



NEUROCARRUS

***Treating Pain
Without Addiction***

Targeted, Non-Opioid
Pain Therapeutics



Opioid prescriptions in England nearly doubled in 10 years - report

The Guardian

2018: The opioid epidemic rages on...

By DEAN REYNOLDS | CBS NEWS | March 6, 2018, 6:45 PM

CDC: Opioid overdoses kill almost 5 people every hour in the U.S.

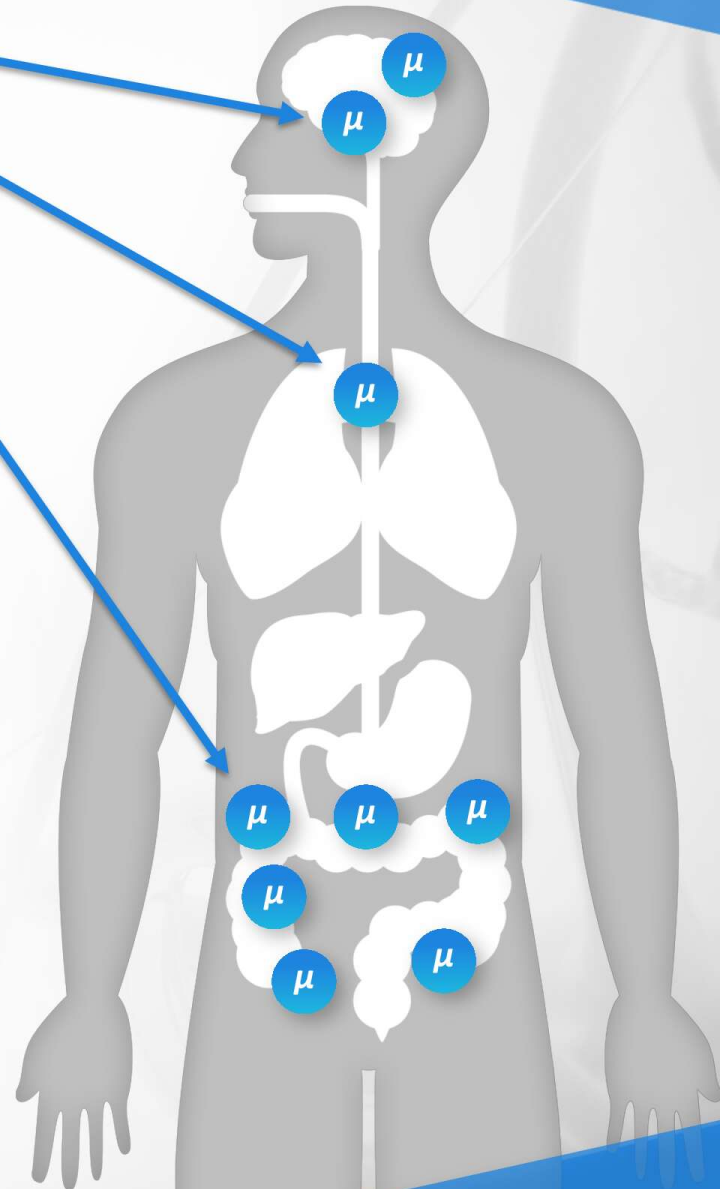
By ASHLEY WELCH | CBS NEWS | March 6, 2018, 2:44 PM

Opioid overdoses spike 30 percent, hospitals report

Opioids block pain through interaction with μ (mu)-receptors.

But interaction with mu-receptors in *the brain* leads to an *addictive euphoria*, among other side effects.

Clearly, we need a *new treatment* for *pain*.



