Drug screening and development using animal disease models

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Drug screening

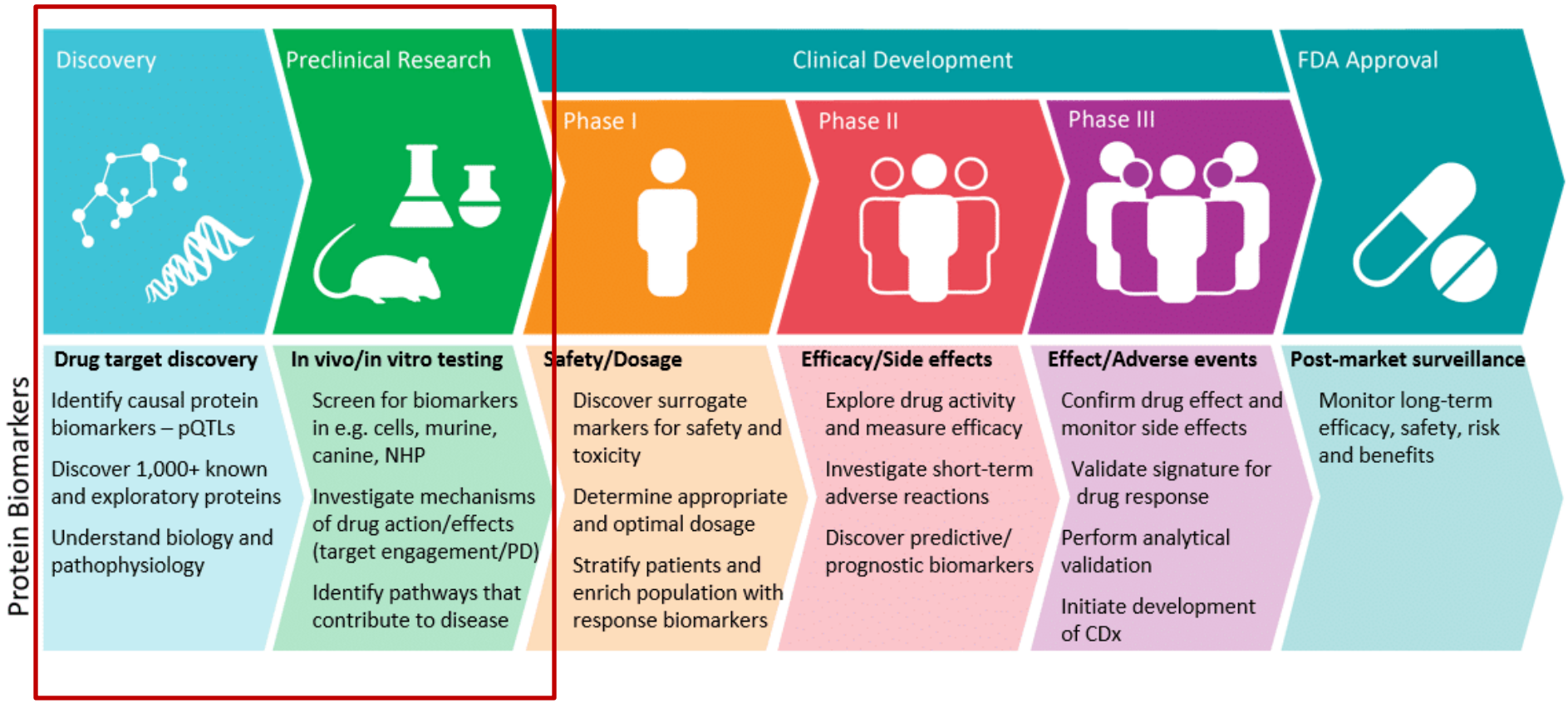
- A process by which potential drugs are identified and optimized before selection of a candidate drug to progress to clinical trials.
- It can involve screening large libraries of chemicals for a particular biological activity in high-throughput screening assays

Basic terms

- **Target:** molecular entity with a biological function
- **Hit:** a chemical having a significant degree of activity at a particular molecular target.
- **Lead:** a chemical having significant activity at a molecular target whose structure is, or is thought to be, readily modified to improve selectivity or toxicological and pharmacokinetic properties necessary for investigation in human).
- **Phenotype:** observable properties of an cell or organism

Markou A et al., *Neuropsychopharmacology* 2009

Where are animals needed?



Animal models are involved in target discovery and preclinical research phases

https://www.olink.com/

Comparison of animal models attributes

Attribute of disease model	Model organism			
	Fly	*Zebrafish*	*Mouse*	*Rat*
Practical issues				
Husbandry infrastructure	$	$	$$$	$$$
Cost per animal per year	$	$	$$$	$$$
Characterized inbred strains	+	-	++++	+++
Outbred laboratory strains	+	+++	++	++
Anatomical similarity	-	+	++	++
Molecular or genetic similarity	+	++	+++	+++
Pathological similarity	-	++	+++	+++
Storage; for example, freezing sperm	No	Yes	Yes	Yes
Molecular biology tools				
Transgenesis*	++	++	++	++
Targeted gene modification*	+	-	++++	+
Transient *in vivo* assays*	++	++++	+	+
Allelic series from TILLING*	+++	++++	++	+
Feasibility of large-scale screens‡	++++	+++	++	+
Affordability of large-scale screens‡	++++	+++	+	-
Sequencing progress§	+++	++	+++	++
Annotation progress§	++	++	++++	++
Cell-biology tools				
Cell lines and tissue culture	++	+	++++	+
Antibody reagents	++	+	++++	++

Lieschke, G. J., & Currie, P. D. *Nature Reviews Genetics* 2007.

Strategies in drug discovery

- Phenotypic drug discovery
- Target-based drug discovery

Phenotypic drug discovery

- a strategy to identify molecules with the ability to alter a cell's or animals phenotype.
- does not rely on knowing the identity of the specific drug target or its hypothetical role in the disease
- Examples :
  - Morphine, Quinine, Paracetamol, Oxytocin, Insulin, Aspirin, Isoniazid, Chlordiazepoxide
  - Drugs for spinal muscular atrophy (SMA), cystic fibrosis (CF), and hepatitis C

Moffat JG et al., *Nat Rev Drug Disc.*, 2017
Swinney DC & Lee JA *F1000Res* 2020

Target-based drug discovery

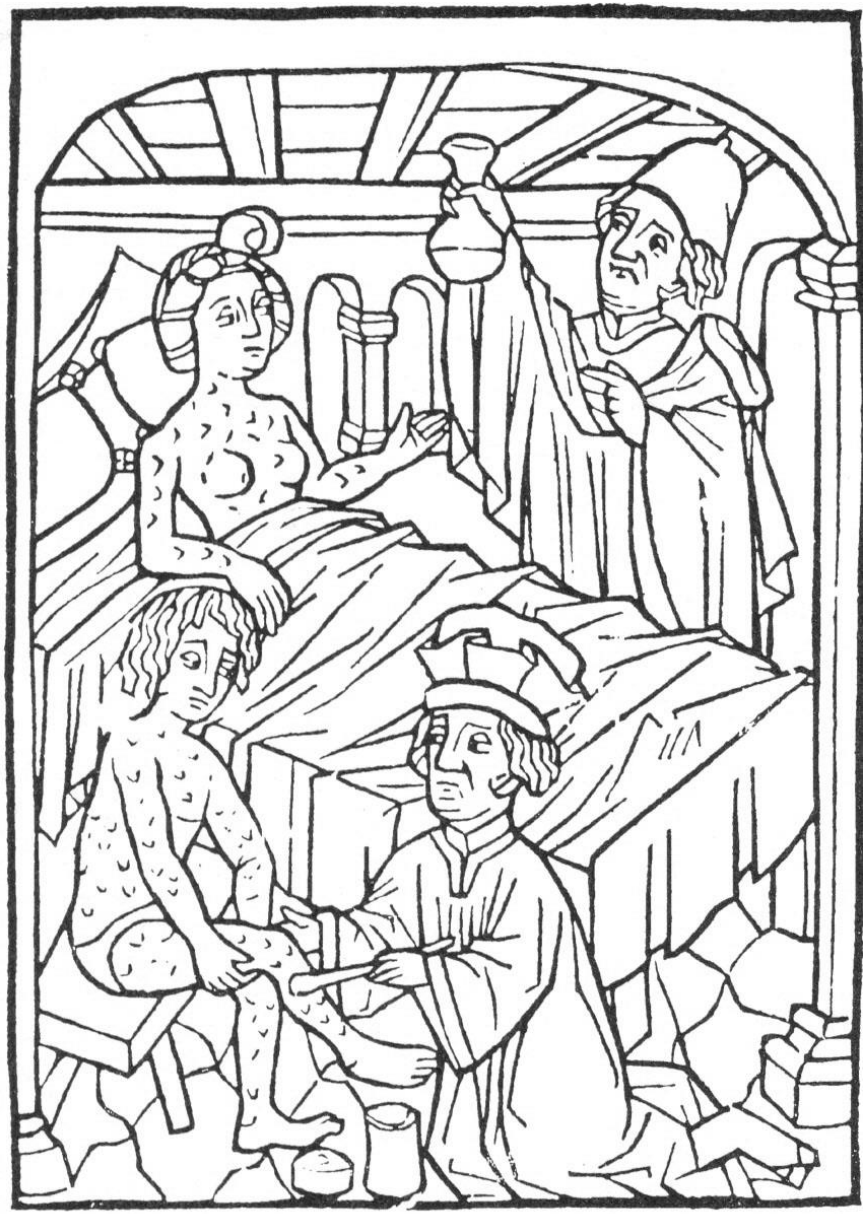
- Also called as Rational drug discovery
- in which the starting point is a defined molecular target that is hypothesized to have an important role in disease
- Due to advances in molecular biology and genomics, a dominant approach to drug discovery in the pharmaceutical industry,
- Examples:
  - 1987: Fluoxetine (SSRI* for depression)
  - 1998: Celecoxib (COX2 inhibitor for pain and inflammation)
  - 2001: Imatinib (bcr-abl inhibitor for cancer)
  - 2003: Omalizumab (anti-IgE for asthma),
  - 2004: Bevacizumab (anti-VEGF for cancer)

Moffat JG et al., *Nat Rev Drug Disc.*, 2017

Phenotypic drug discovery case studies

Syphilis

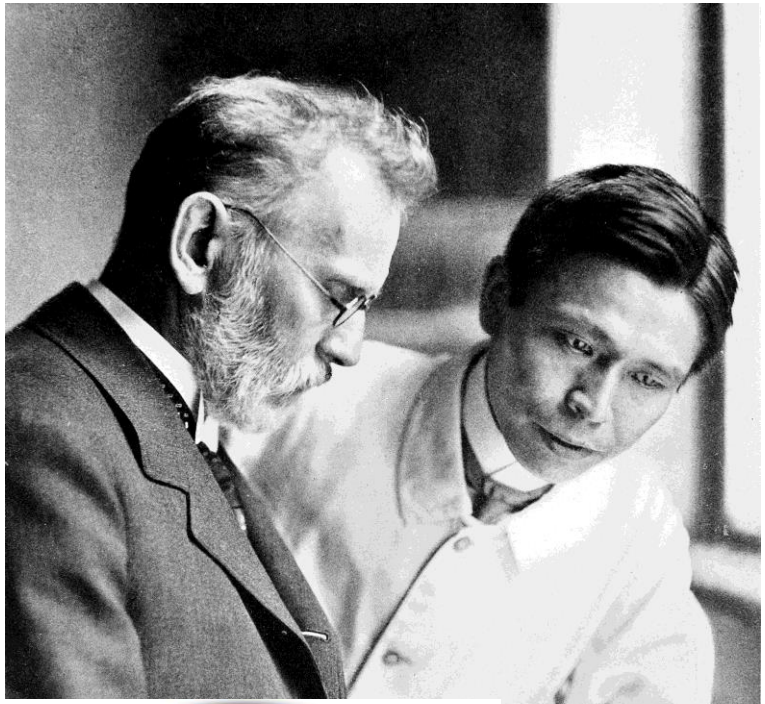
- Sexually transmitted infection
- Caused by bacterium *Treponema pallidum*
- Symptoms include
  - Skin lesion in infected site
  - Skin rashes

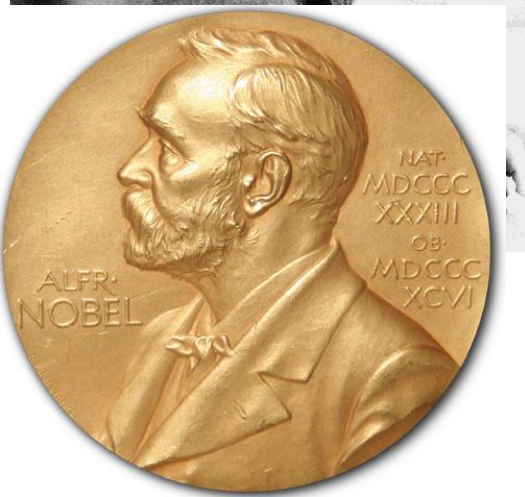


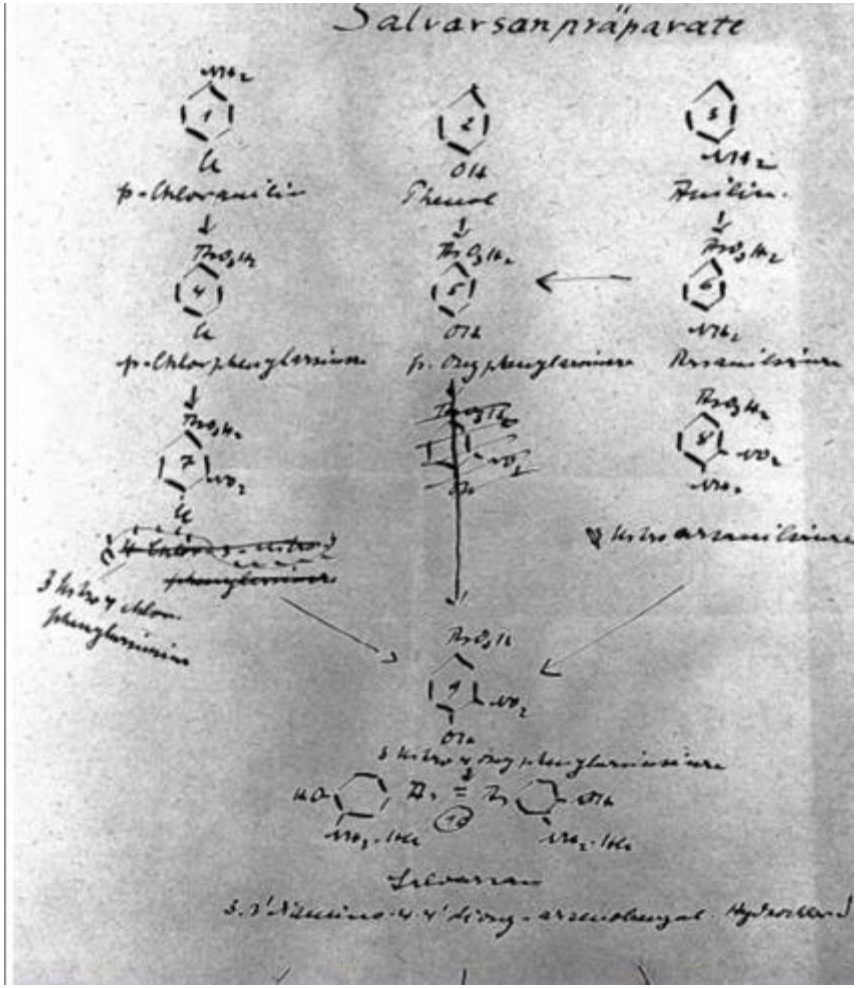
https://www.niaid.nih.gov/diseases-conditions/syphilis

Drug screen for syphilis

Paul Ehrlich & Sahachiro Hata







Drug screen for Syphilis

- mice were intraperitoneally injected with blood containing *Trypanosoma* bacterium
- Inject each mice with a candidate compounds (arsenic-derivatives)
- Several 100s of compounds were screened
- Blood were tested daily for bacterium

Ehrlich P & Hata S *Springer* 1910

Drug screen for Syphilis in rabbits

Tabelle I. Verlauf des Mäuserecurrens bei verschieden starker Infektion.

Tage nach der Infektion	Tödliche Infektion mit stark virulentem Stamm				Starke Infektion mit stark virulentem Stamm				Mäßige Infektion mit stark virulentem Stamm			Schwächere Infektion mit schwächerem Stamm	
	a	b	c	d	a	b	c	d	a	b	c	a	b
1	+(3–5)	+(3–5)	+(5–7)	+(3–5)	+($\frac{1}{1-2}$)	+($\frac{1}{2}$)	+ (1)	+($\frac{1}{1\ 2}$)	+($\frac{1}{3}$)	+($\frac{1}{5}$)	+($\frac{1}{10}$)	+ w.	+ w.
2	+ + +	+ + +	+ + +	+ + +	+ (8)	+ (8–10)	+ (10)	+ (8–10)	+ (8)	+ (8)	+ (5)	+ (4)	+ (2)
3	+ + +	+ + + +	+ + + +	+ + + +	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ +	+ +	+ +
4	+ + +	tot	+ + + +	+ + + +	+ + + +	+ + +	+ + + +	+	—	—	—	—	+ w.
5	+		tot	+ +	tot	—	+ w.	+ w.	—	—	—	—	+ s. w.
6	+			tot		—	+ w.	+ s. w.	—	—	—	—	—
7	+ w.					—	+	+	—	+	+ w.	—	—
8	+ s. w.					+	+	+	+	+ w.	+	+ w.	+
9	+					+	+	+	+	+	+	+ w.	+
10	+					+ w.	tot	+ w.	—	—	—	+ s. w.	—
11	+ +					—		—	—	+ w.	—	+ s. w.	—
12	tot					+		—	—	+ w.	—	—	—
13						+		—	+	—	+	+ w.	+
14						—		—	+ w.	—	+ s. w.	+	+
15						—		—	+	—	+ w.	—	—
16						+		—	—	—	—	—	—
17						+		—	—	—	+ w.	—	+ w.
18						—		—	—	+ s. w.	—	—	—
19						—		—	—	+ w.	—	—	—
20						—		—	—	+	—	—	+ w.
21						—		—	—	—	+ w.	+ s. w.	+
22						—		—	—	—	+	—	—
23						—		—	—	—	—	+ w.	+ w.
24						—		—	—	—	—	—	—
25						—		—	—	+ s. w.	—	—	—
26						—		—	—	+	+ s. w.	—	—
27						—		—	—	—	—	+ s. w.	—
28						—		—	—	—	—	+	—
29						—		—	—	—	—	+ s. w.	+ w.
30						—		—	—	—	—	—	—
31						—		—	—	—	—	—	—
32						—		—	—	—	—	—	—
33												—	—
34												+	—
35												+	—
36												—	—
37												—	—

