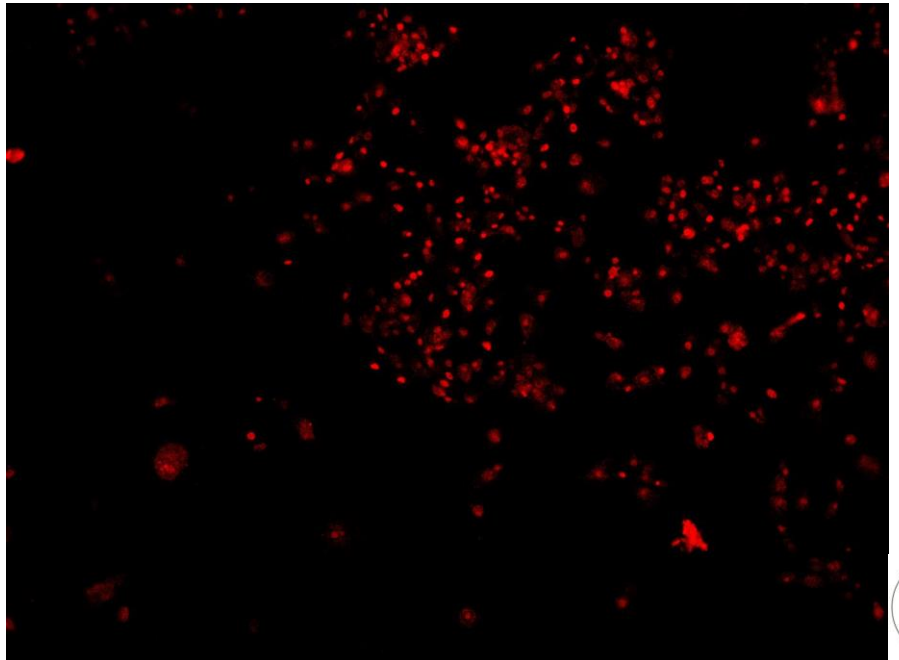




Cervical Cancer

Treated





unpublished

Conclusion

hCG-β is a promising target for cancer therapeutics **because**:

- Only expressed in cancer, so will cause no harm to the patient
- Is ubiquitous in broad range of cancers, potentially a universal treatment for cancers
- Morpholino therapeutics allows hCG-β to go from an undruggable target to druggable target

Fighting Back Against Cancer

Onco-Tools
Cure cancer without harming patients