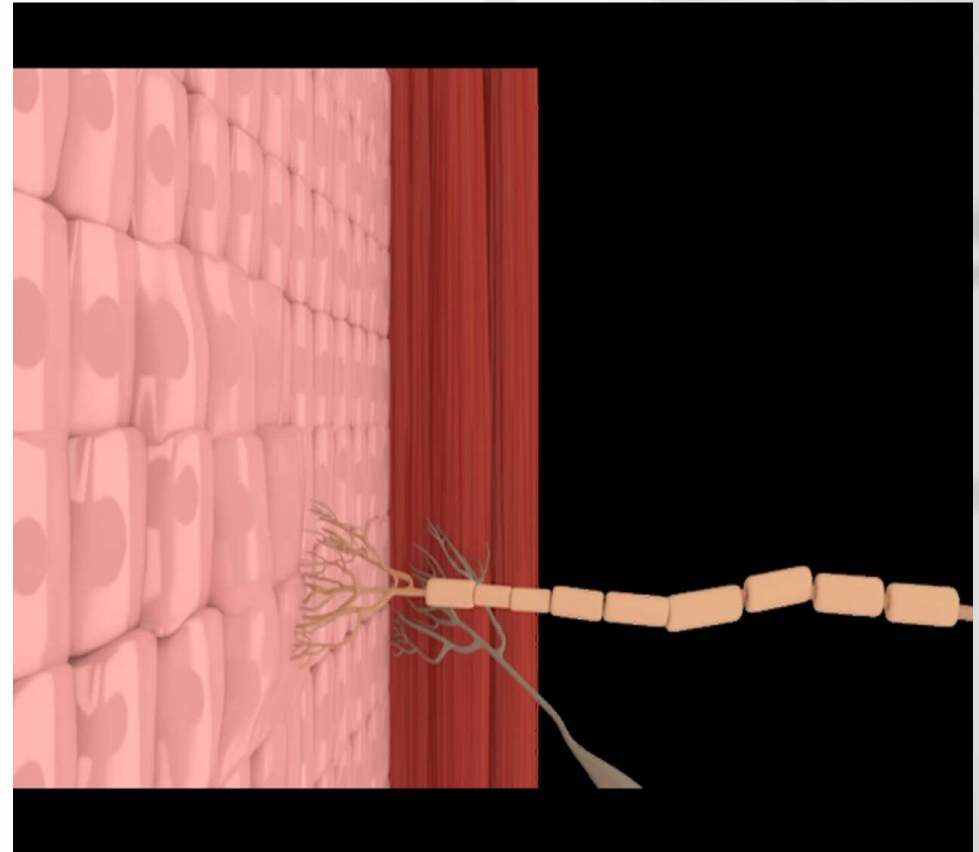
BOTOX freezes motor neurons...

...what if we could freeze pain neurons?

With N-001, we can.