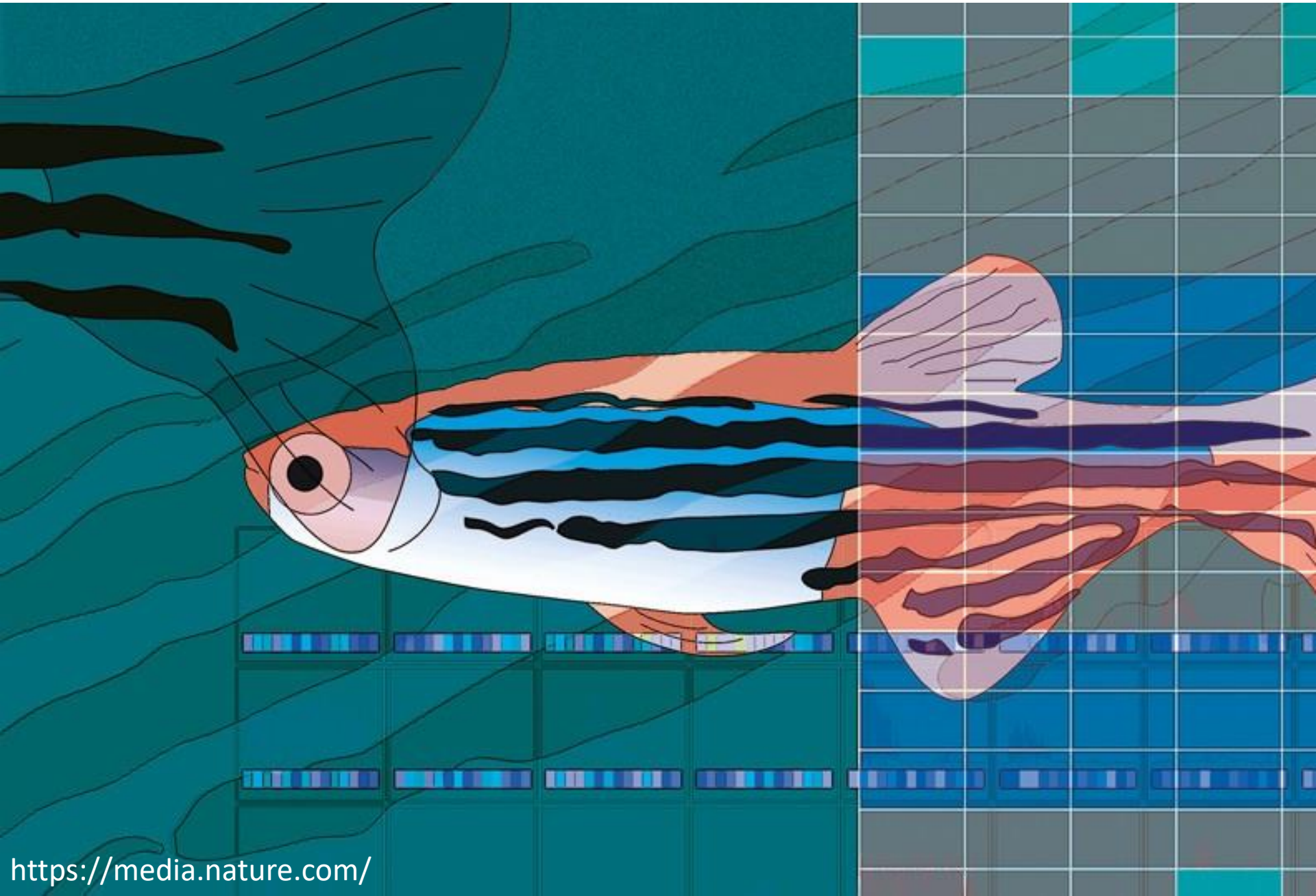
Ehrlich P & Hata S *Springer* 1910

Drug screen for Syphilis

- Compound 606, arsphenamine was able to reduce bacterial load in animals
- Was eventually tested in humans
- Marketed as Salvarsan in 1910 - first effective chemotherapeutic drug

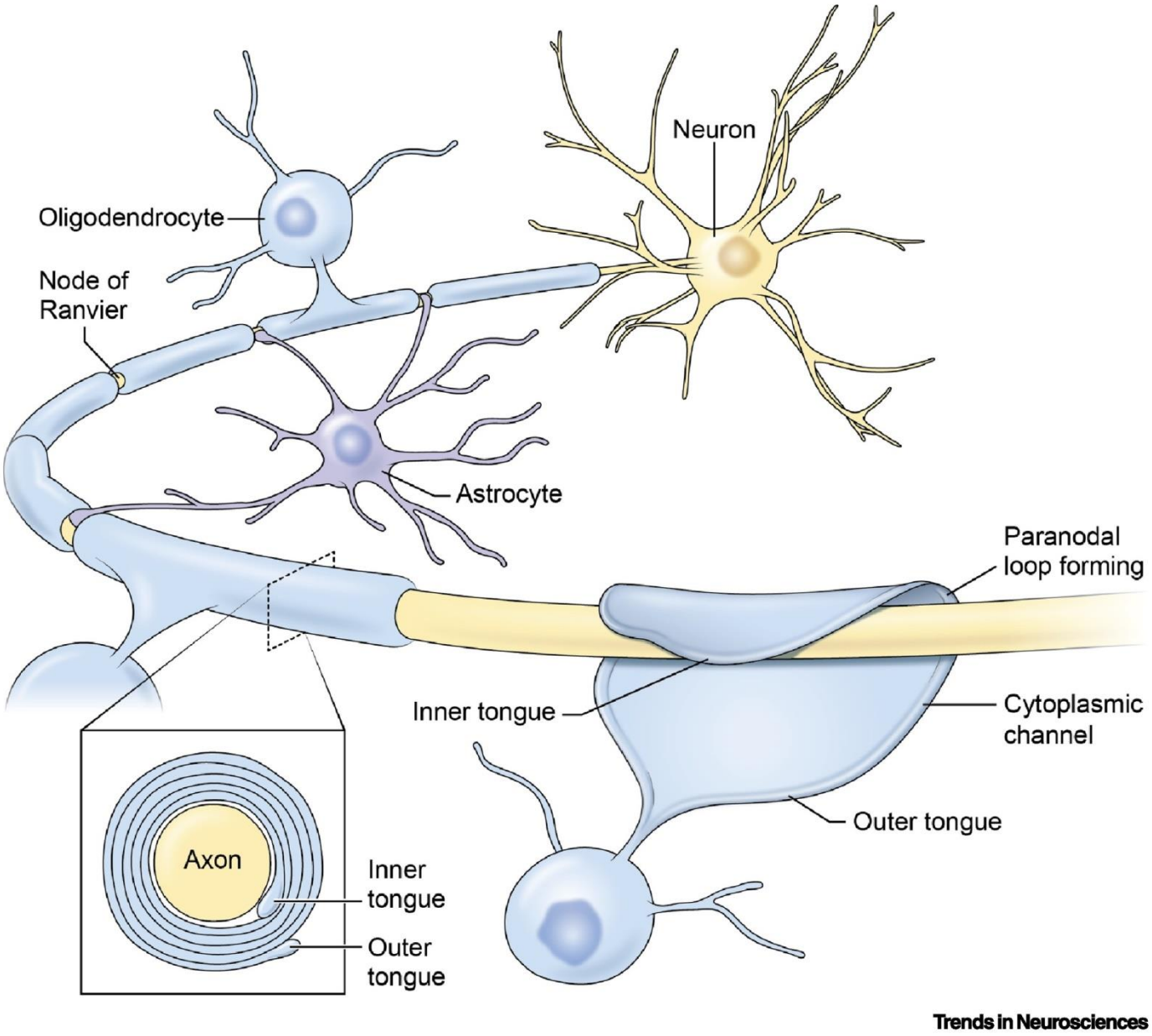
Ehrlich P & Hata S *Springer* 1910

Zebrafish in phenotypic drug screen



https://media.nature.com/

Oligodendrocytes myelinate axons



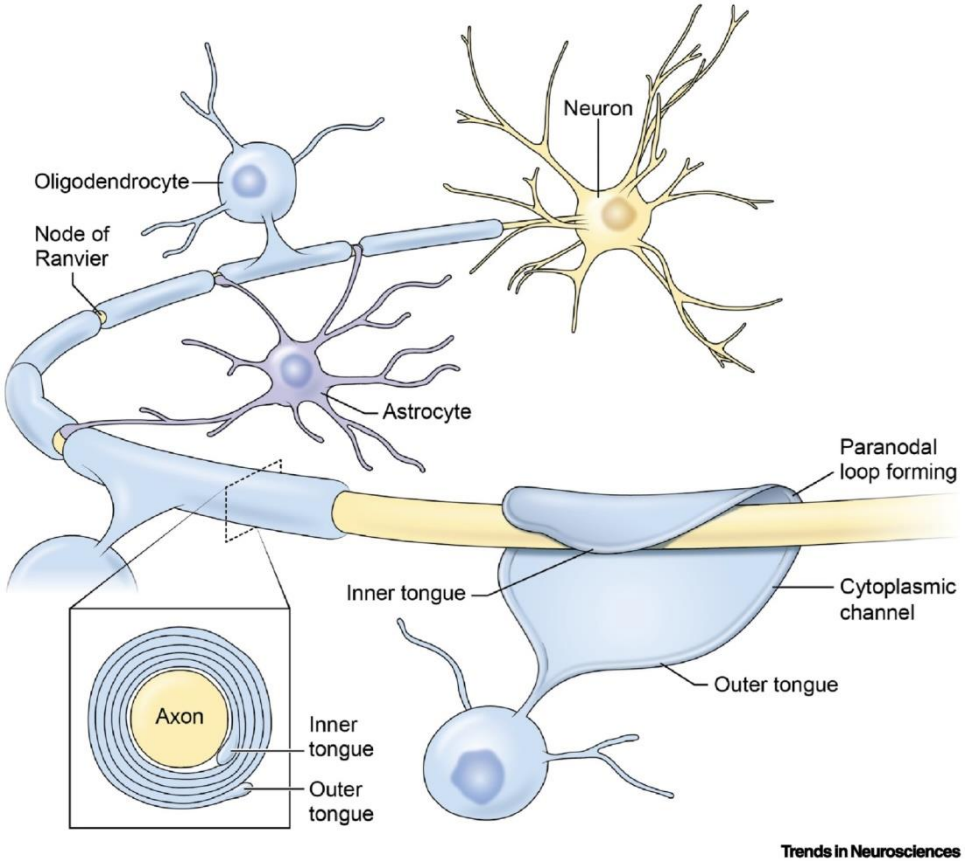
Fields RD & Dutta D *Trends Neurosci.* 2019

Myelination-associated diseases and disorders

- age-related cognitive decline
- neonatal hypoxia
- childhood leukodystrophies
- autism
- schizophrenia
- multiple sclerosis
- motor neuron disease
- Huntington’s disease
- Alzheimer’s disease

Early JJ et al., *eLife* 2018

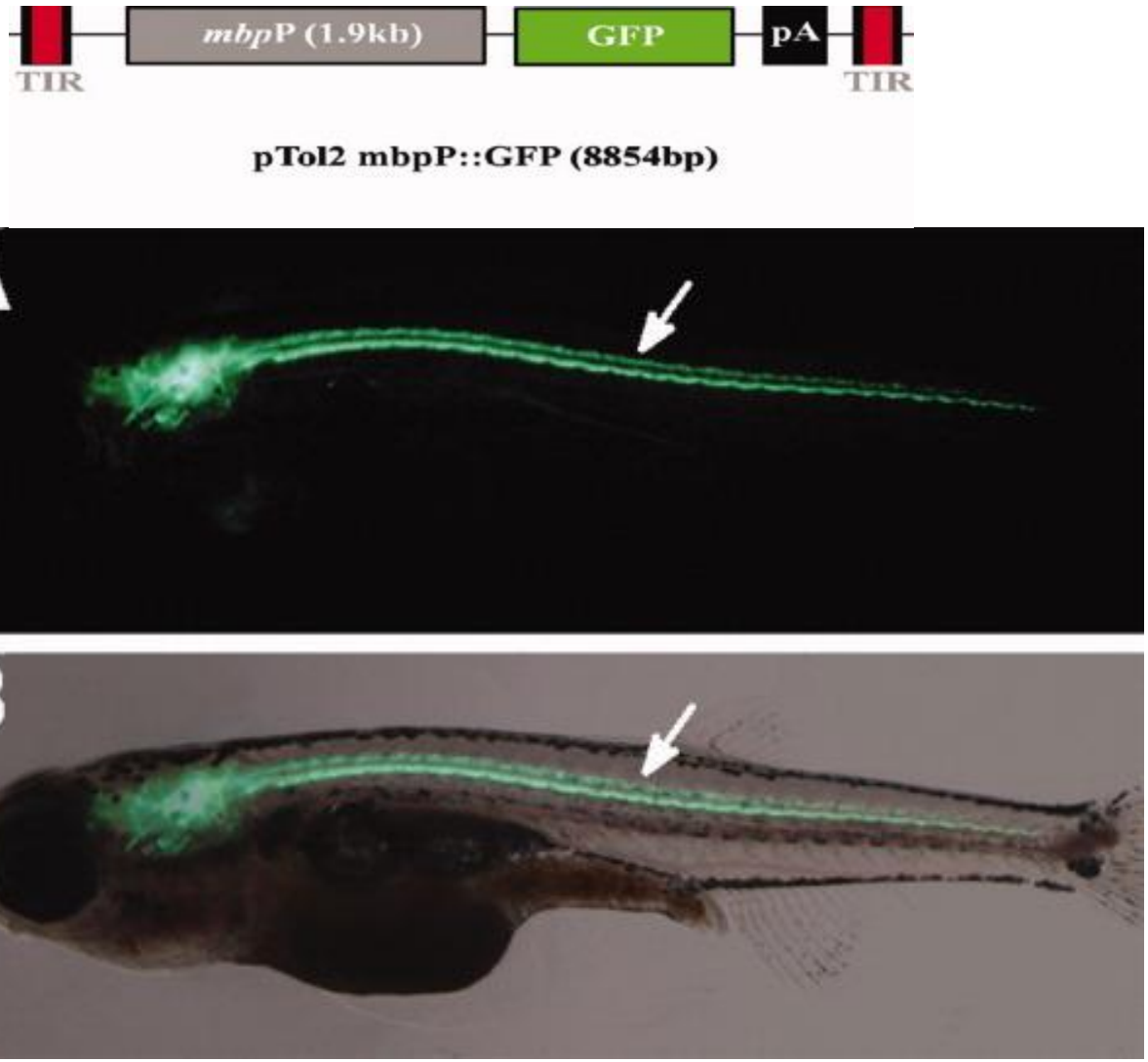
Oligodendrocytes myelinate axons



Find a drug that can increase olidodendrocytes or myelination with aim to treat myelination-associated diseases

Fields RD & Dutta D *Trends Neurosci.* 2019

Transgenic zebrafish can label oligodendrocytes



Jung SH et al., *Dev Dyn* 2010

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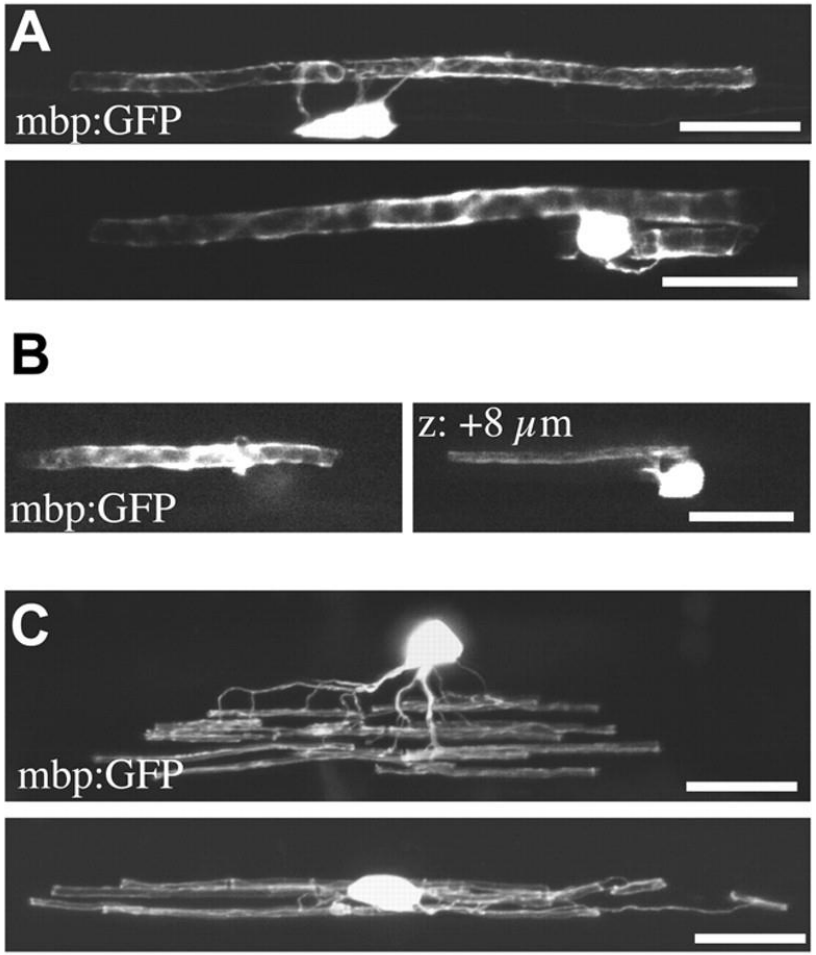
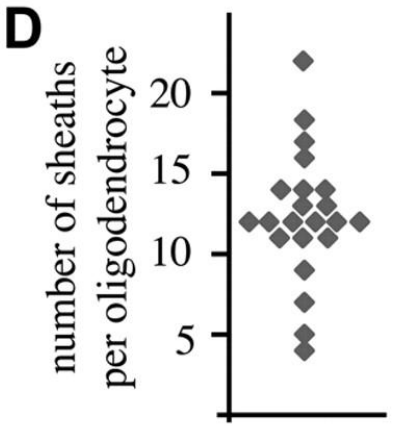
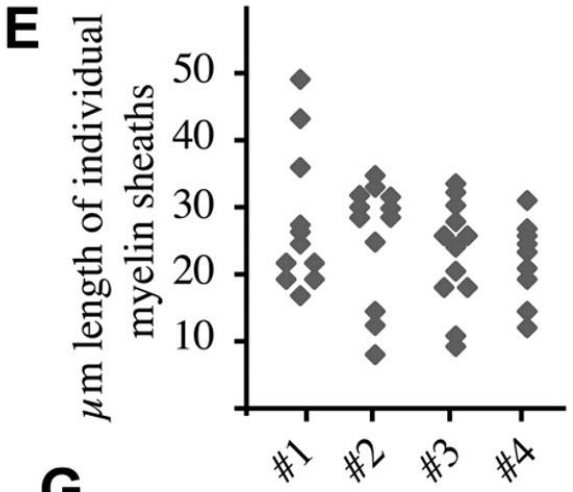
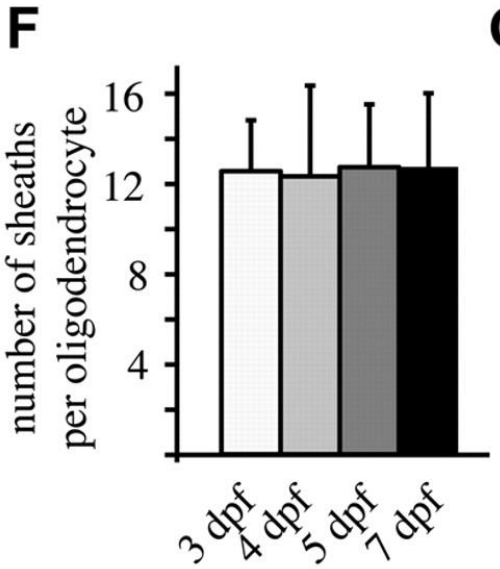
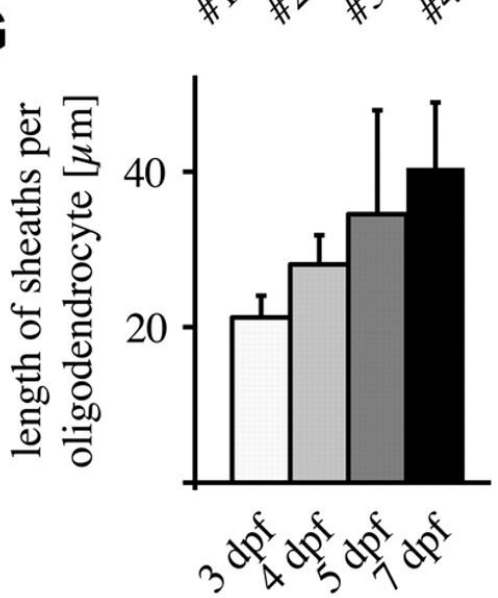




