



Long Island
Section 303



Quality 4.0
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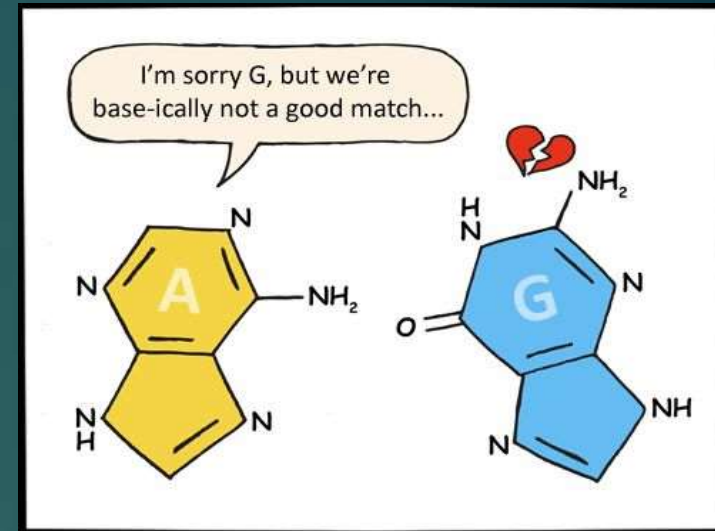
Adapting Quality Assurance to Nucleic Acid Based Treatments

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*borrowed from Pinterest "Science Cartoons"; not for public distribution



What Constitutes a Nucleic Acid Based Treatment/Therapeutic???

▶ DNA:

- ▶ DNA vaccines
- ▶ Oligonucleotides
 - ▶ Treatments
 - ▶ Adjuvants

▶ RNA:

- ▶ RNA vaccines
- ▶ RNA interference (RNAi)
 - ▶ Short-interfering RNAs (siRNAs)/microRNAs (miRNAs)
- ▶ RNA activation (RNAa)
 - ▶ Short-interfering RNAs (siRNAs)/microRNAs (miRNAs)
 - ▶ Short-activating RNAs (saRNAs)/anti-genome RNAs (agRNAs)

RNA Interference (RNAi): powerful tool to reduce expression of a protein linked to a condition

▶ Short interfering RNAs (siRNAs)

- ▶ Require exquisite sequence complementarity to its messenger RNA (mRNA) target site – typically single gene targets.

▶ MicroRNAs (miRNAs)

- ▶ Only require partial sequence complementarity to its mRNA target (called a “seed sequence”) – can target multiple genes.

Background: RNAi and RNAa

A Messenger RNA (mRNA)



B + ssRNA Virus Genome



Active Research into RNAi-Based Cosmetic Products

siRNA or miRNA	Gene Target	Skin Care Application
miRNA	TYR	Whitening
siRNA	MITF	Whitening
siRNA	p53	Whitening
miRNA inhibitor	miR-29	Anti-wrinkle
miRNA	HYAL	Moisturizing
siRNA	Androgen receptors	Hair care
siRNA	Tbx21	Hair care
miRNA inhibitor	miR-31	Hair care
miRNA	LSD1/2	Anti-aging

Taken from review article: Zhang et al., 2013, *J. Cosmet. Sci.*

Active Research into RNAi-Based Pharmaceuticals

miRNA Therapeutics in Development			
Therapeutic miRNA	Disease	Company	Status
Miravirsen	Hepatitis C virus (HCV) treatment	Santaris Pharma	Enrolled in Phase II clinical trials
MRX34	Treatment for multiple cancers	Mirna Therapeutics	Enrolled in Phase I clinical trials; currently halted
RG-101	HCV treatment	Regulus Therapeutics	AntagomiR technology in development
RG-012	Alport Syndrome treatment	Regulus Therapeutics	Initiating Phase II clinical trials
MGN-1374	Post-myocardial infarction treatment	miRagen Therapeutics	Enrolled in pre-clinical trials; targets miR-15 and miR-195
MGN-2677	Vascular disease treatment	miRagen Therapeutics	Targets miR-143 and miR-145
MGN-4220	Cardiac fibrosis treatment	miRagen Therapeutics	Targets miR-29
MGN-4893	Polycythemia treatment	miRagen Therapeutics	Targets miR-451
MGN-5804	Cardiometabolic disease treatment	miRagen Therapeutics	Targets miR-378
MGN-6114	Peripheral arterial disease treatment	miRagen Therapeutics	Targets miR-92
MGN-9103	Chronic heart failure treatment	miRagen Therapeutics	Targets miR-208