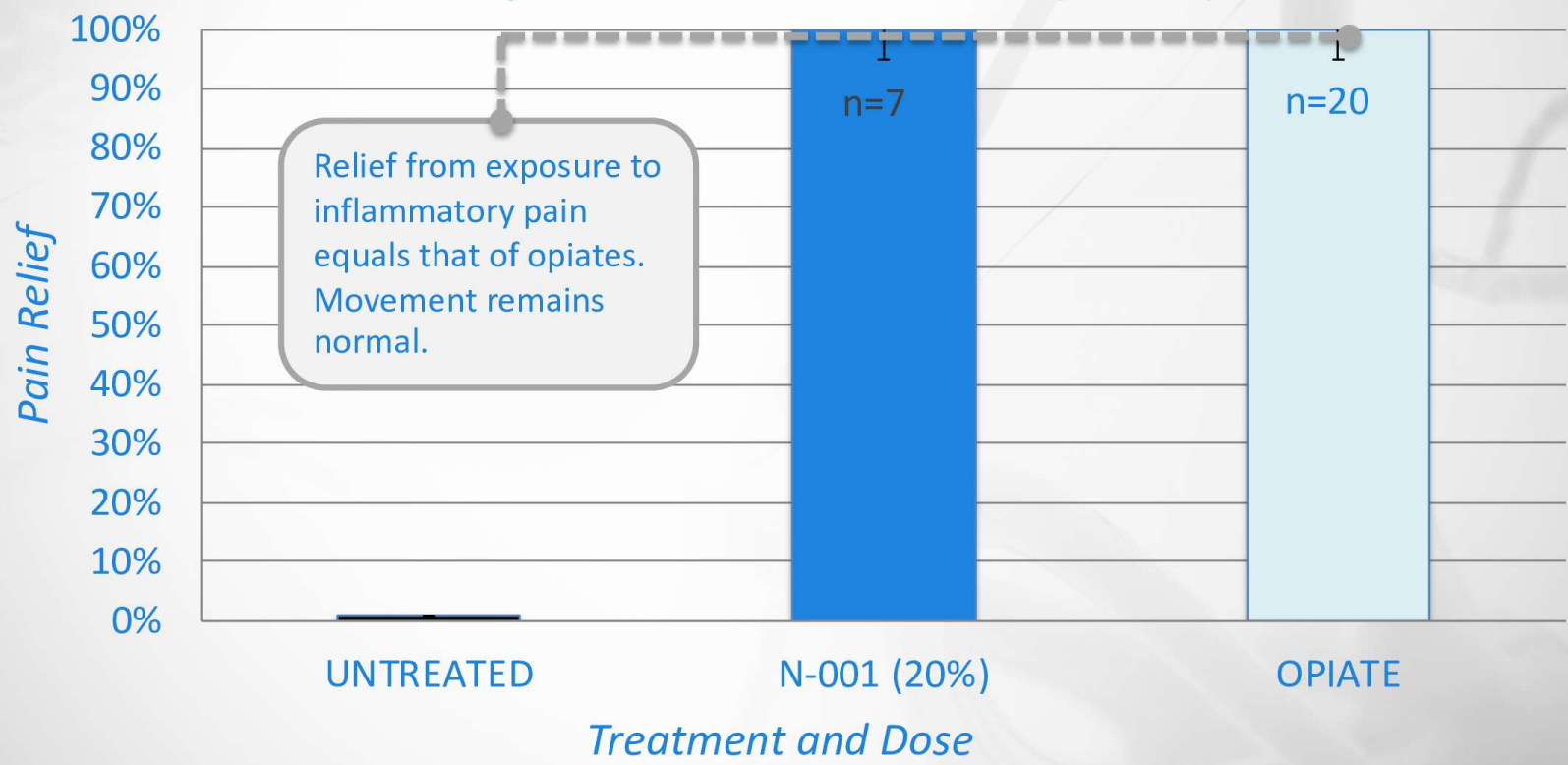
N-001 Profile

- A rationally designed protein that targets pain (sensory) neurons
- Disrupts pain signaling in neuronal axon through modification of cytoskeletal actin
- Locally administered at site of pain by injection or topical application
- Uses our novel drug delivery system, capable of transporting other drugs to targeted neurons



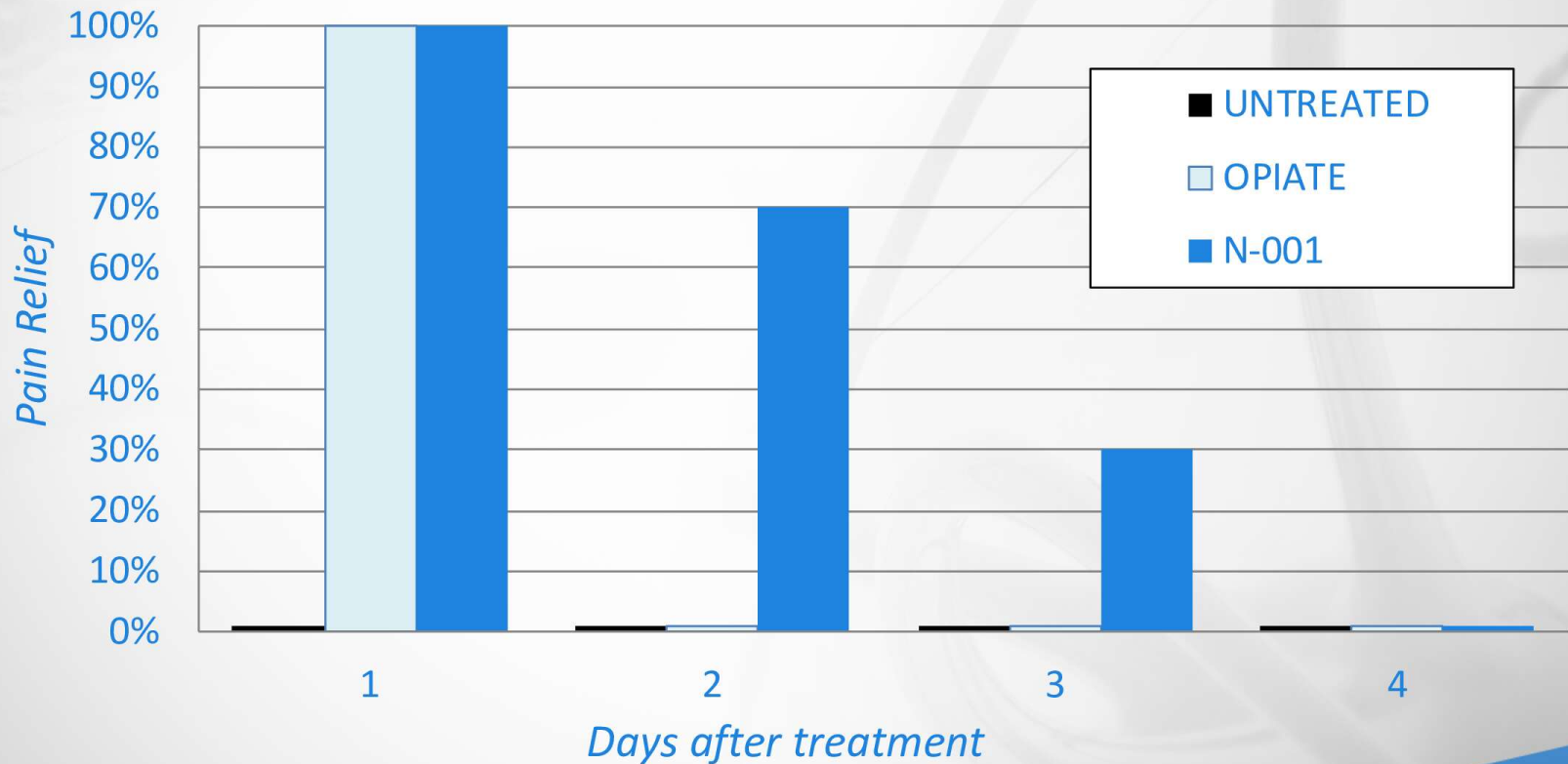
N-001 *eliminates* acute pain.