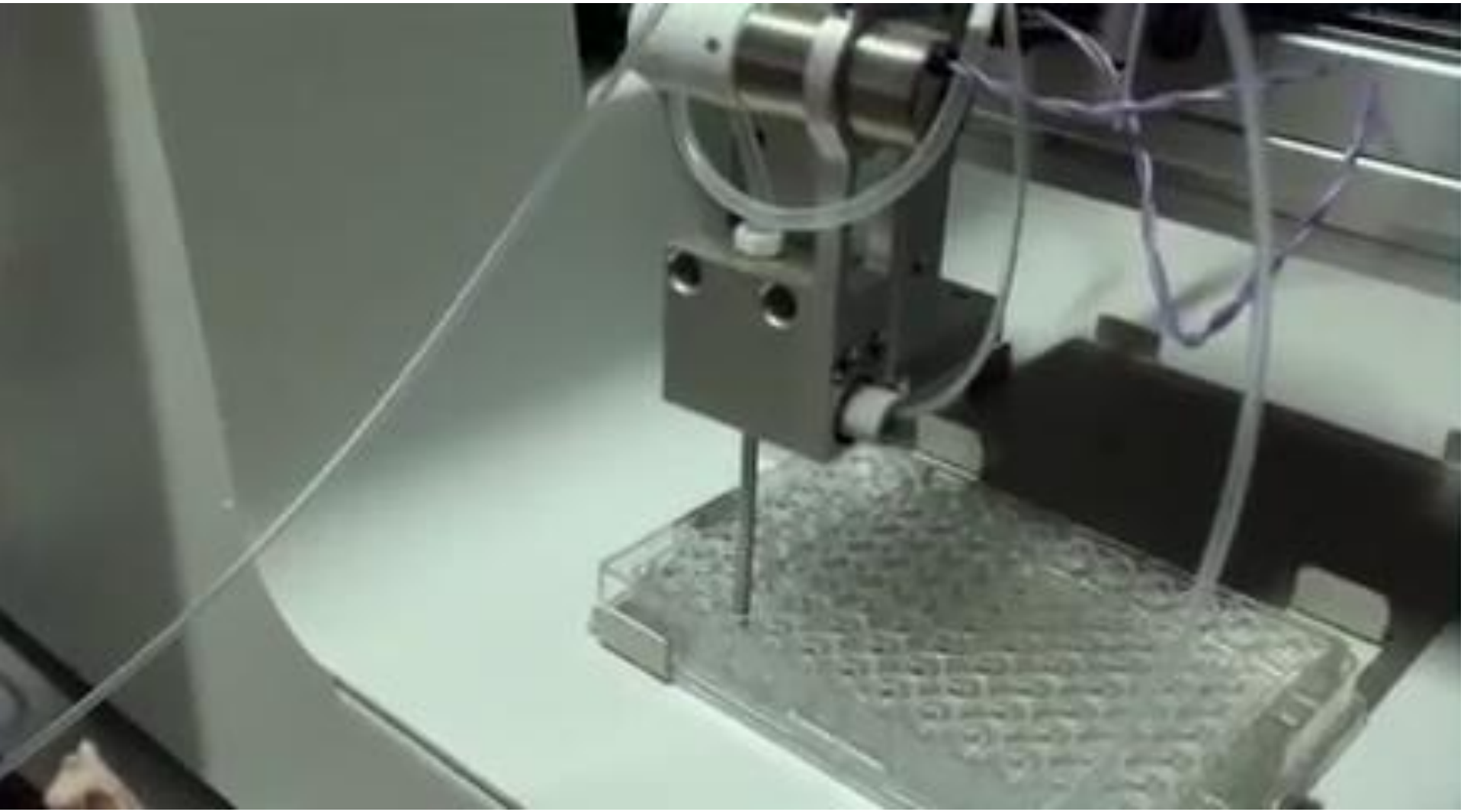






Almeida RG et al., *Dev* 2011

Automated-imaging of transgenic zebrafish larvae



https://youtu.be/t9fcRQWcUrU?t=2591

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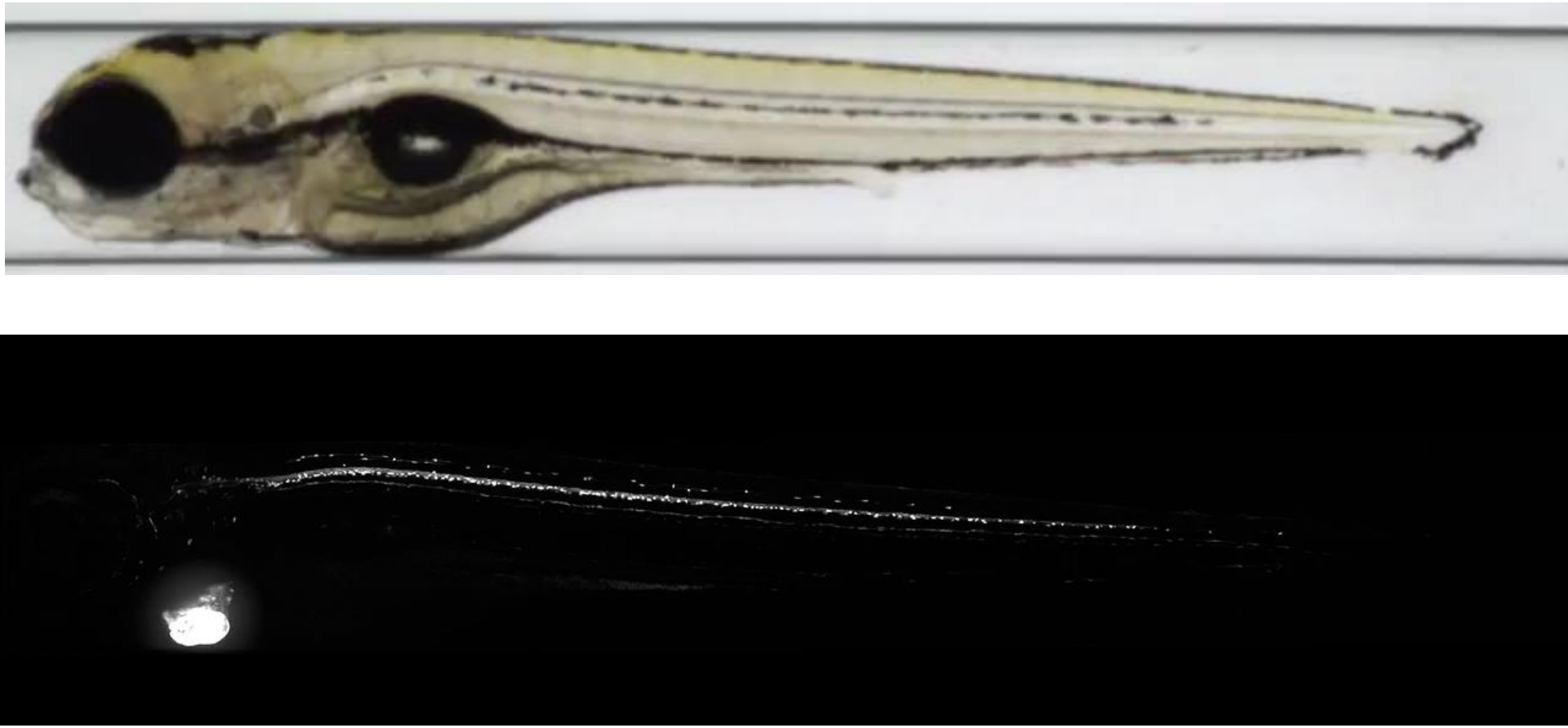
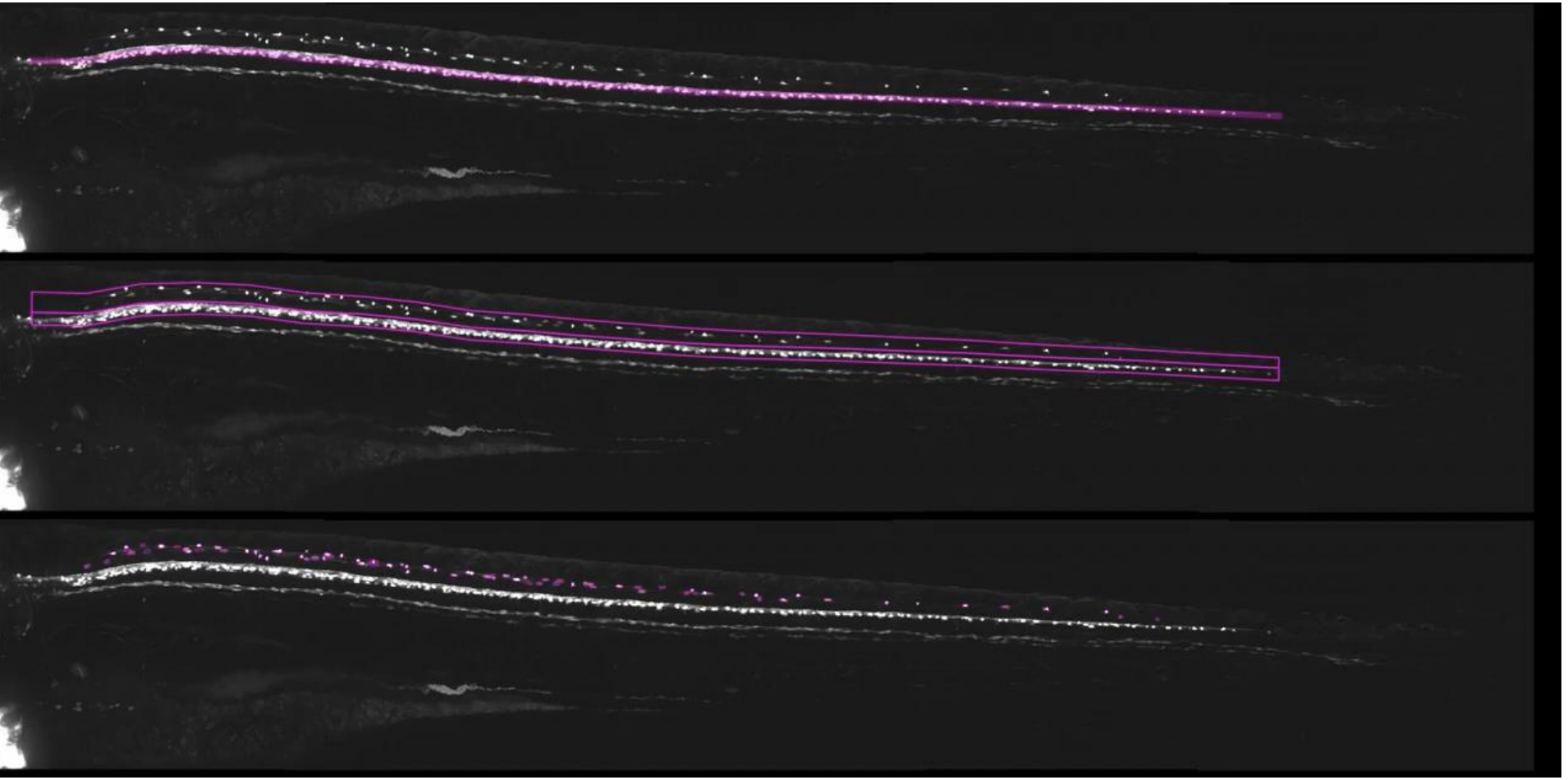


Early JJ et al., *eLife* 2018

Automated-image analysis to identify oligodendrocytes



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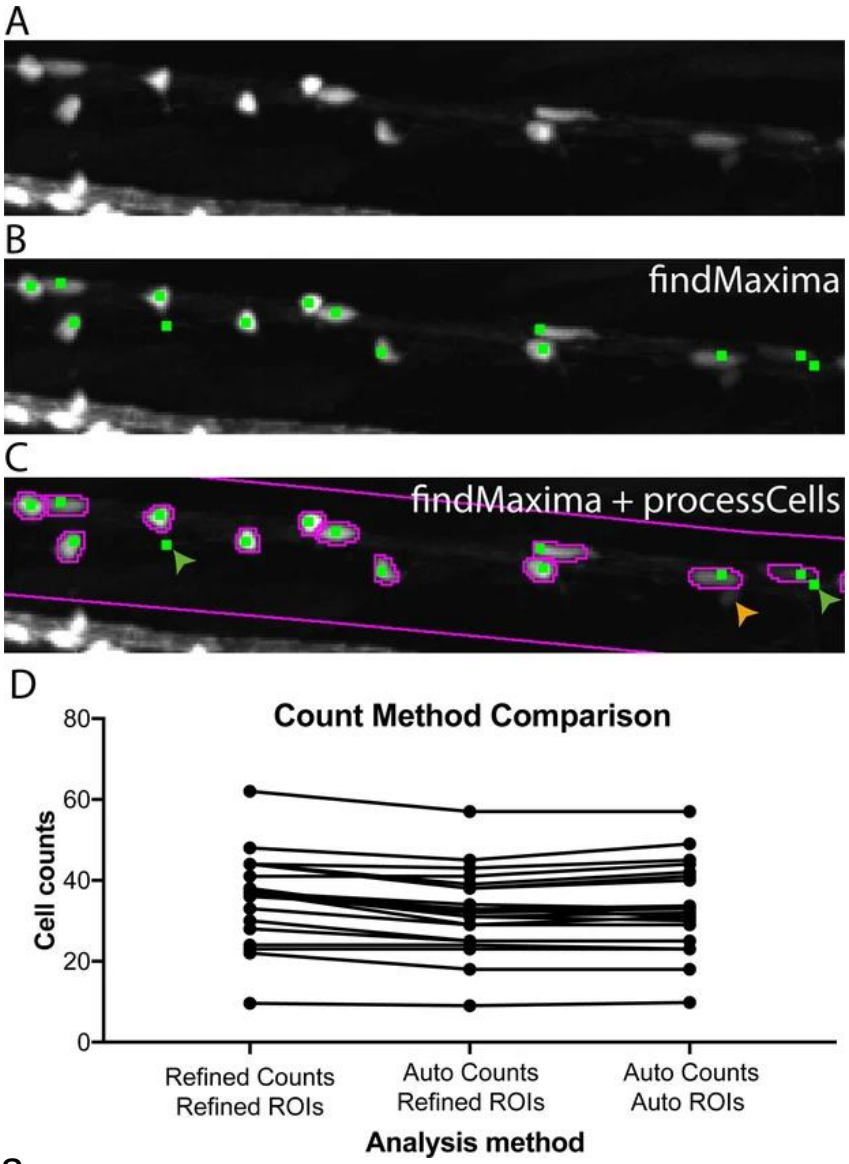
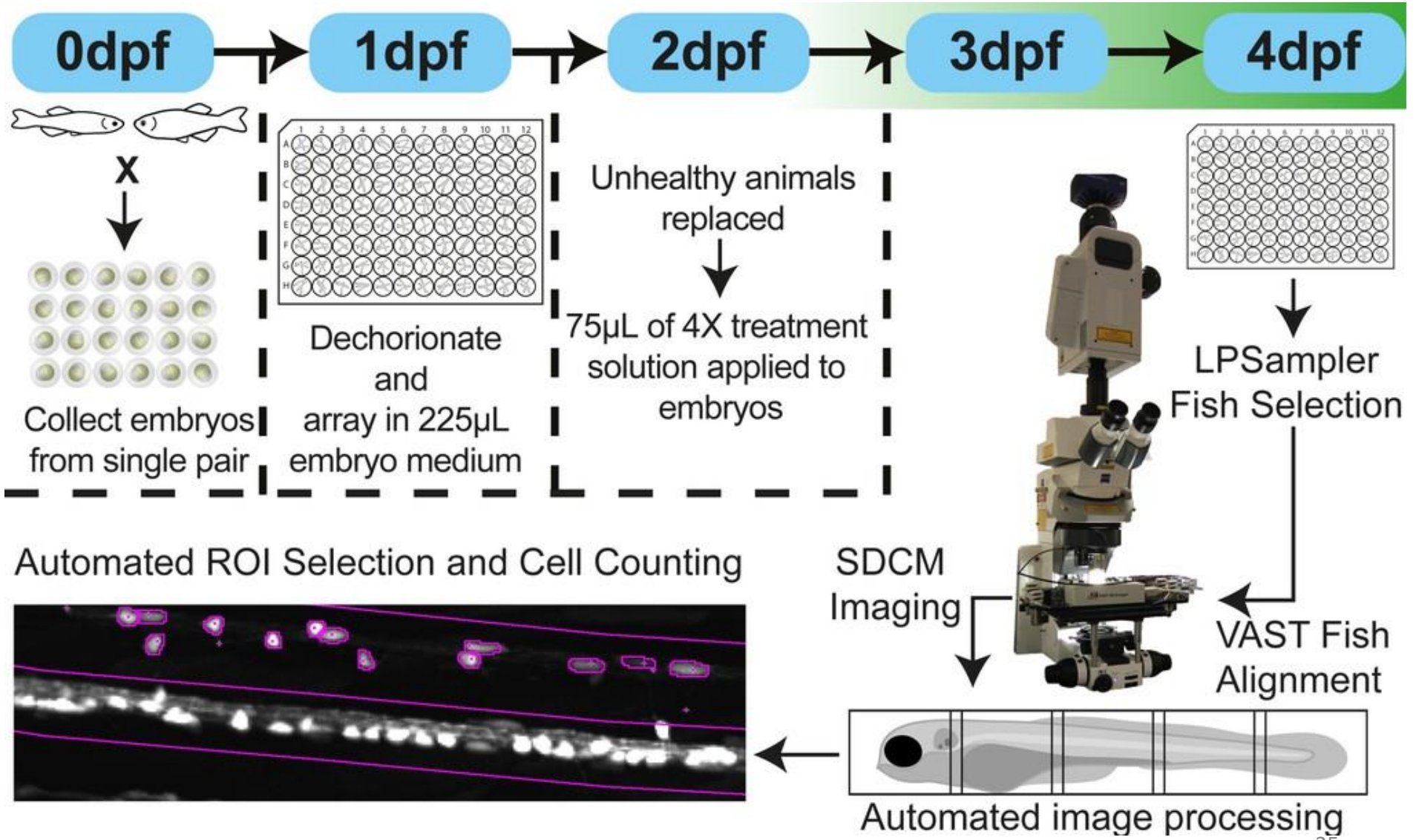


Early JJ et al., *eLife* 2018

Large scale chemical screen to identify oligodendrocyte regulators



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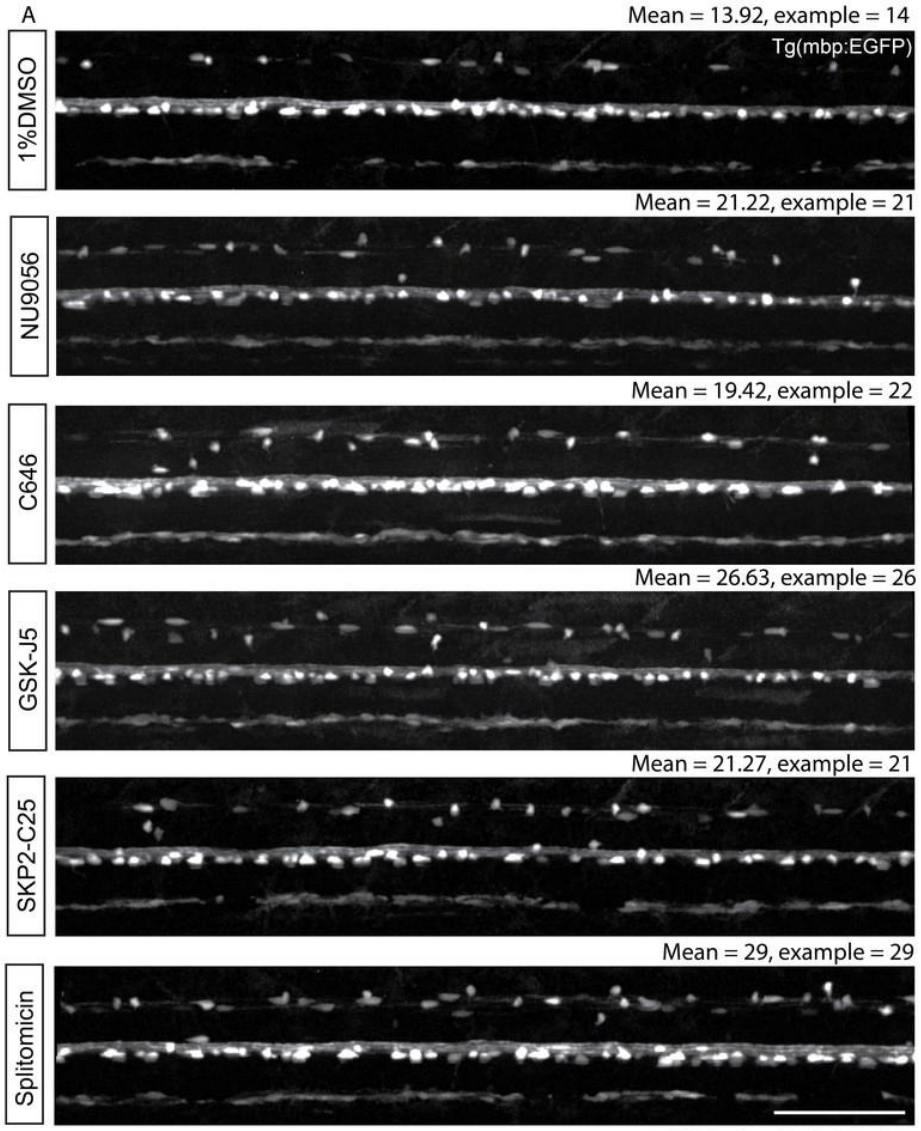