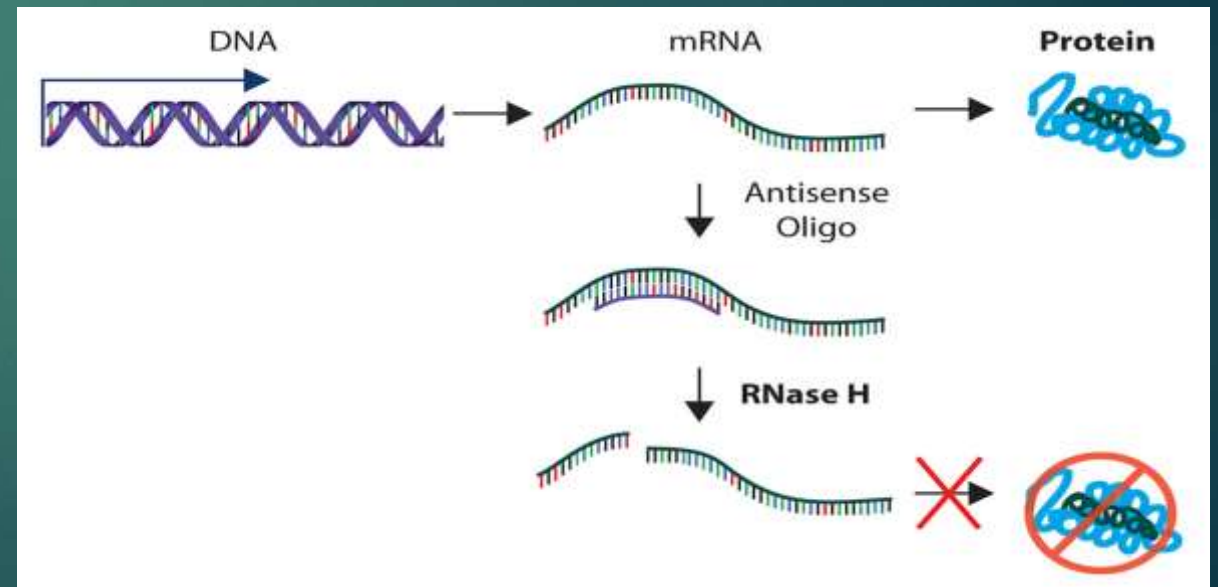
Taken from review article: Chakraborty et al., 2017, *Molecular Therapy: Nucleic Acids*

FDA Approved Oligonucleotide Drugs – so far, there are 3...

- ▶ 1) **Vitravene**: (Ionis Pharmaceuticals) anti-viral drug against cytomegalovirus in immunocompromised
 - ▶ Delivery: intraocular injection
- ▶ 2) **Kynamro**: (Ionis Pharmaceuticals) treatment for familial hypercholesterolemia
 - ▶ Delivery: Sub-cutaneous injection
- ▶ 3) **Macugen**: (OSI Pharmaceuticals and Pfizer) anti-angiogenic treatment for age-related macular degeneration
 - ▶ Delivery: intraocular injection

** As of 2016, there were more than 135 oligonucleotide based drugs enrolled in clinical trials in the US.

MODE
OF
ACTION



** borrowed from Integrated DNA Tech website **

Oligonucleotides – it's hard when you're too small for PCR

- ▶ Oligonucleotides are chemically synthesized...therefore, they are manufactured like a small molecule drug
- ▶ Oligonucleotides have complex mechanisms of action once delivered in vivo...therefore, they behave like biologic therapies
- ▶ The Food and Drug Administration (FDA) has released recommendations when it comes to regulating oligonucleotide based treatments and therapies and their drug approval
 - ▶ Lack of firm regulations...
- ▶ Disagreement between the FDA and its European counterpart, the European Medicines Agency (EMA) about how to regulate oligonucleotide therapeutics

Quality Assurance: What are the Issues to Contemplate...

- ▶ **Oligonucleotide synthesis** – characterization
- ▶ **Safety** – off-target effects
 - ▶ Sequence-specific: also affects unintended targets
 - ▶ Sequence-non-specific
- ▶ **Efficacy** – assay validation and corroboration
- ▶ **Delivery** – systemic vs. local
- ▶ ***In vivo* Stability**
 - ▶ Protect nucleic acids from degradative enzymes

Oligonucleotide Synthesis:

- ▶ DNA and RNA oligonucleotides are mostly manufactured by “solid phase synthesis” → gradual synthesis of short nucleotide strands tethered to beads
- ▶ QC/QA:
 - ▶ Mass spectrometry – confirm molecular weight
 - ▶ Sequencing – confirm the desired nucleotide sequence
 - ▶ Capillary gel electrophoresis (CGE) or polyacrylamide gel electrophoresis (PAGE) – confirm the chain length
 - ▶ Spectroscopy – confirm the concentration of the oligonucleotide