N-001 Vs. Opioid In Formalin Pain Test (Mouse)



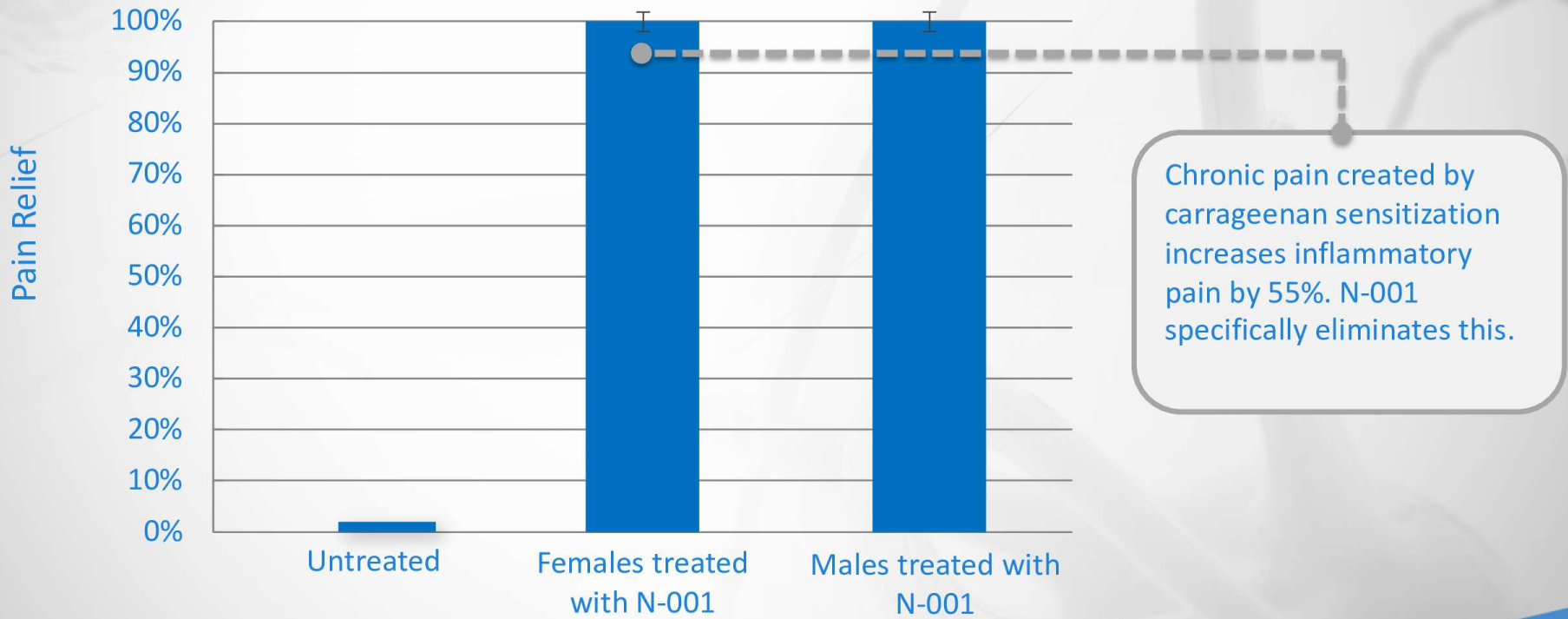
N-001 decreases pain for *longer* than opiates.