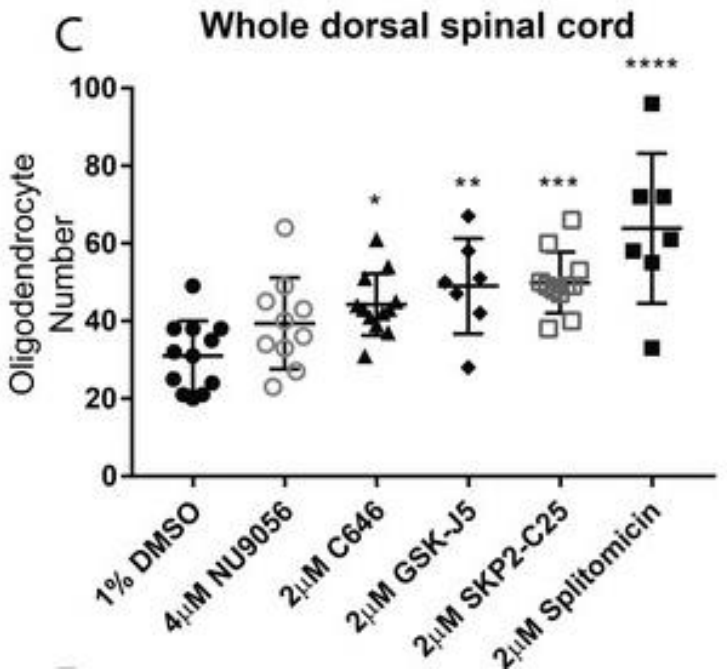

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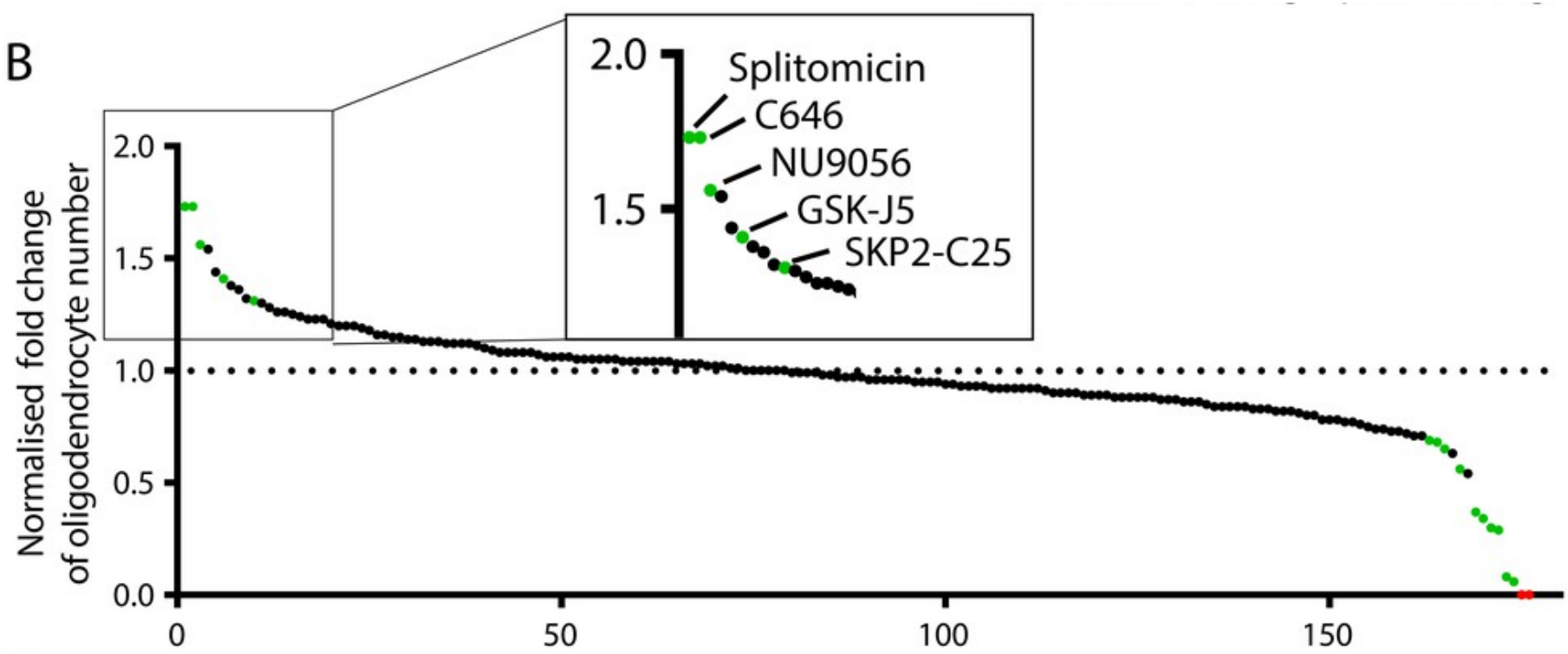
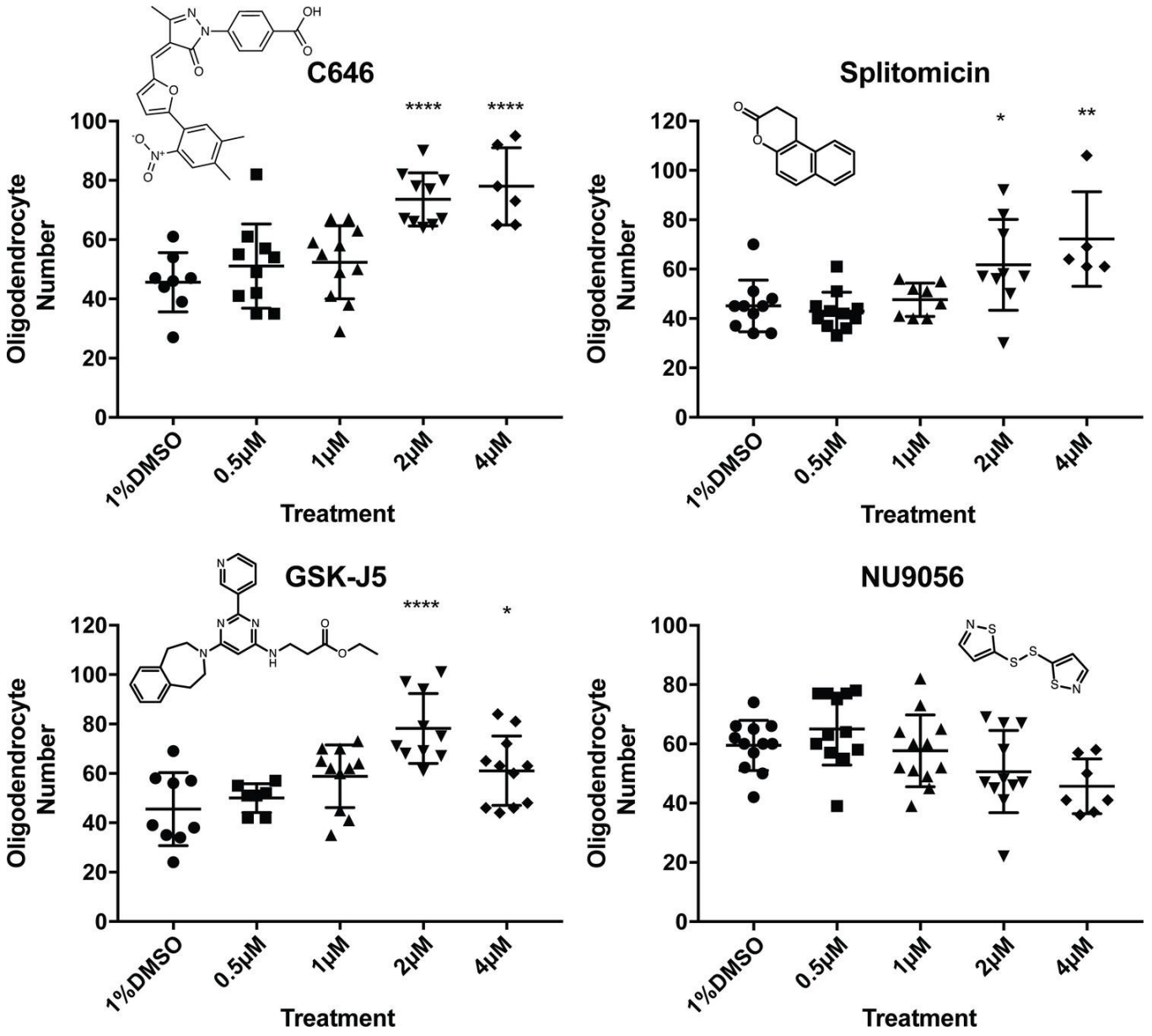


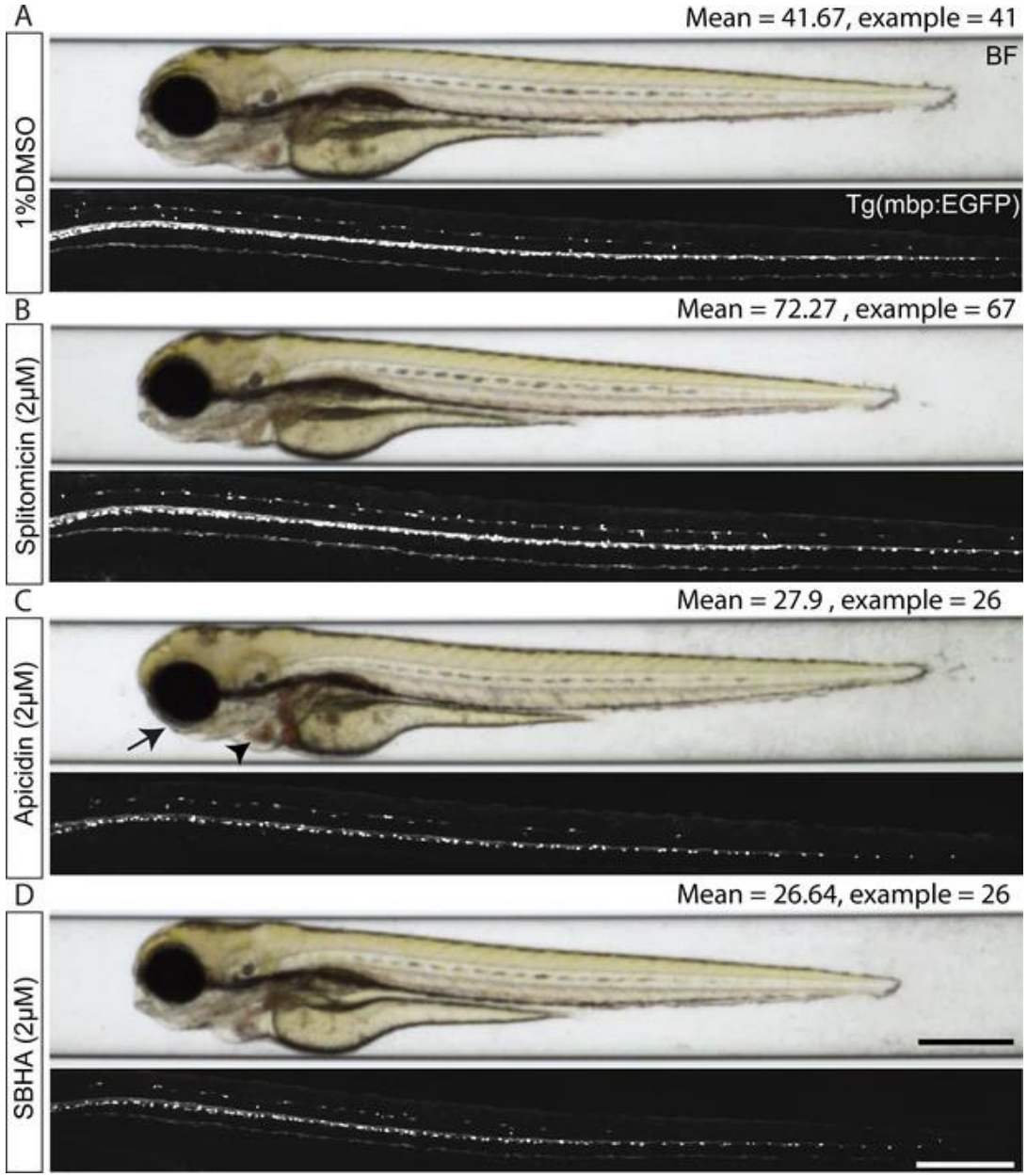
Early JJ et al., *eLife* 2018

Optimization of drug concentrations



Early JJ et al., *eLife* 2018

Drug safety issues can be monitored



Early JJ et al., *eLife* 2018

Summary

- Transgenic zebrafish larvae can be used in phenotypic drug screening
- Novel compounds with therapeutic potential can be identified without prior knowledge of targets

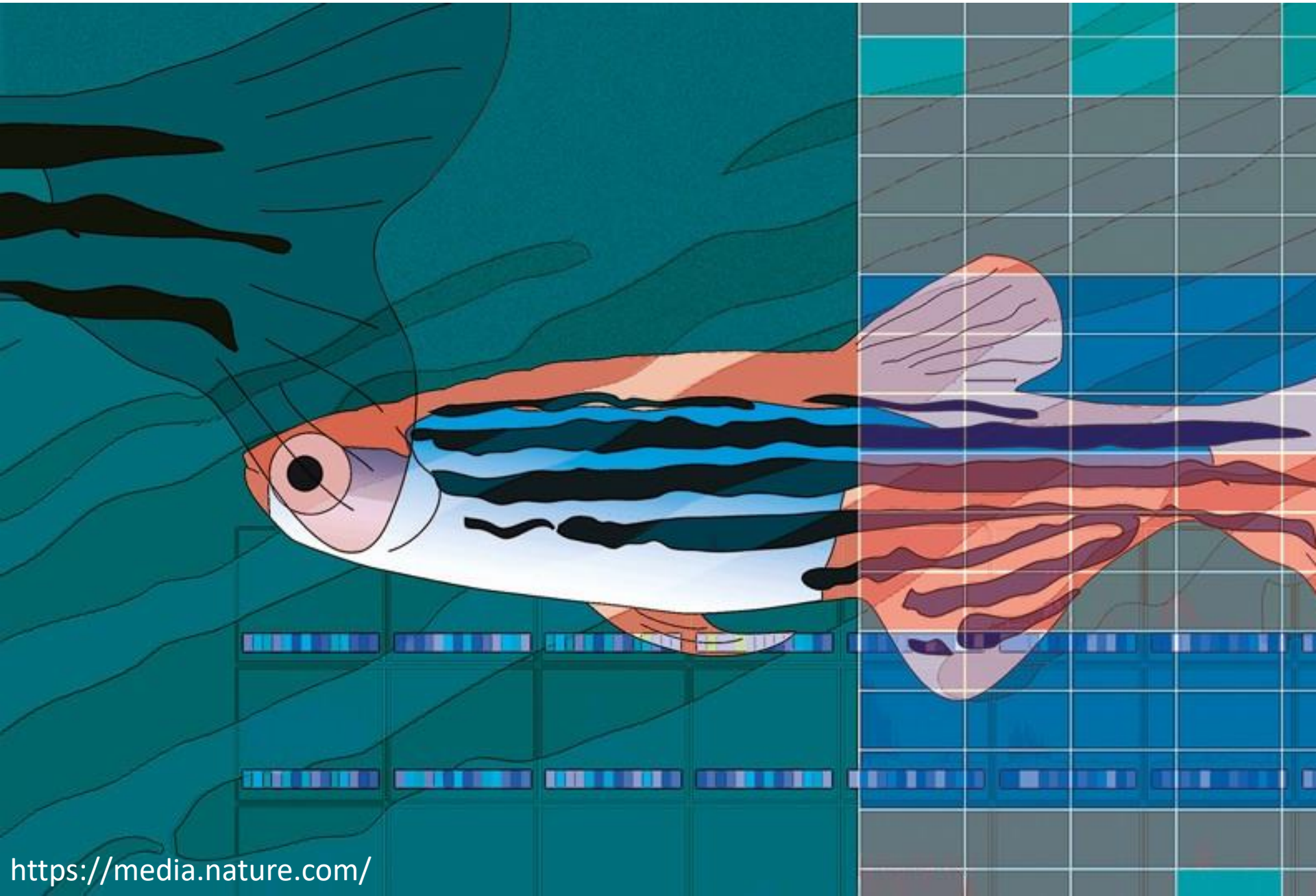
Early JJ et al., *eLife* 2018

Phenotypic drug discovery - Summary

- Animal models with robust phenotypes can be used in PDD.
- PDD allows unbiased selection of drug candidates without prior assumptions as to how the candidate will work.
- Target is identified at later stages

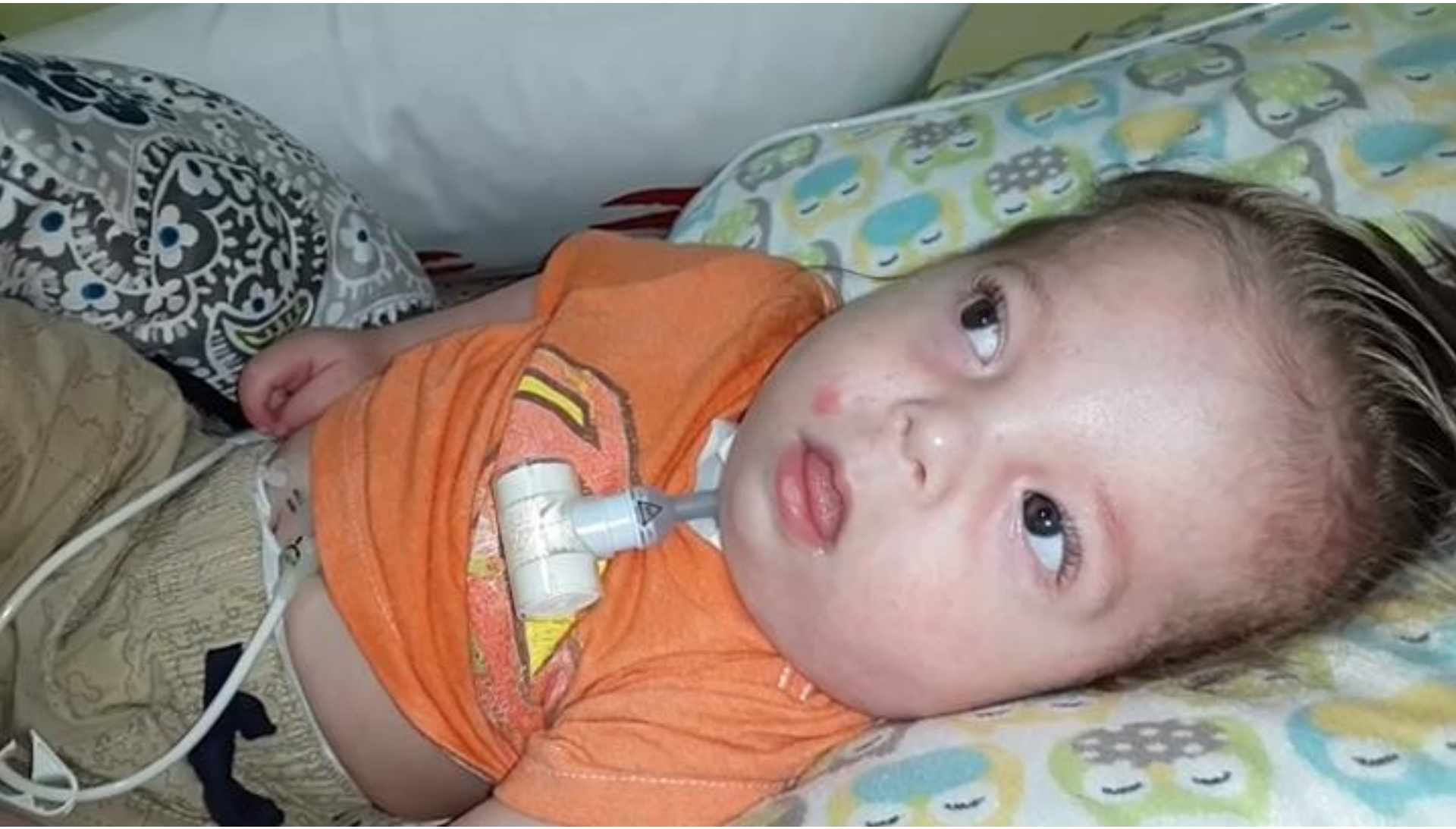
Target-based drug discovery case studies

Zebrafish model in Dravet syndrome



https://media.nature.com/

Dravet syndrome – childhood epilepsy



https://www.youtube.com/watch?v=LwTNt1RFIWE&

Dravet syndrome – childhood epilepsy

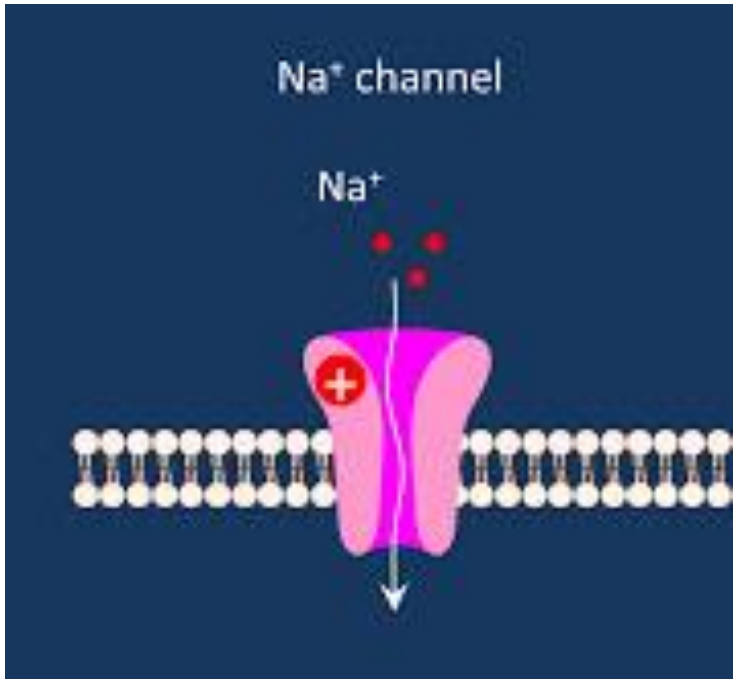
- Dravet syndrome is a rare disorder of pediatric epilepsy
- 1:40000 live births (in UK)
- Seizures triggered by fever
- persistent drug-resistant seizures
- severe intellectual disability
- impaired social development

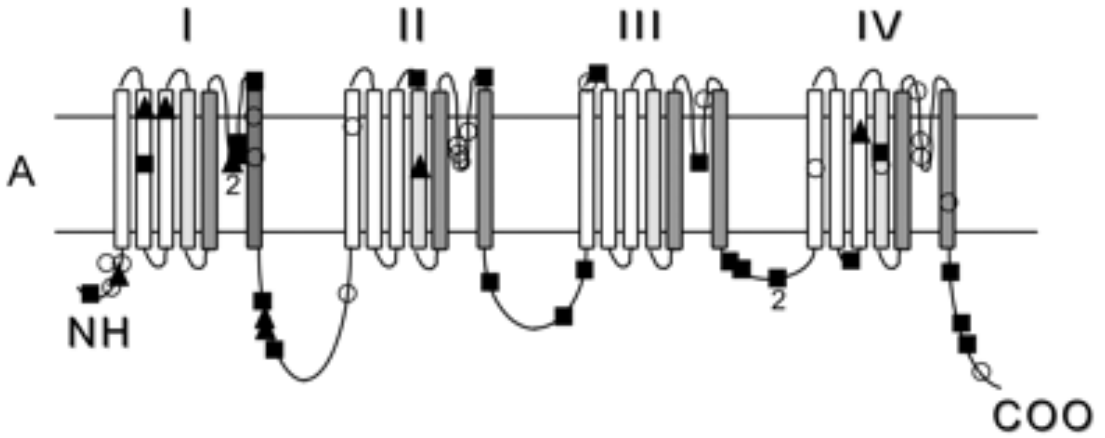
- FDA approved drugs:
  - 2018: Epidiolex (cannabadiol)
  - 2018: Stiripentol (in combination with valproate and clobazam)
  - 2020: Fenfluramine (associated with valvular heart disease and pulmonary arterial hypertension.