Efficacy:

- ▶ Must have assays that validate the oligonucleotide therapeutic is effective
- ▶ Molecular assay: is the mRNA and/or protein targets being reduced (i.e. RNAi) or augmented (i.e. RNAa)?
 - ▶ Example: Western blot assay probing for target protein
- ▶ Bio-activity assay: is the reduction or augmentation in protein levels actually ameliorating the condition?
 - ▶ Example: Melanogenesis assay for skin pigmentation disorders

Safety:

- ▶ 1) Sequence specific → unintended targets

5' – ACTGAACTTTGGATCCG – 3'

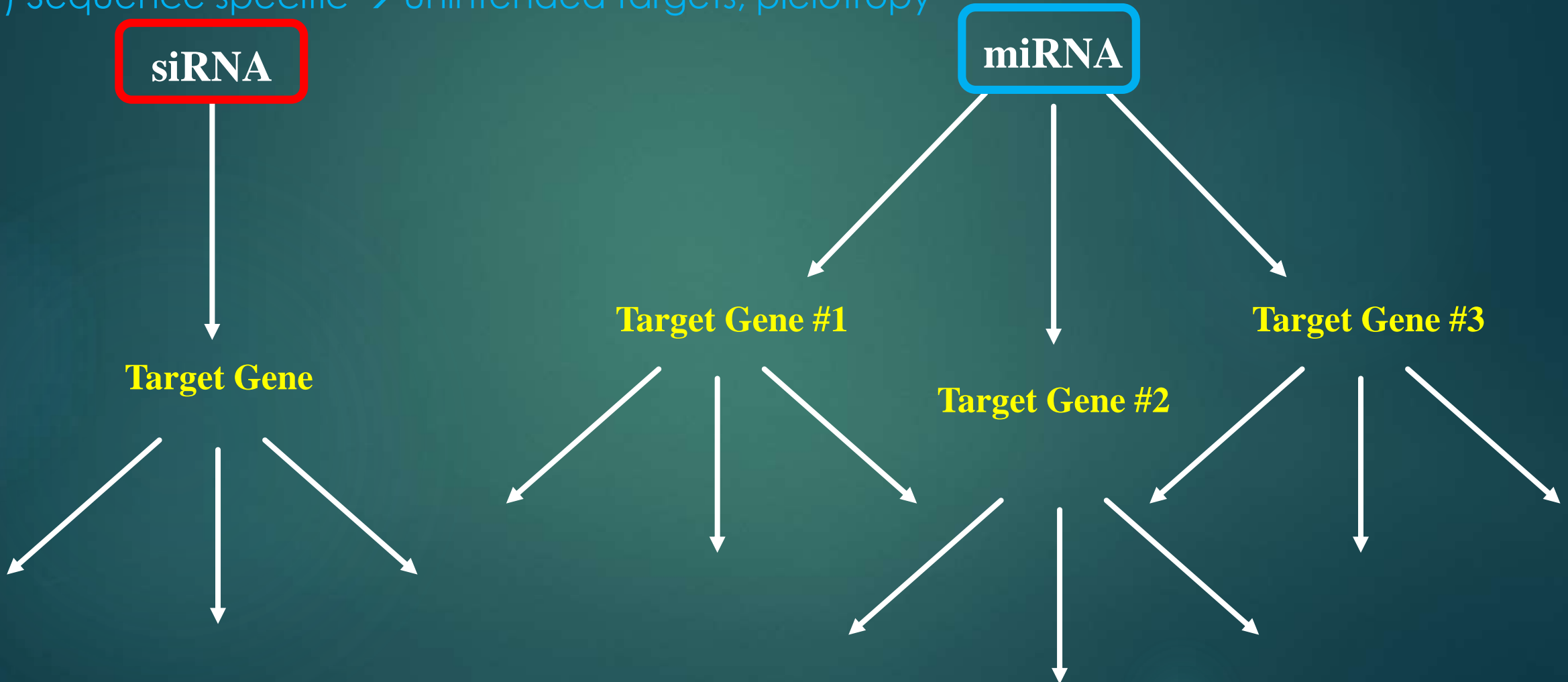
3' – TGACTTGAAAACCTAGGC – 3'