N-001 Duration In Formalin Pain Test (Mouse)



N-001 *eliminates* chronic pain.

N-001 Chronic Pain Test (Mouse), Carageenan Sensitized





Global Pain Treatment Market (Pharmaceutical):
\$32 billion

U.S. Diabetic Neuropathy patient population:
15 million

U.S. Osteoarthritis patient population:
30 million

PCT REQUEST		For receiving Office use only	
<p>The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.</p>		International Application No.	
		International Filing Date	
		Name of receiving Office and "PCT International Application"	
		Applicant's or agent's file reference (if desired) (12 characters maximum) 579814	
Box No. I	TITLE OF INVENTION		
	ENGINEERED CLOSTRIDIUM BOTULINUM TOXIN ADAPTED TO DELIVER MOLECULES INTO SELECTED CELLS		
Box No. II	APPLICANT		<input checked="" type="checkbox"/> This person is also inventor
<p>Name and address: (family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</p> PAVLIK, Benjamin J. 2203 Vine St. APT 8 Lincoln, Nebraska 68503 United States of America		<p>Telephone No.</p> <p>Facsimile No.</p> <p>Applicant's registration No. with the Office</p>	
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Form PCT/RO/101 (first sheet) (16 September 2012)

See Notes to the request form

Intellectual Property

Exclusive license for all fields of use (drug and delivery system)

PCT filed in 8 countries

Repurposed bacterial toxins for human therapeutics

Benjamin J. Pavlik¹, Kevin E. Van Cott¹ and Paul H. Blum²

www.nature.com/scientificreports

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Retargeting the *Clostridium botulinum* C2 toxin to the neuronal cytosol

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Benjamin J. Pavlik¹, Elizabeth J. Hruska², Kevin E. Van Cott¹ & Paul H. Blum^{1,2}

Many biological toxins are known to attack specific cell types, delivering their enzymatic payloads to the cytosol. This process can be manipulated by molecular engineering of chimeric toxins. Using toxins with naturally unlinked components as a starting point is advantageous because it allows for the development of payloads separately from the binding/translocation components. Here the *Clostridium botulinum* C2 binding/translocation domain was retargeted to neural cell populations by deleting its non-specific binding domain and replacing it with a *C. botulinum* neurotoxin binding domain. This fusion protein was used to deliver fluorescently labeled payloads to Neuro-2a cells. Intracellular delivery was quantified by flow cytometry and found to be dependent on artificial enrichment of cells with the polysialoganglioside receptor GT1b. Visualization by confocal microscopy showed a dissociation of payloads from the early endosome indicating translocation of the chimeric toxin. The natural *Clostridium botulinum* C2 toxin was then delivered to human glioblastoma A172 and synchronized HeLa cells. In the presence of the fusion protein, native cytosolic enzymatic activity of the enzyme was observed and found to be GT1b-dependent. This retargeted toxin may enable delivery of therapeutics to peripheral neurons and be of use in addressing experimental questions about neural physiology.

Naturally occurring neurotoxins have long been used to study neural physiology, and the exploitation of modified biological neurotoxins as drug delivery systems is expanding^{1,2}. These toxin-based delivery systems are multi-domain proteins that bind target cells and translocate material (payloads) across the lipid bilayer into the cytosol of the targeted cell. These systems are altered AB-type toxins, consisting of a payload domain (A) and a binding/translocation domain (B). The A and B domains can be covalently linked by a polypeptide or disulfide bond that is later cleaved during the translocation step³⁻⁵. Non-covalently linked (binary) A and B toxin domains are transcribed and translated independently and associate prior to exerting toxicity. These binary systems have recently been studied in the context of payload delivery to cancer cells⁶. It is advantageous from a protein engineering perspective to design separately expressed molecules because binding/translocation and payload modules can then be developed independently. The *Clostridium botulinum* C2 toxin (C2) is not a neurotoxin, but it has a binary AB toxin design and been shown to deliver a variety of engineered payloads in a non-specific manner to a variety of cells⁷⁻¹⁴. It was not known if the binary AB-type C2 toxin structure could be used as a platform to introduce a new binding specificity and deliver molecular payloads. Here it was hypothesized that by replacing the C2 toxin binding domain with a *C. botulinum* neurotoxin (BoNT) serotype C1 binding domain (C1 H_{CD}), the engineered B domain and payload could be expressed separately, combined and enable targeting of neural cells, while preserving the normal C2 translocation process.