There is a need for a better drug..

Baraban SC et al., *Nat Comm* 2013

Dravet syndrome patients have mutations in *SCN1A* gene

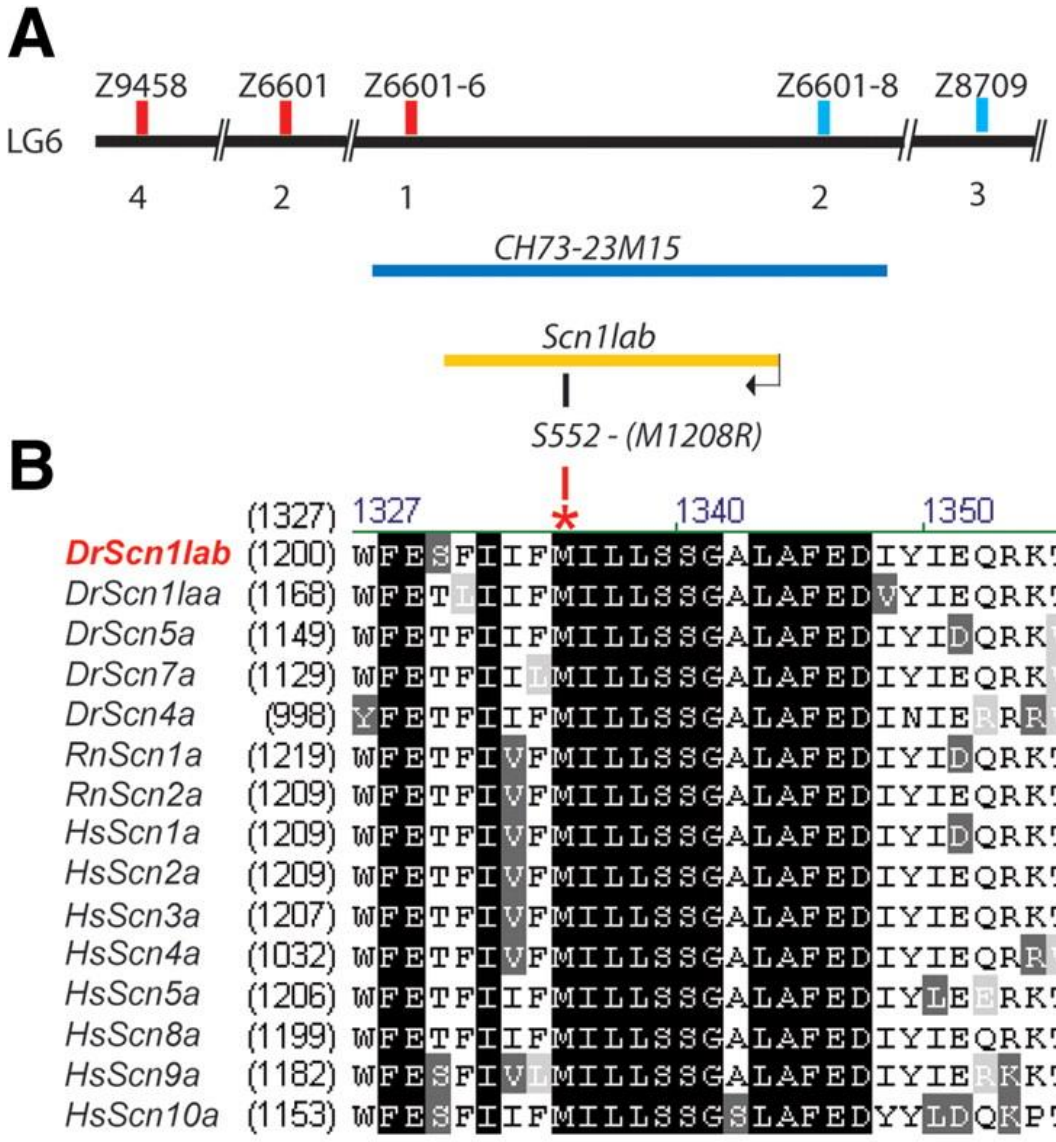




Voltage-gated sodium channel α-subunits

Harkin LA et al., *Brain* 2007

SCN1A is conserved in Zebrafish



Schoonheim PJ et al., *J. Neurosci* 2010

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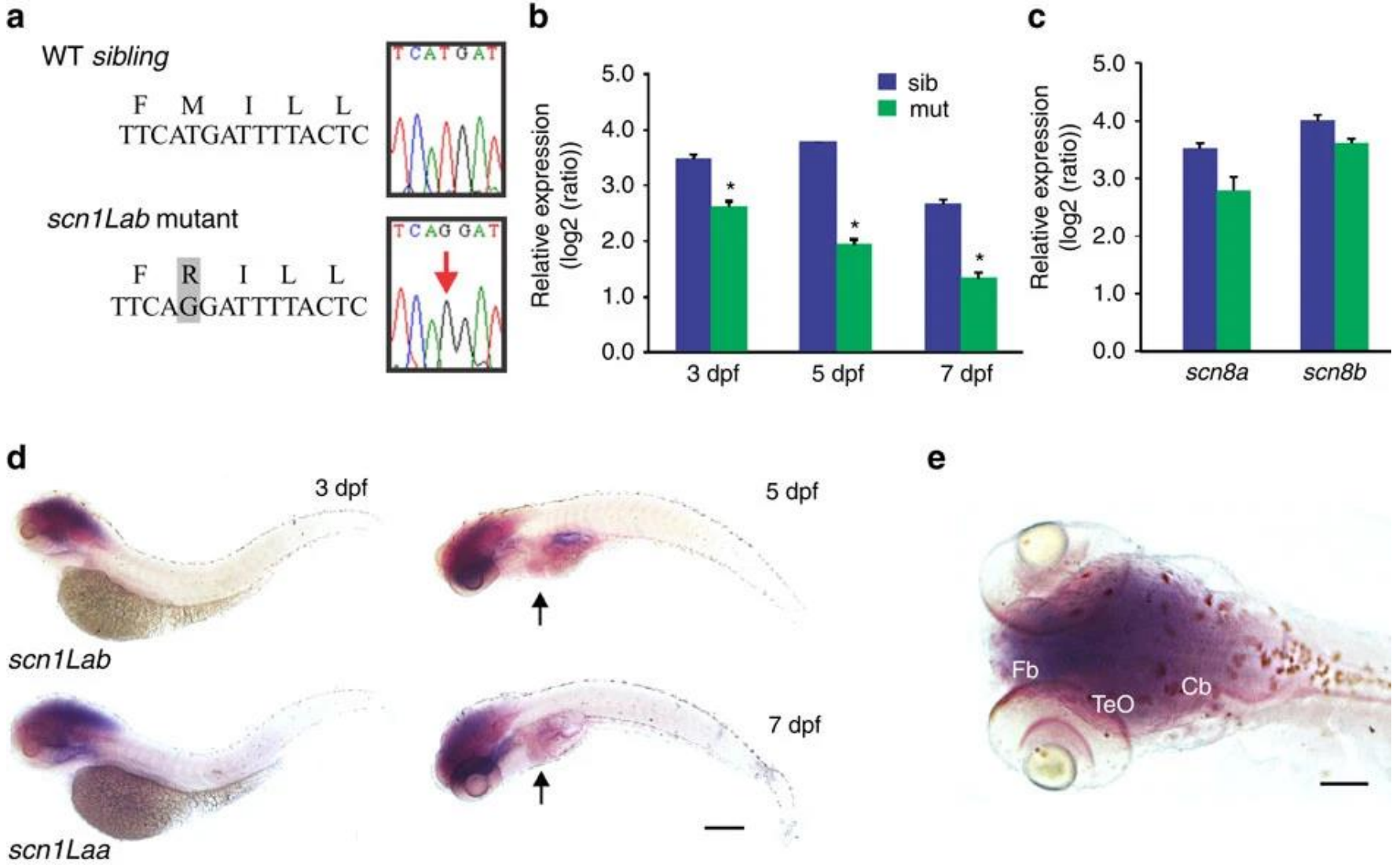
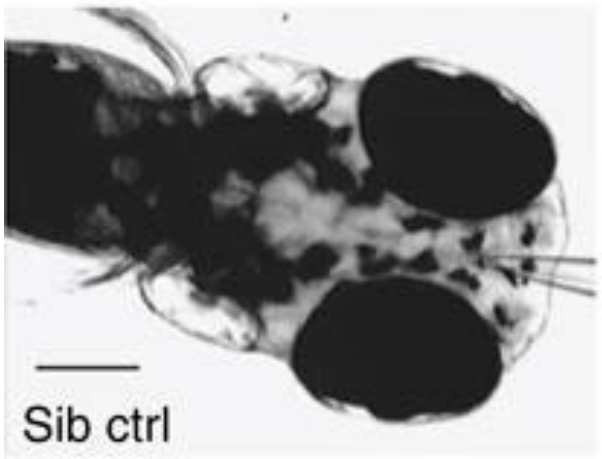
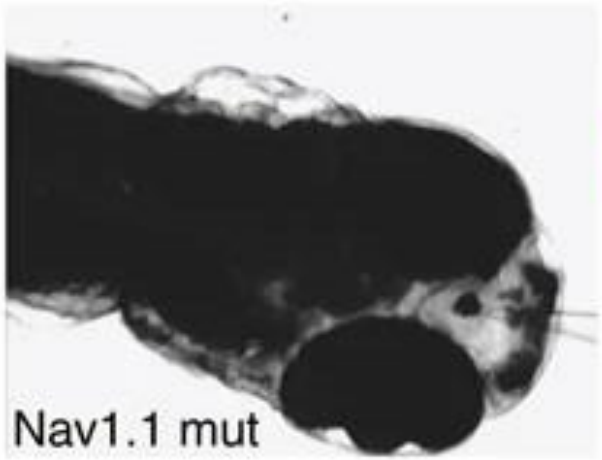
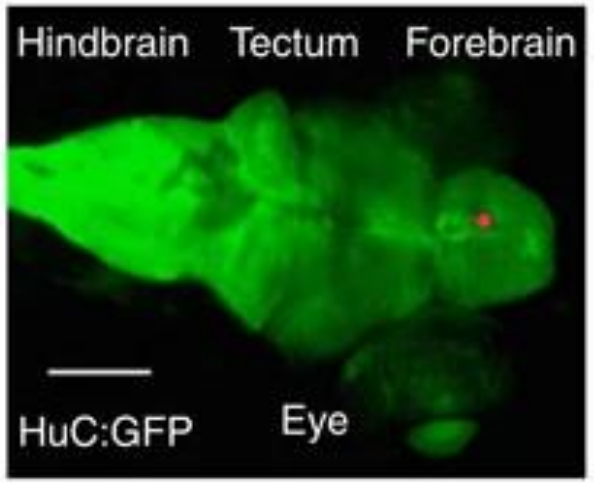


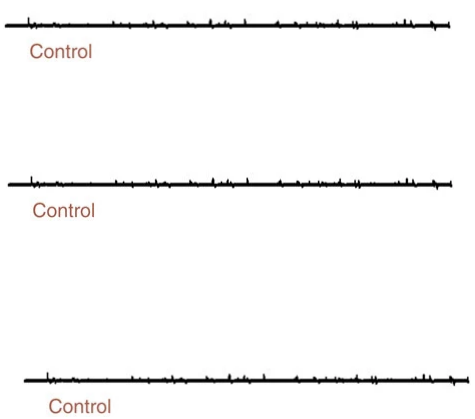
Baraban SC et al., *Nat Comm* 2013

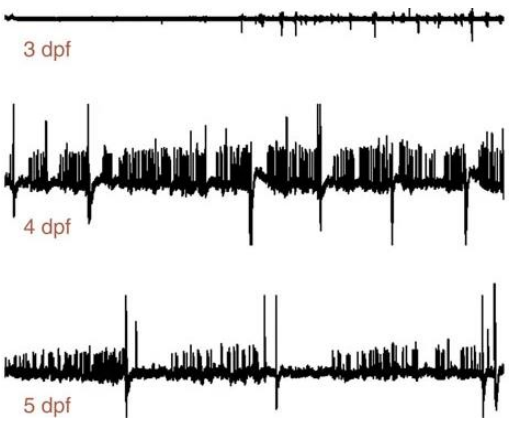
scn1a mutant develop seizures





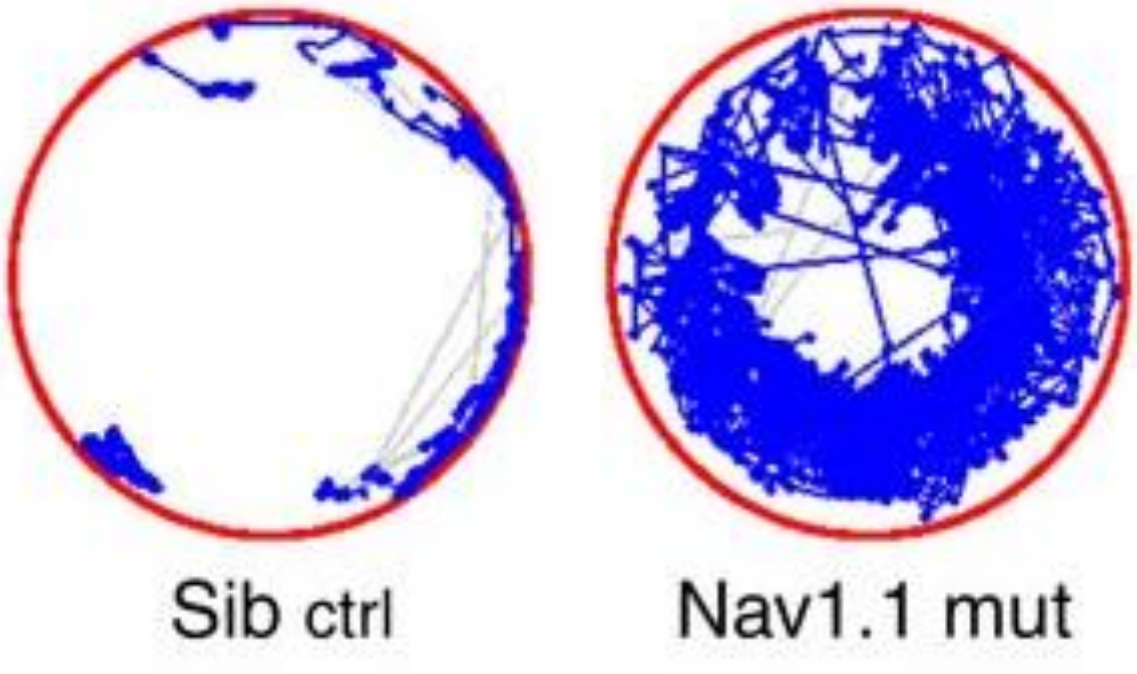






Baraban SC et al., *Nat Comm* 2013

scn1a mutant swimming behaviour

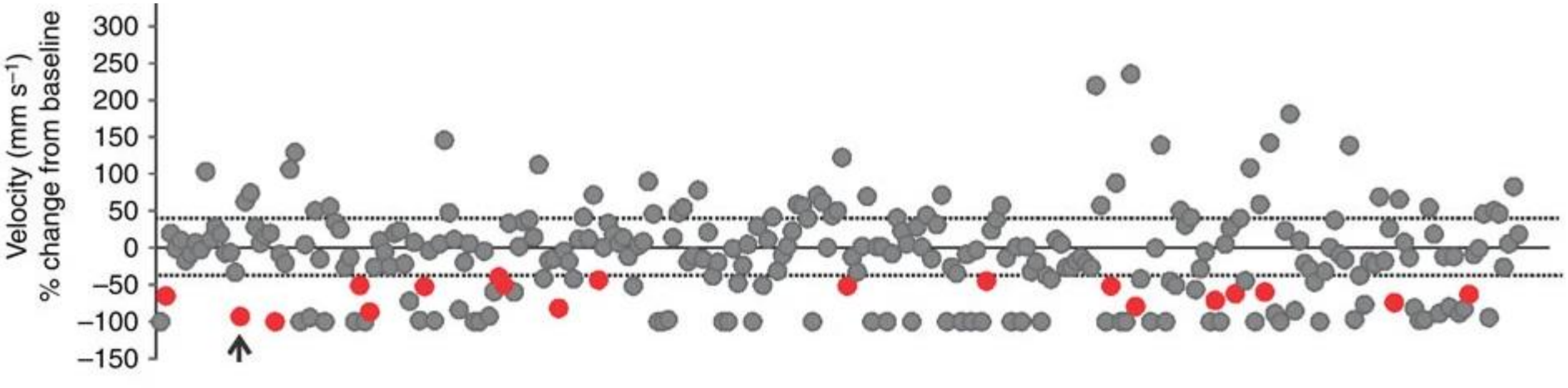


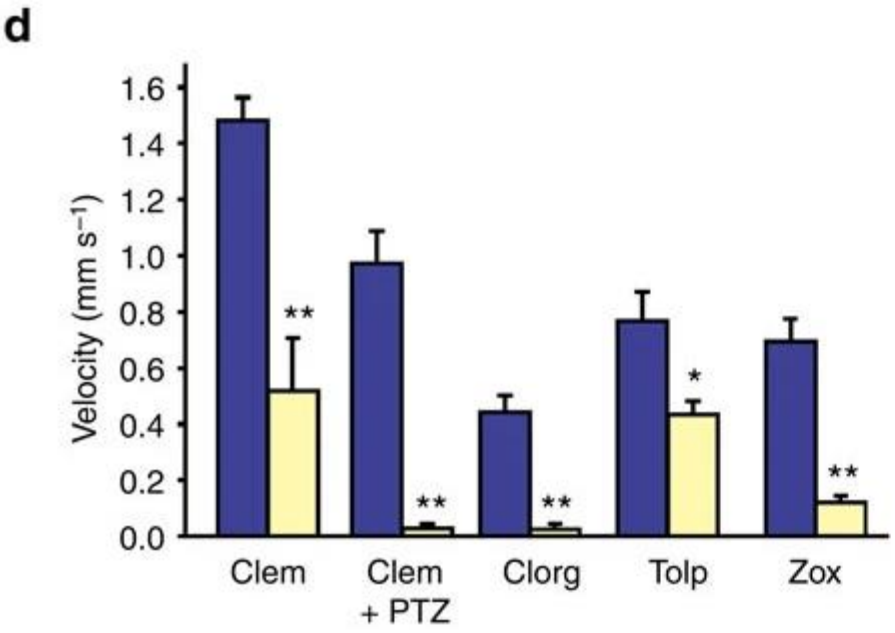
scn1a mutant larvae exhibit elevated swim activity