- ▶ 2) Sequence non-specific → innate immune response

Double-stranded RNA triggers innate IR

Nucleic Acid Safety Issues

- ▶ 1) Sequence specific → unintended targets, pleiotropy



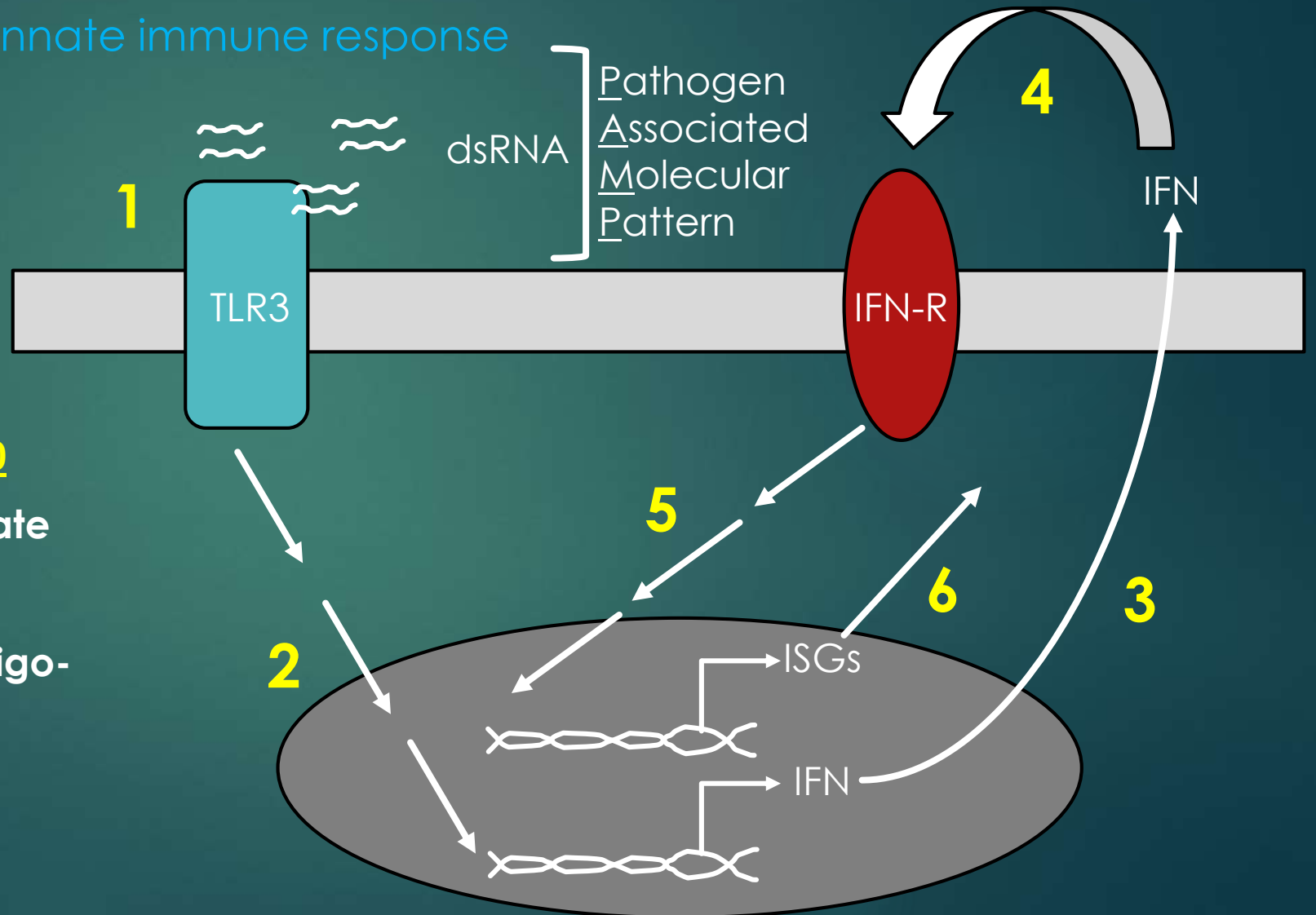
Nucleic Acid Safety Issues

▶ 2) Sequence non-specific → innate immune response

▶ INTERFERON (IFN)

▶ **Threshold for triggering the innate immune response:**

- Usually dsRNA molecules >30 nucleotides in length will stimulate the innate immune response
- siRNAs, miRNAs, and other oligonucleotides are typically 18-27 nucleotides in length



Delivery & *In vivo* Stability:

- ▶ 1) **Current vehicles:**
 - ▶ Liposomes – the most favored and widely used
 - ▶ Nanoparticles
- ▶ 2) **Systemic delivery** – introduced into circulation, i.e. intravenous injection
 - ▶ MAJOR ISSUE: targeting
- ▶ 3) **Topical delivery** – applied as a cream, lotion, or other solution directly to the skin
 - ▶ MAJOR ISSUE: penetration