The native C2 toxin is composed of two separate proteins. The B domain protein (C21) binds target cells and translocates the A domain (C2), the payload. The A domain is an ADP-ribosyltransferase that causes cell rounding and apoptosis initiated by ADP-ribosylation of cytoplasmic actin¹⁵⁻¹⁷ (Fig. 1a). C21 monomers are proteolytically processed to remove a 20 kDa segment from the N-terminus, which activates the binding/translocation domain into C21a¹⁸. C21a monomers then spontaneously oligomerize and bind the cell surface via interactions with asparagine-linked glycans on the cell membrane^{11,18}. The A domain, C2, binds to the C21a oligomers and the C21a/C2 complex is internalized by clathrin and Rho-dependent mechanisms^{17,19,20}. Acidification of the early endosome causes membrane pore formation by C21a oligomers, through which C21 is transported into the cytoplasm^{21,22}.

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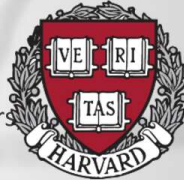
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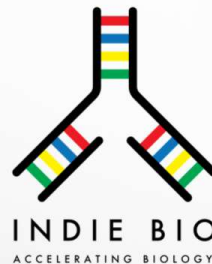
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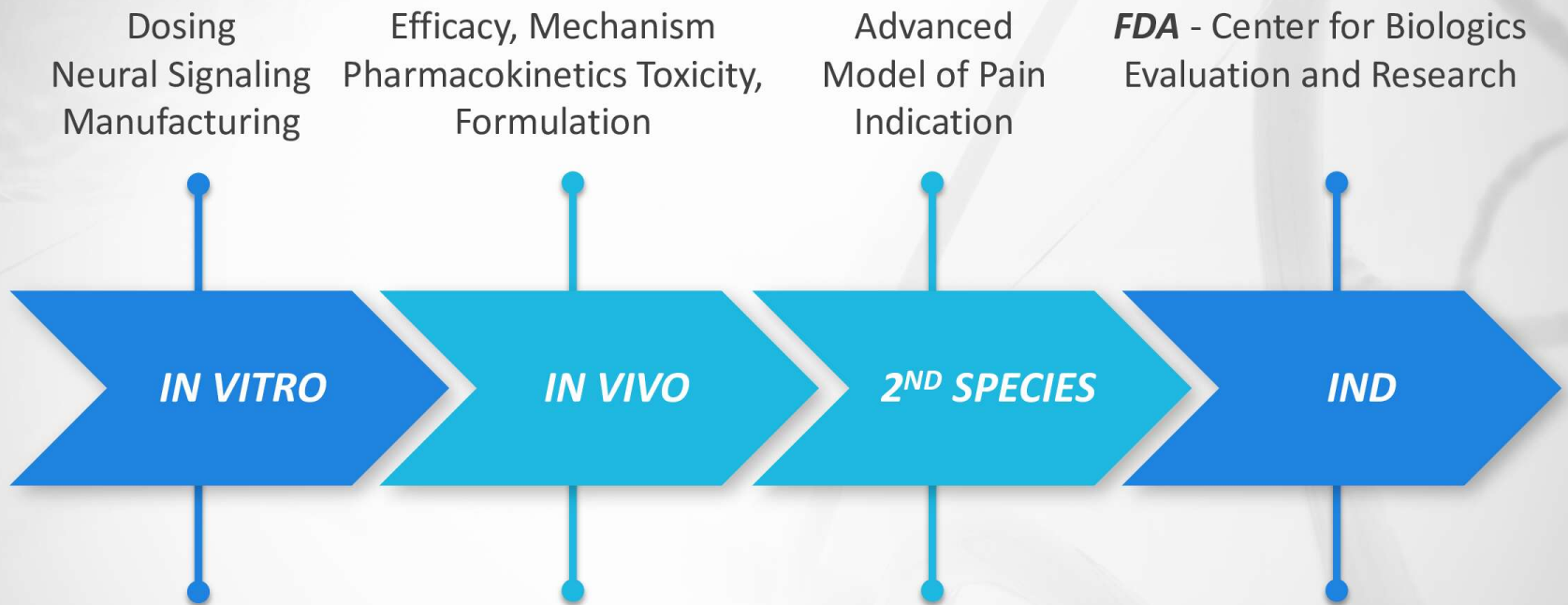
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