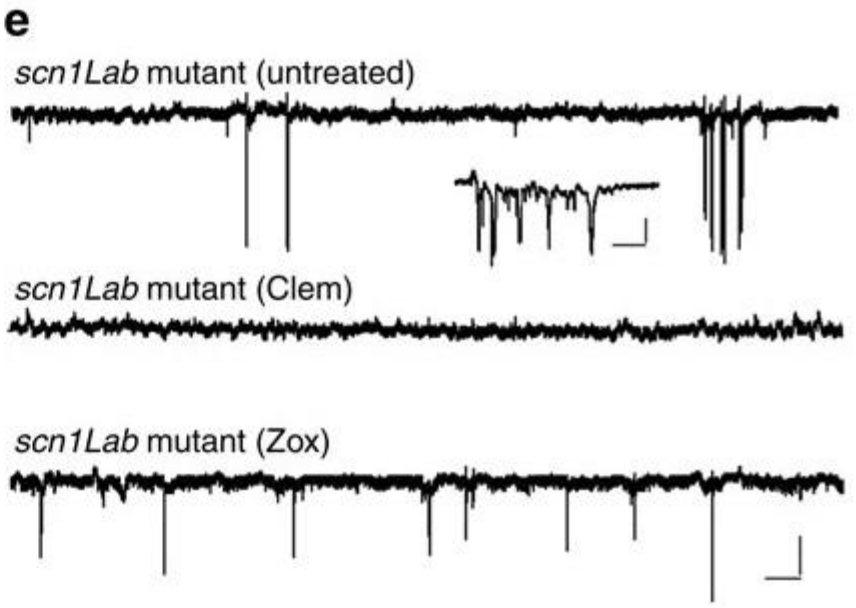
To find a drug that can rescue swimming behaviour

Baraban SC et al., *Nat Comm* 2013

Drug screen based on swimming behaviour

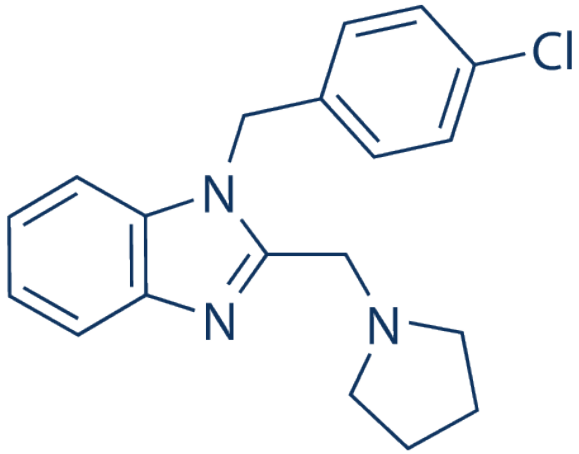






Baraban SC et al., *Nat Comm* 2013

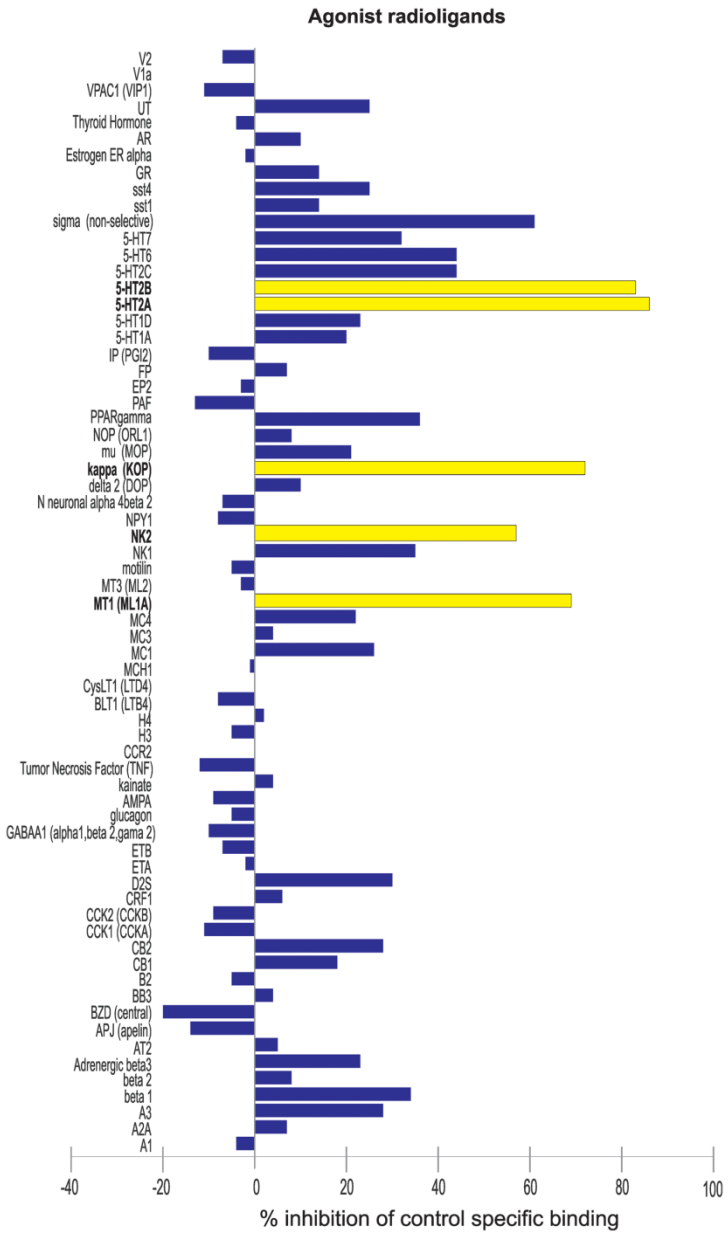
Clemizole, a novel drug for Dravet syndrome?



- Clemizole is a histamine H_1-receptor antagonist
- first developed in the 1950s to treat itching
- **BUT** antihistamines are known to aggravate paediatric epilepsies
- So how does Clemizole works?

Baraban SC et al., *Nat Comm* 2013

What is Clemizole's target?

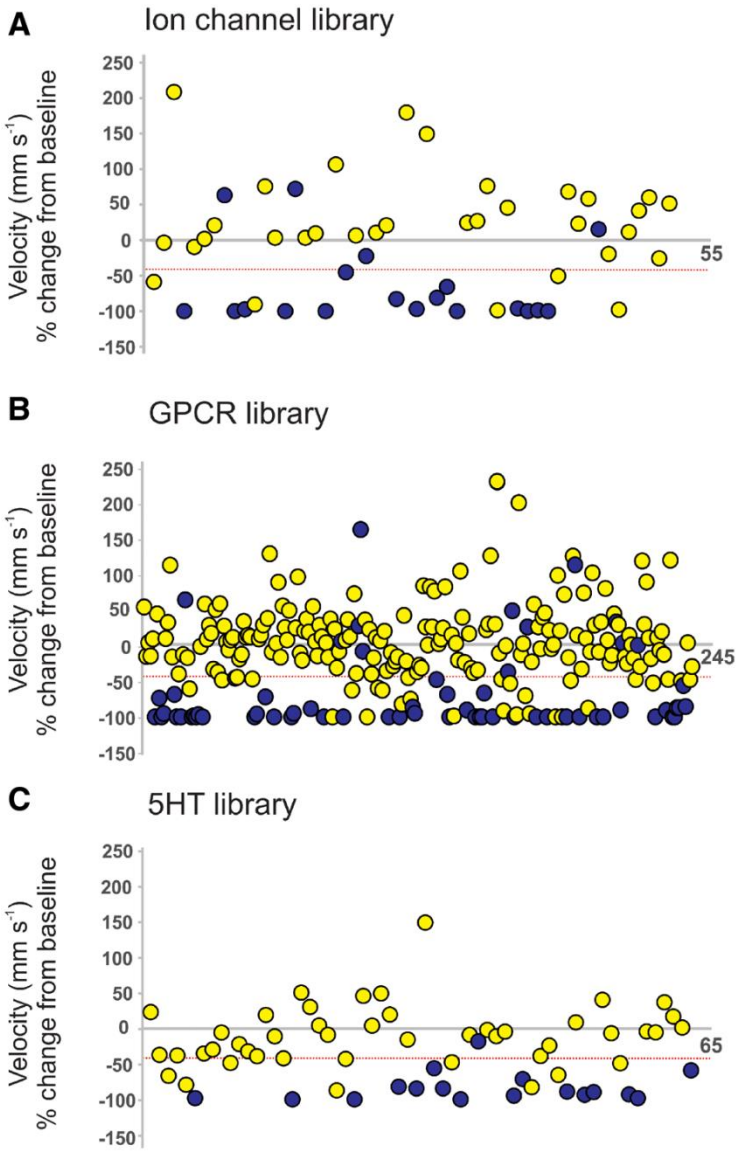


Griffin A et al., *Brain* 2017

Clemizole binding targets

- H1 receptor
- HTR2A
- HTR2B
- ion channel modulators and
- G-protein-coupled receptors (GPCRs).

Novel drug targets for Dravet syndrome

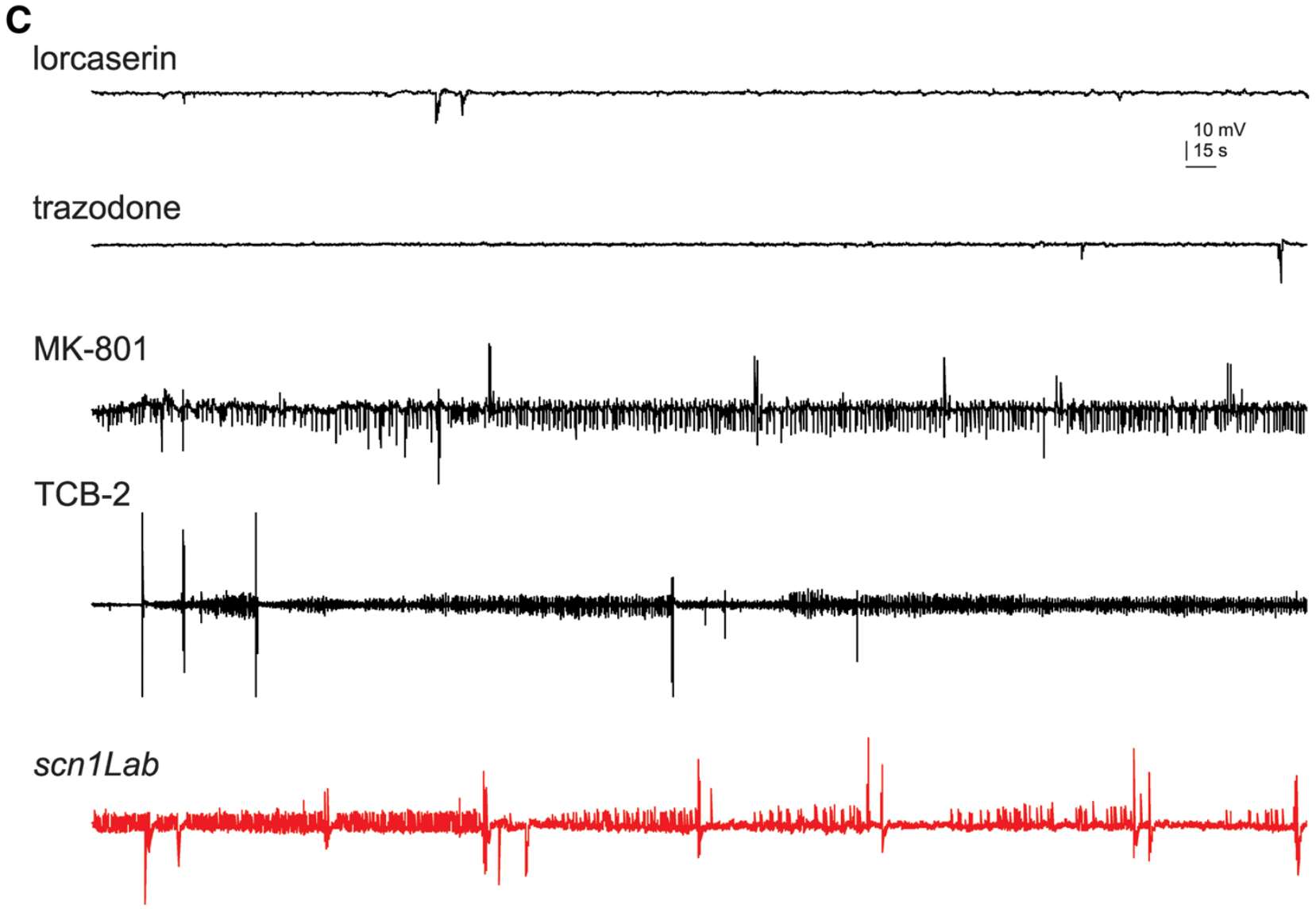


Belviq® (lorcaserin) is an FDA-approved HTR2C agonist prescribed for chronic weight management.

Desyrel® (trazodone) is also an FDA-approved antidepressant commonly prescribed for sleep disorders.

Griffin A et al., *Brain* 2017

Novel drug targets for Dravet syndrome



Griffin A et al., *Brain* 2017

Lorcaserin is currently in Phase 3 clinical trial

A Study of Lorcaserin as Adjunctive Treatment in Participants With Dravet Syndrome (MOMENTUM 1)

The safety and scientific validity of this study is the responsibility of the study sponsor and investigators. Listing a study does not mean it has been evaluated by the U.S. Federal Government. Know the risks and potential benefits of clinical studies and talk to your health care provider before participating. Read our disclaimer for details.

ClinicalTrials.gov Identifier: NCT04572243

Recruitment Status : Recruiting
First Posted : October 1, 2020
Last Update Posted : December 17, 2020

See **Contacts and Locations**

Sponsor:
Eisai Inc.

Information provided by (Responsible Party):
Eisai Inc.

Study Details | **Tabular View** | **No Results Posted** | Disclaimer | How to Read a Study Record

Study Description

Go to

Brief Summary:
The primary purpose of the study is to demonstrate that lorcaserin has superior efficacy compared to placebo on percent change in frequency of convulsive seizures per 28 days in participants with Dravet syndrome.

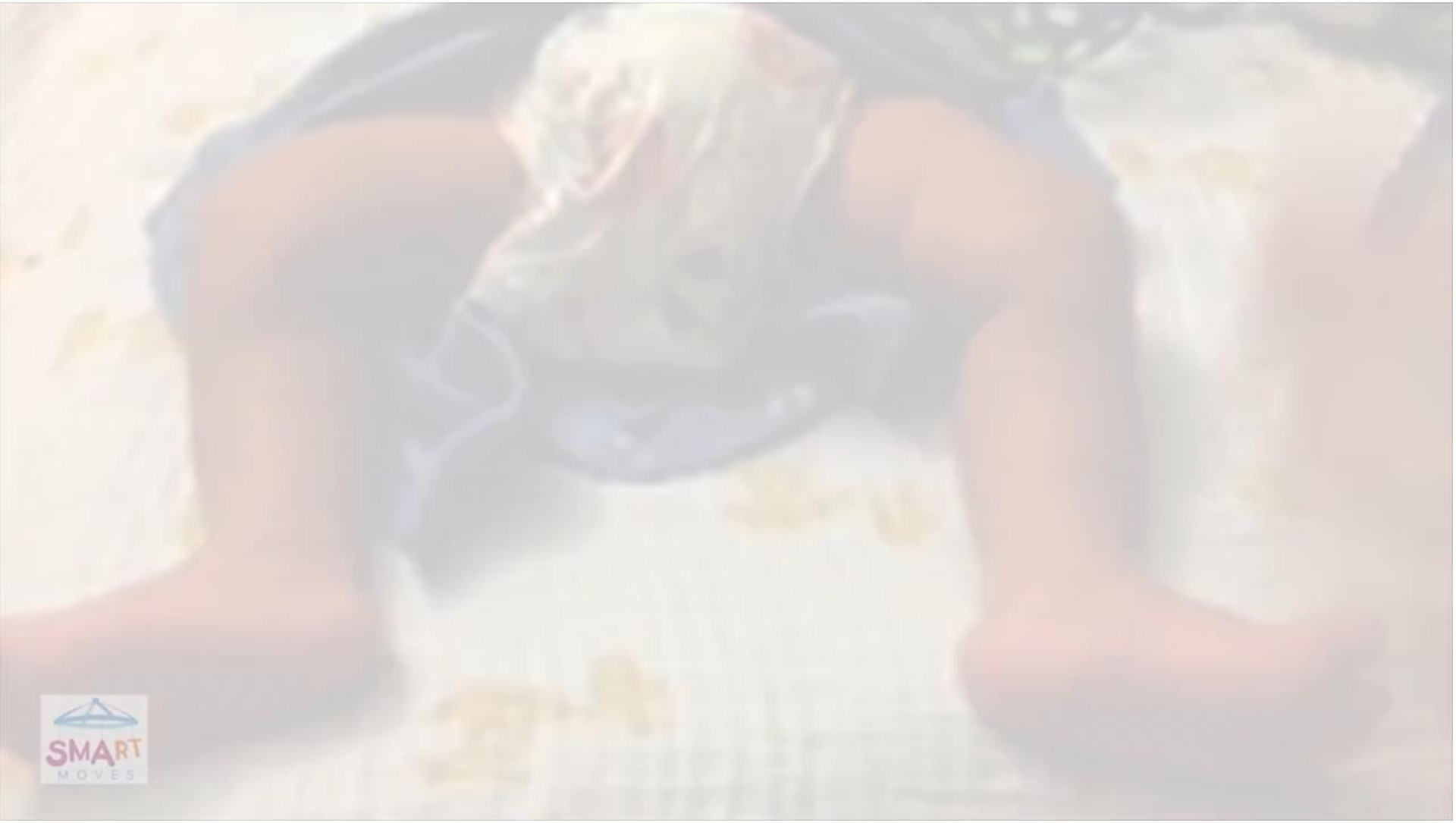
Condition or disease	Intervention/treatment	Phase
Epilepsies, Myoclonic	Drug: Placebo Drug: Lorcaserin	Phase 3

Summary

- Zebrafish models enable rapid drug screening and discovery
- Modulation of serotonergic signalling as a potent suppressor of seizure activity in Dravet Syndrome
- Animal model provide a rapid path from preclinical discovery in zebrafish to potential clinical treatments for Dravet syndrome

Griffin A et al., *Brain* 2017

Spinal Muscular Atrophy (SMA)



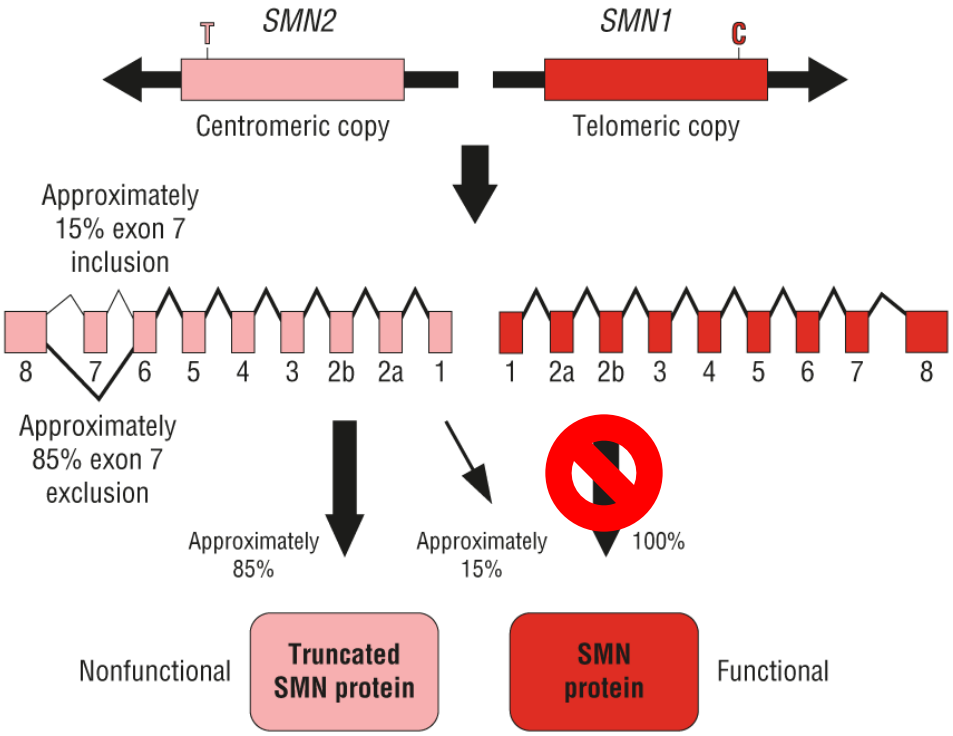
https://www.youtube.com/watch?v=8pYll96rcyc

Spinal Muscular Atrophy (SMA)

- group of neuromuscular hereditary diseases
- autosomal recessive disease
- 1 in 10,000 live births
- Progressive loss of motor neurons
  - Motor neurons control skeletal muscle activity such as speaking, walking, breathing, and swallowing.
  - The loss of motor neurons causes progressive muscle weakness and loss of movement due to muscle wasting (atrophy).