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 - ▶ First target tissue of IV injected – LIVER
 - ▶ Bio-distribution study:
 - ▶ Therapeutic + Tracker Molecule
 - ▶ Tracker Molecule selection is critical
- ▶ 3) **Topical delivery**
 - ▶ MAJOR ISSUE: penetration



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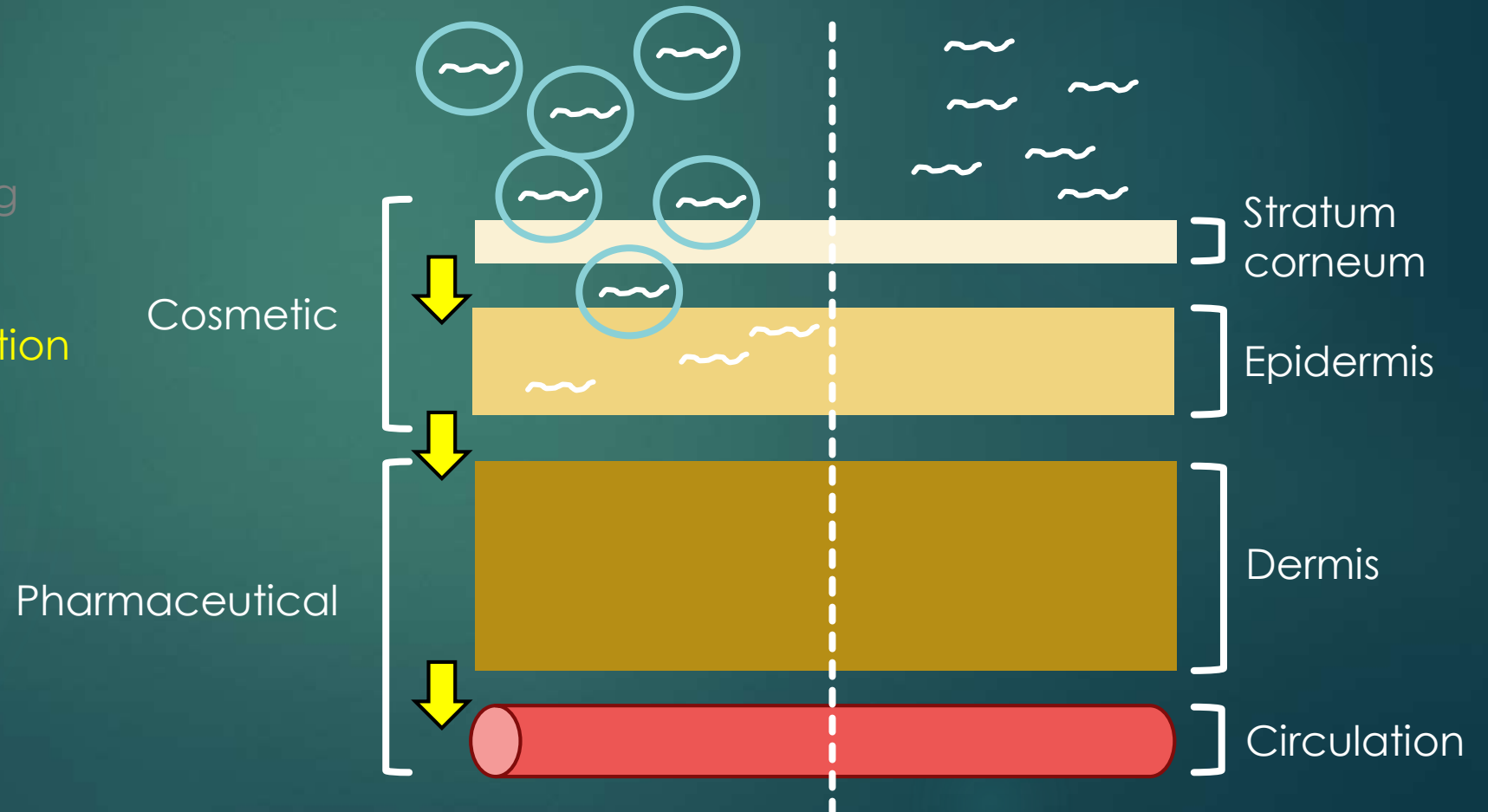
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SUMMARY

- ▶ Oligonucleotide based therapeutics are actively being investigated by both the cosmetic and pharmaceutical industries
- ▶ Many pharmaceutical and cosmetic products soon to reach the market
- ▶ Federal regulatory guidelines are still murky
- ▶ Due diligence validation, stability, QC/QA testing will be necessary to ensure DNA/RNA oligonucleotide efficacy and safety

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