Kolb SJ et al., *JAMA* 2011

Genetics of SMA

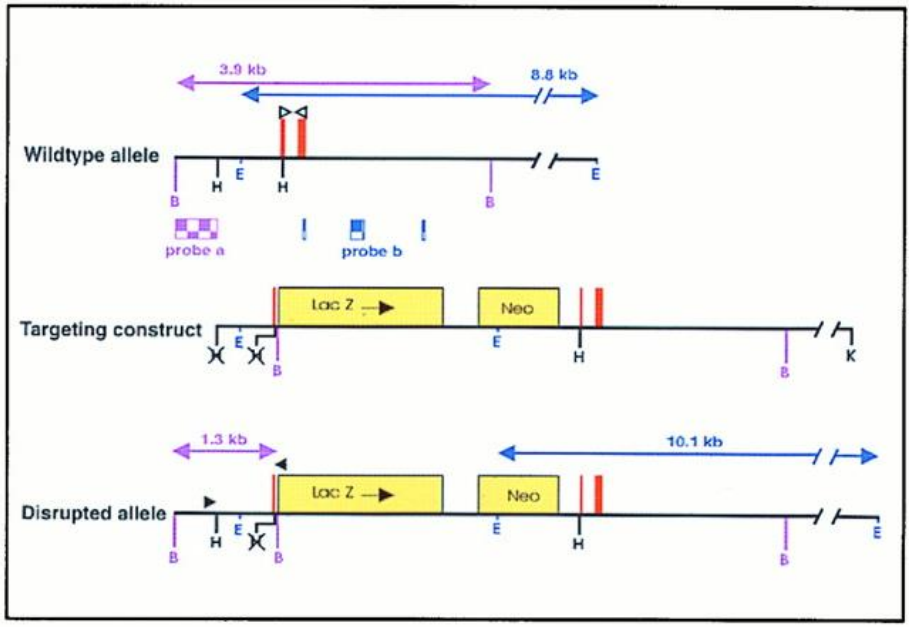


- SMA patients have mutations in Survival Motor Neuron 1 (*SMN1)* gene and lack SMN1 protein
- SMN is essential for spliceosomal snRNP biogenesis
- *SMN2* gene encode majorly a truncated SMN protein due to alternative splicing

Kolb SJ et al., *JAMA* 2011

Mice model of SMA?

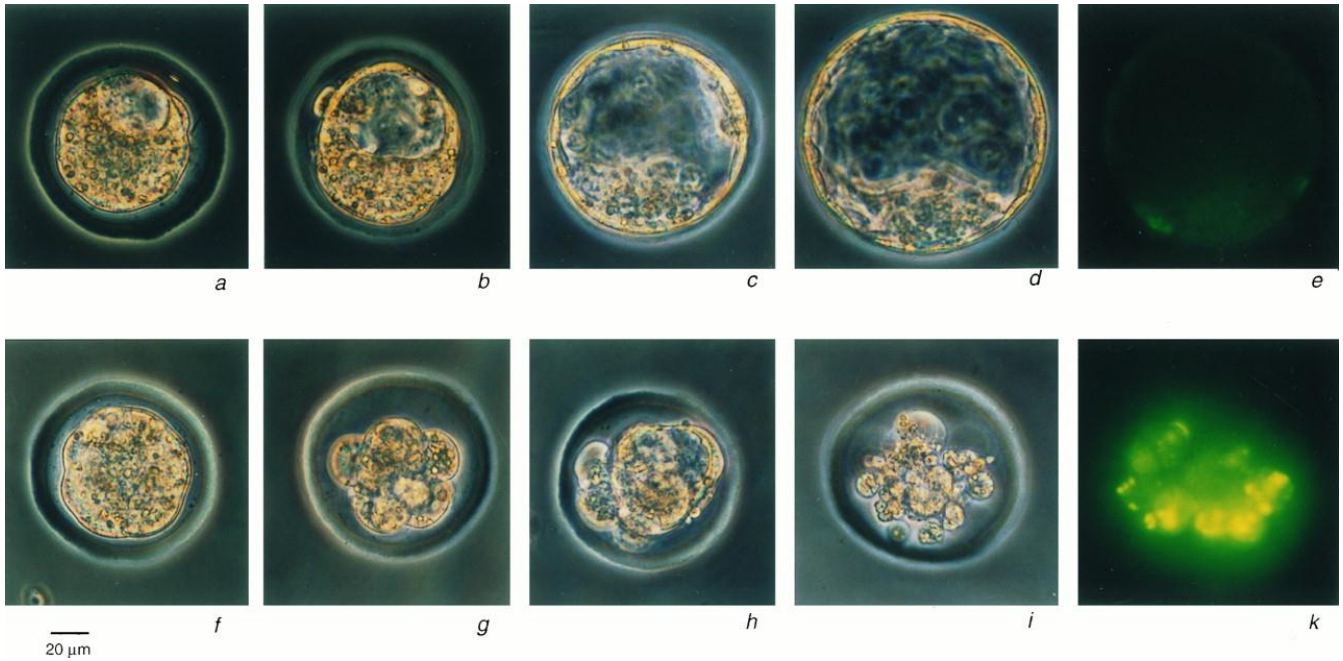
```
hu   MAMssgGSGGGVPEQEDSVLFRRGTGQSDDSDIWDDTALIKAYDKAVASFKHALKNGDIC   60
mo   MAM---GSGGAGSEQEDTVLFRRGTGQSDDSDIWDDTALIKAYDKAVASFKHALKNGDIC   57

hu   ETSGKPKTTPKRKPAKKNKSQKKNTAASLQQWKVGDKCSAIWSEDGCIYPATIASIDFKR   120
mo   ETPDKPKGTARRKPAKKNKSQKKNATTPLKQWKVGDKCSAVWSEDGCIYPATITSIDFKR   117

hu   ETCVVVYTGYGNREEQNLSDLLSPICEVANNIEQNAQENenESQVSTDESENSRSPGNKS   180
mo   ETCVVVYTGYGNREEQNLSDLLSPTCEVANSTEQNTQEN--ESQVSTDDSEHSSRSLRSK   175

hu   DNIKPKSAPWNSFLPPPPPMPGPRLGPGKPGLKFNGPPPPPPPPPPPHLLSCWLPPFPSGP   240
mo   AHSKSKAAPWTSFLPPPPPMPGSGLGPGKPGLKFNGPPPPPPLPPPPFLPCWMPPFPSGP   235

hu   PIIPPPPPICPDSLDDADALGSMLISWYMSGYHTGYYMGFRQNQKEGRCSHSln         294
mo   PIIPPPPPISPDCLDDTDALGSMLISWYMSGYHTGYYMGFRQNKKEGKCSHTn-         288
```



- Mice has only one *SMN* gene
- What about *SMN* knockout mice?
- Does it develop SMA?

Schrank B et al., *PNAS* 1997

Mice model of SMA



- *SMN* -/- embryos cannot transition to the blastocyst stage
- Mice *SMN* -/- are embryonic lethal
- So, how to make a SMA mice model?

Schrank B et al., *PNAS* 1997

SMN2 in SMA?

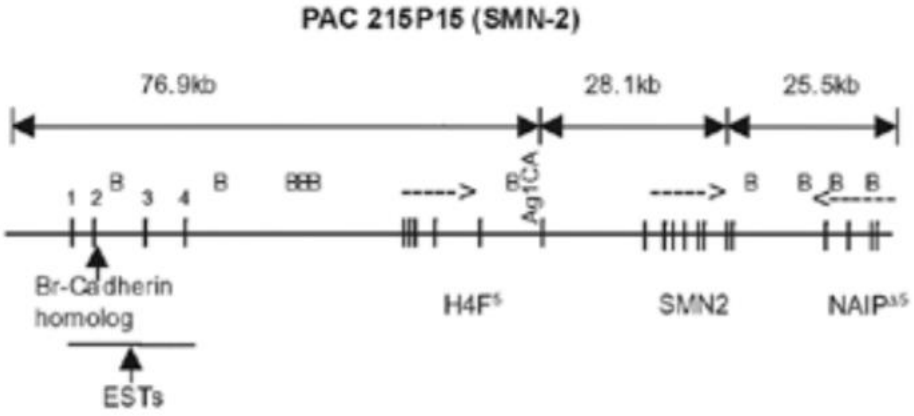
Type	Age at Onset	Highest Function	Natural Age at Death	*SMN2* No.
0	Prenatal	Respiratory support	<1 mo	1
1	0-6 mo	Never sit	<2 y	2
2	<18 mo	Never stand	>2 y	3, 4
3	>18 mo	Stand alone	Adult	
3a	18 mo-3 y	Stand alone	Adult	3, 4
3b	>3 y	Stand alone	Adult	4
4	>21 y	Stand alone	Adult	4-8

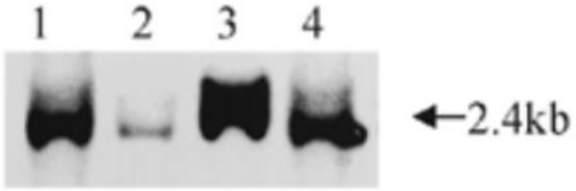
SMA patients with *SMN1* mutations and with higher copies of *SMN2* can survive relatively longer

Kolb SJ et al., *JAMA* 2011

Mice model of SMA

- What happens if we introduce human *SMN2* gene into *SMN* -/- mice?

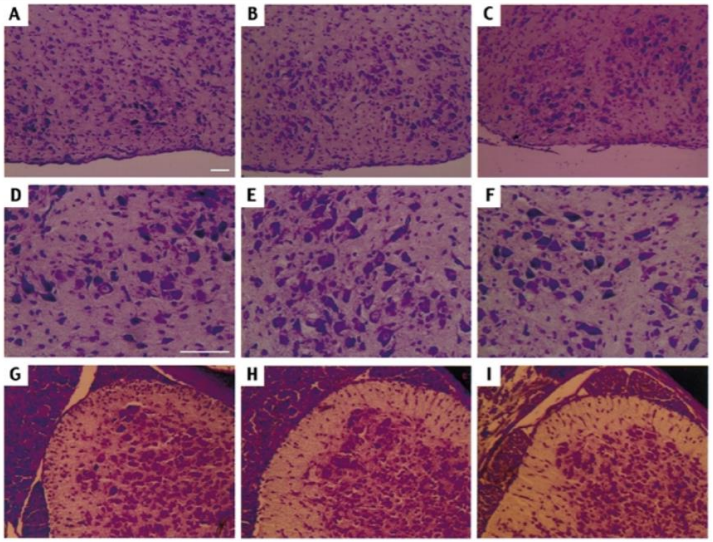




1,4: Normal human DNA 4 copies
2: Founder mice one copy of SMN2
3: Founder mice 8 copy

Monani UR et al., *Human Mol Genetics* 2000

Mice model of SMA



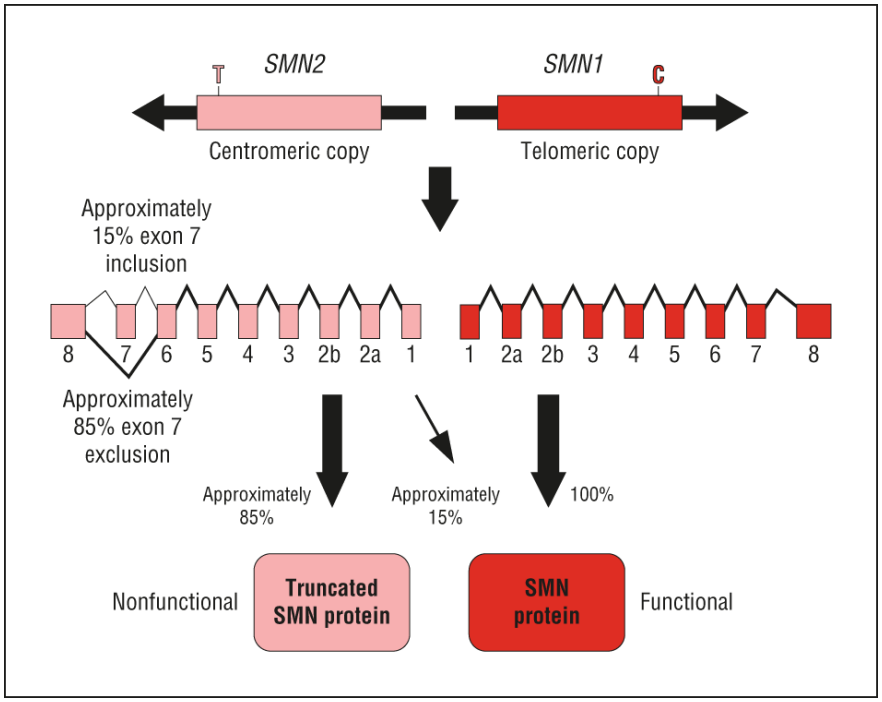
	Low copy *Smn*$^{-/-}$;*SMN2*	High copy *Smn*$^{-/-}$;*SMN2*	*Smn*$^{+/+}$;*SMN2*
Facial motor neurons			
P1	3936 ± 95 (n = 5)	4002 ± 641 (n = 2)	4105 ± 390 (n = 3)
P3–5	1709 ± 189 (n = 4)	2617 ± 173 (n = 4)	2981 ± 152 (n = 4)
Spinal motor neurons			
P1	1876 ± 143 (n = 6)	2186 ± 83 (n = 2)	2299 ± 290 (n = 2)
P3–5	1113 ± 36 (n = 4)	1614 ± 85 (n = 4)	1740 ± 123 (n = 3)

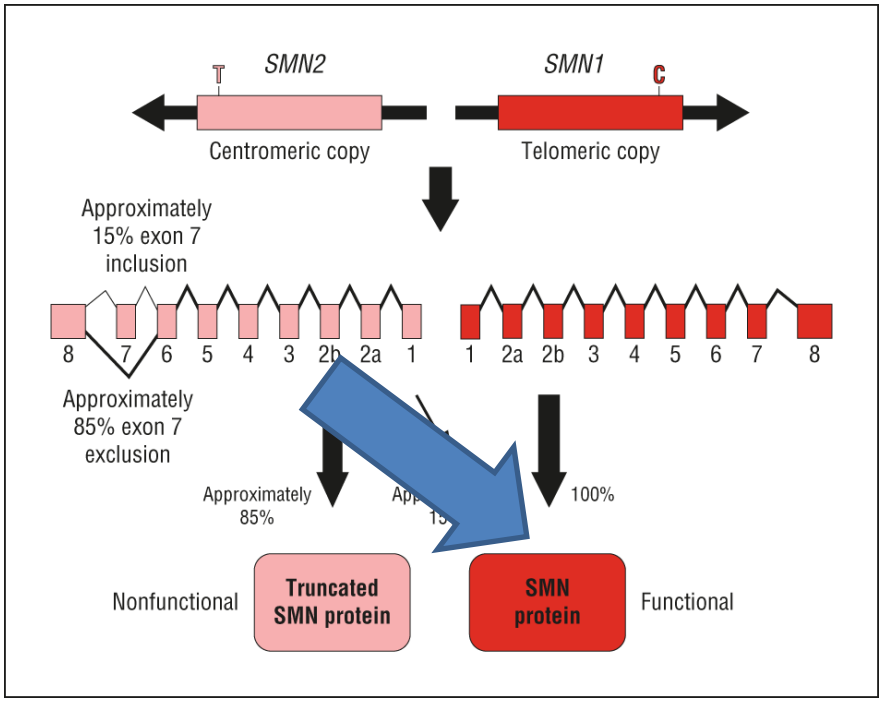
- *SMN* -/- with low copy h*SMN2* die at P4-6
- *SMN* -/- with high copy h*SMN2*
  - survive to adulthood
  - Do not show loss of motor neurons

Monani UR et al., *Human Mol Genetics* 2000

Mice model of Spinal Muscular Atrophy (SMA)

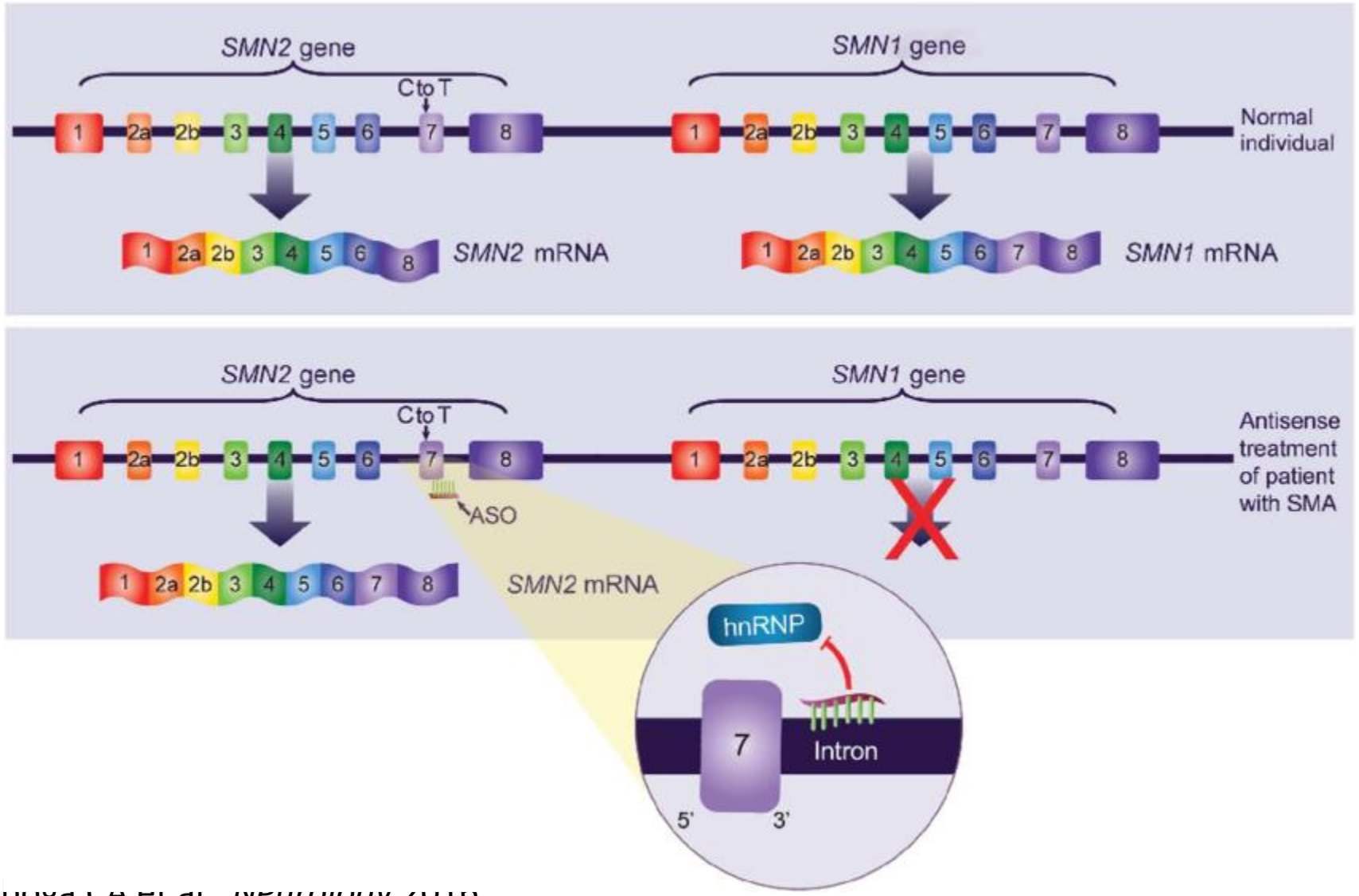
- But *SMN2* majorly encodes a truncated protein due to alternate splicing
- So how to make *SMN2* gene transcripts to be translated to full-length protein?





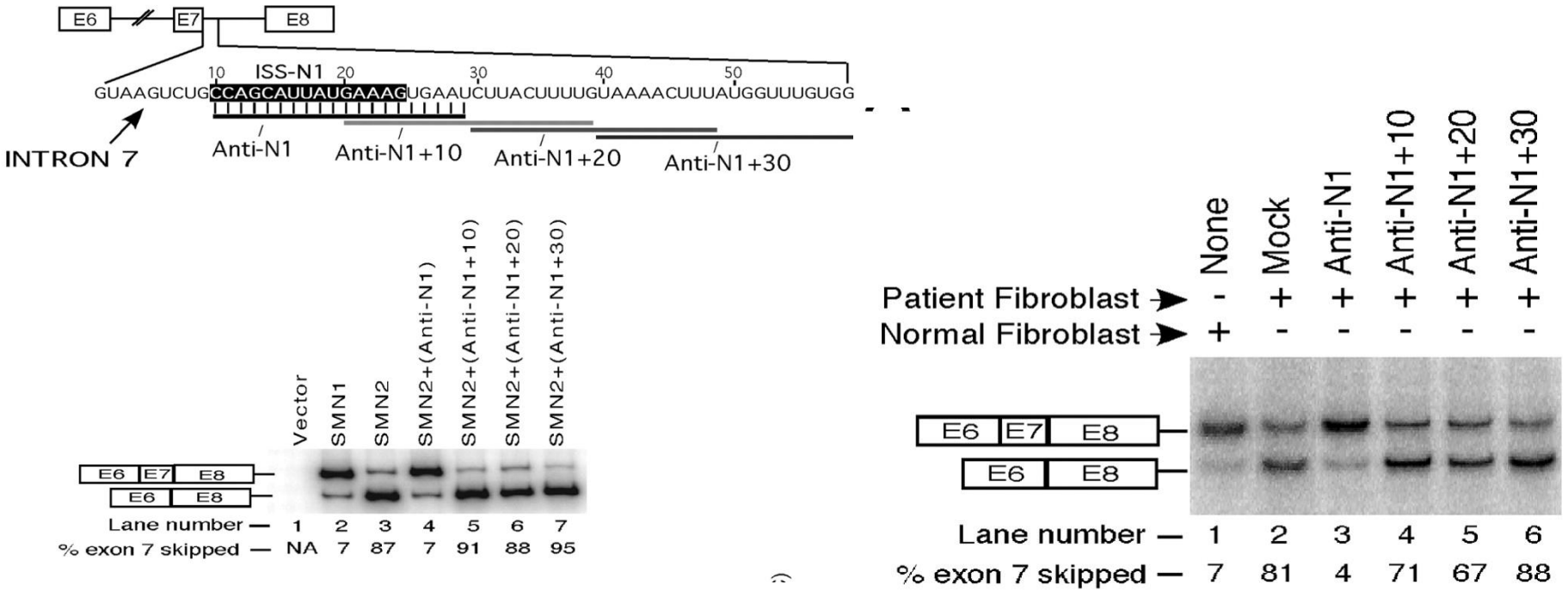
Kolb SJ et al., *JAMA* 2011

Can antisense oligonucleotides induce *SMN2* exon 7 inclusion?



Chiriboga CA et al., *Neurology* 2016

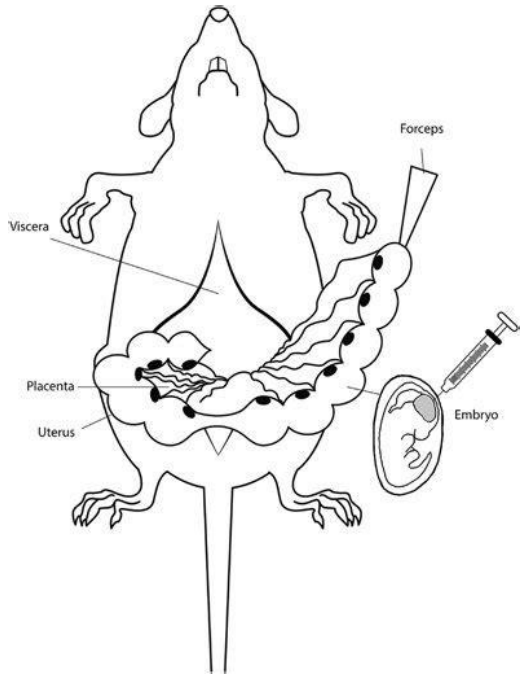
Antisense oligonucleotides in SMN2 splicing



- Antisense oligonucleotides targeting *SMN2* intron 7 splicing silencer N1 (ISS-N1) can promote exon 7 inclusion
- Can this approach work in mice model of SMA?

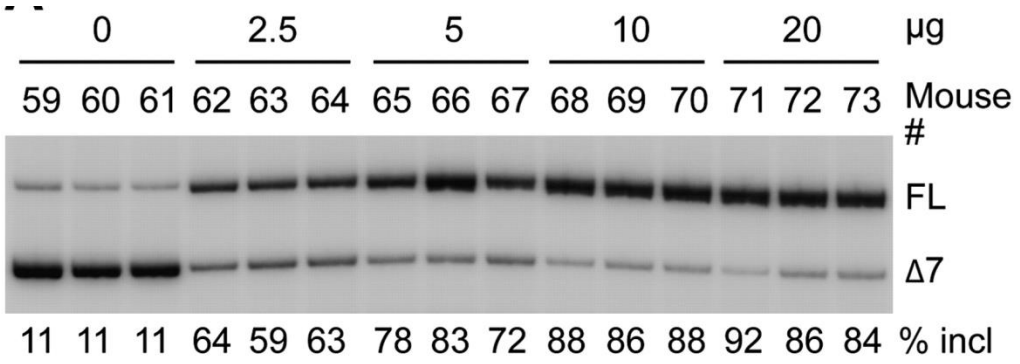
Singh NK et al., *MCB* 2006

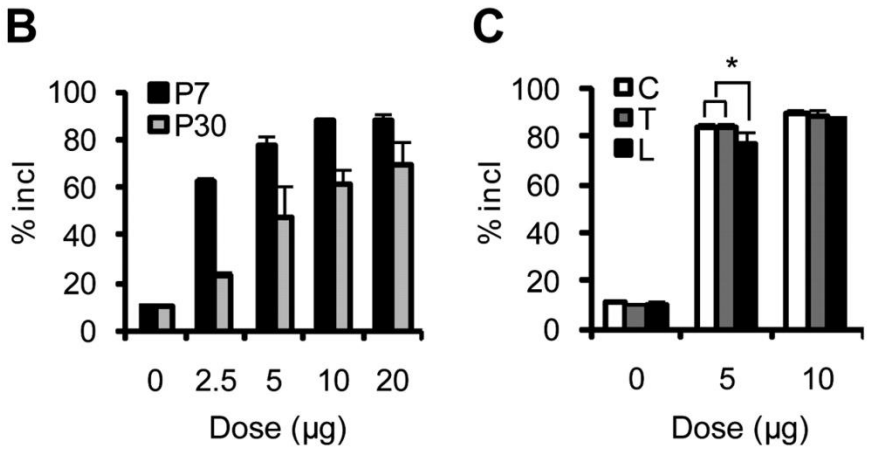
Antisense oligonucleotides in SMN2 splicing



intracerebroventricular injection of ASO in embryonic day 15 (E15) of *SMN* -/- ; *hSMN2*

RT-PCR of lumbar spinal cord samples

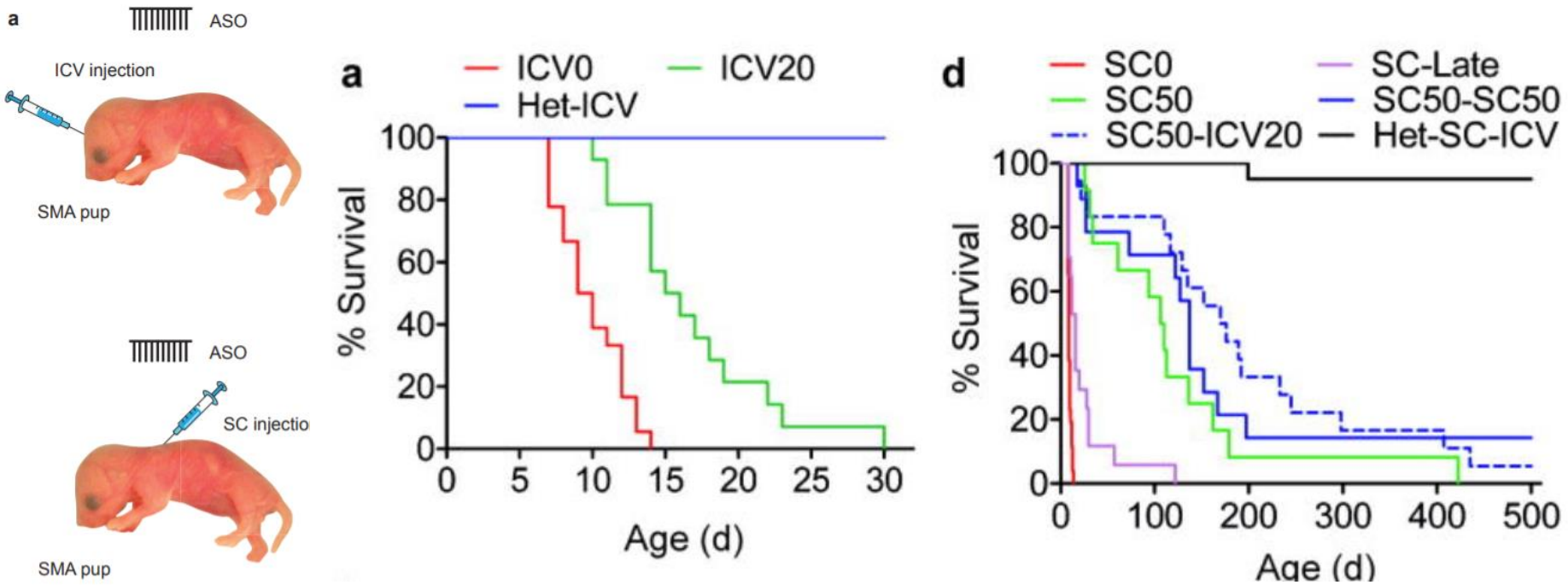




Embryonic administration of ASO can promote full length *SMN2* expression in spinal cord

Hua Y et al., *Genes & Dev* 2010

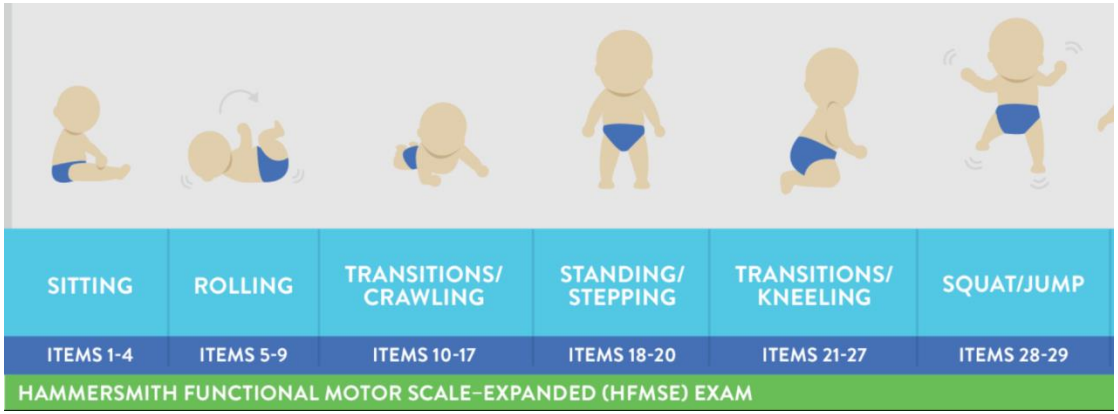
Antisense oligonucleotides in SMN2 splicing

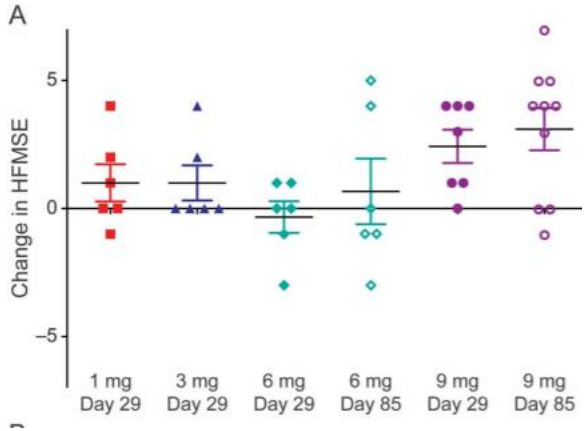


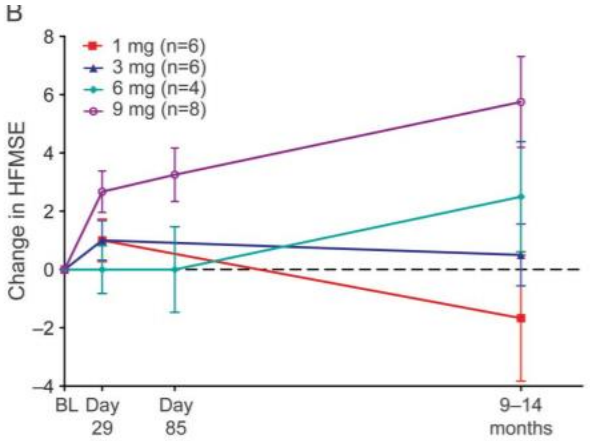
- **Subcutaneuos (SC) ASO injections increase median survival**
- **ASO action in peripheral tissues is essential for survival**

Hua Y et al., *Nature* 2011

Nusinersin – first FDA/EMA approved ASO for SMA







Chiriboga CA et al., *Neurology* 2016

Summary

- Animal models can play an important role in target-based drug discovery
- Nusinersen, the first FDA/EMA approved splice-switching drug for SMA was developed using mice model of SMA.

Are animals needed for drug development?

Some lessons from the past

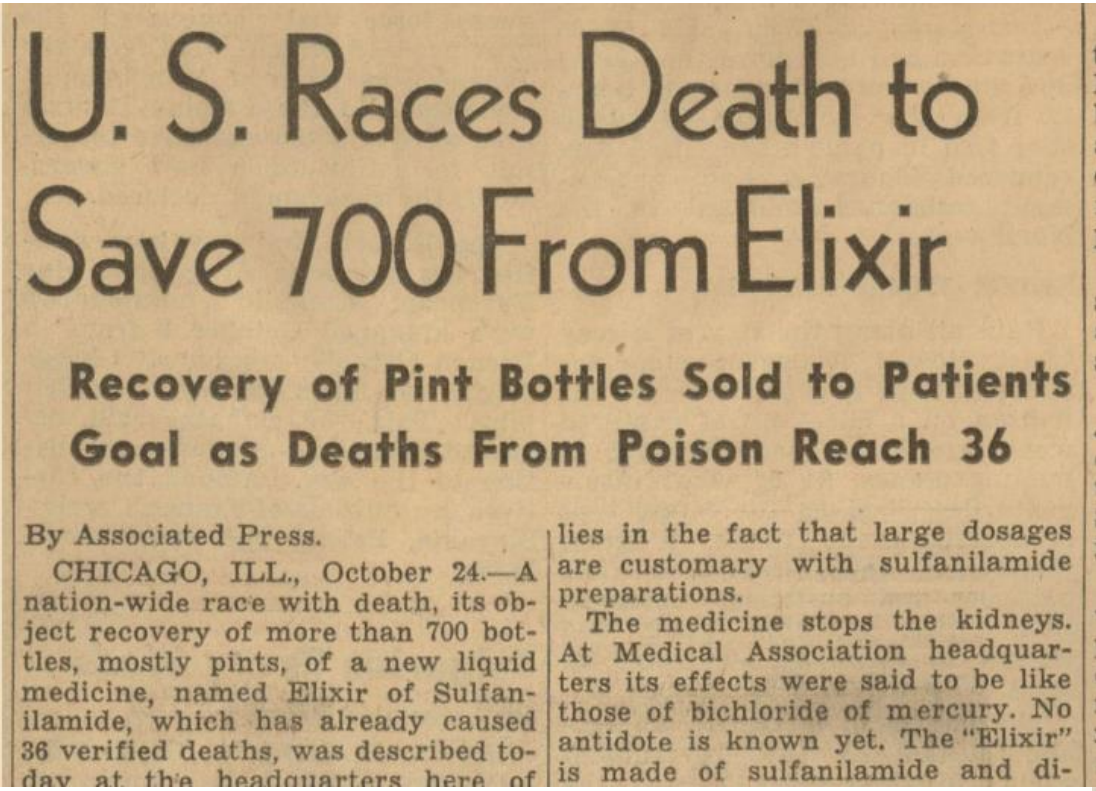
- Sulfanilamide tragedy in USA
- Thalidomide tragedy in 46 countries..

Elixir Sulfanilamide tragedy

- Sulfanilamide, a drug used to treat *Streptococcal* infections common in children
- Used safely in tablet and powder form
- In 1937, demand for liquid form of the drug increased
- Diethylene glycol was used as solvent to dissolve Sulfanilamide
- Liquid form of sulfanilamide was distributed without prior testing
- Problem started…

https://www.fda.gov/files/about%20fda/published/The-Sulfanilamide-Disaster.pdf

Elixir Sulfanilamide tragedy



U. S. Races Death to Save 700 From Elixir

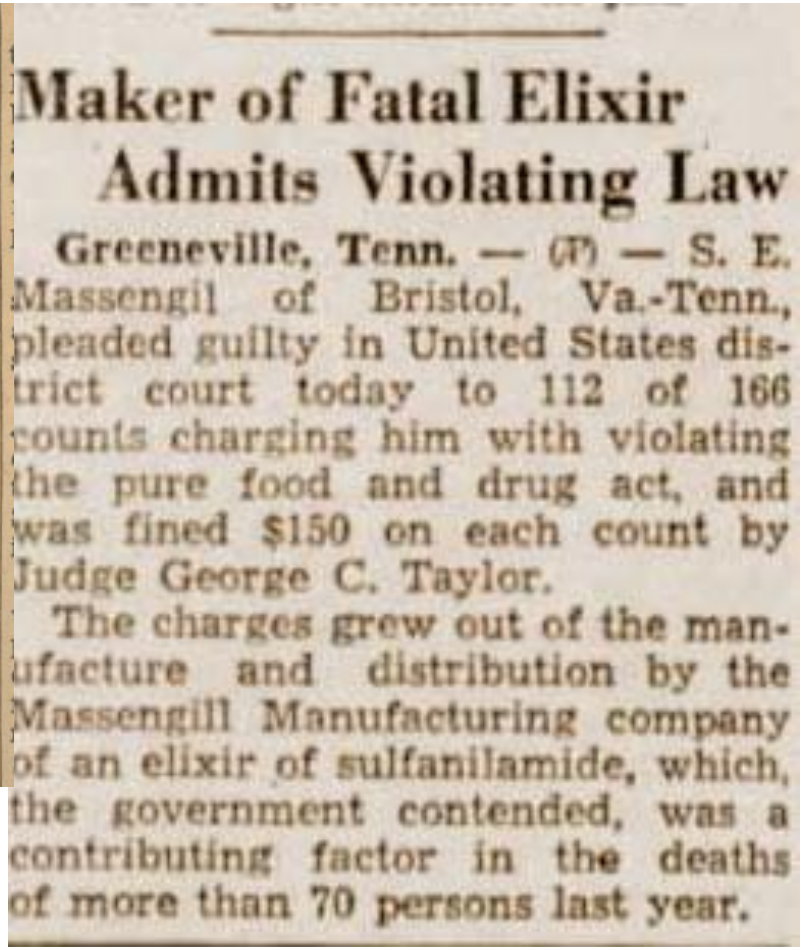
Recovery of Pint Bottles Sold to Patients Goal as Deaths From Poison Reach 36

By Associated Press.

CHICAGO, ILL., October 24.—A nation-wide race with death, its object recovery of more than 700 bottles, mostly pints, of a new liquid medicine, named Elixir of Sulfanilamide, which has already caused 36 verified deaths, was described today at the headquarters here of

lies in the fact that large dosages are customary with sulfanilamide preparations.

The medicine stops the kidneys. At Medical Association headquarters its effects were said to be like those of bichloride of mercury. No antidote is known yet. The "Elixir" is made of sulfanilamide and di-



Maker of Fatal Elixir Admits Violating Law

Greeneville, Tenn. — (AP) — S. E. Massengil of Bristol, Va.-Tenn., pleaded guilty in United States district court today to 112 of 166 counts charging him with violating the pure food and drug act, and was fined $150 on each count by Judge George C. Taylor.

The charges grew out of the manufacture and distribution by the Massengill Manufacturing company of an elixir of sulfanilamide, which, the government contended, was a contributing factor in the deaths of more than 70 persons last year.

https://www.toxicology.org/

Elixir Sulfanilamide tragedy

- 100s of people who consumed the drug died due to kidney failure
- 1938 Federal Food, Drug, and Cosmetic Act was passed in USA

https://www.fda.gov/files/about%20fda/published/The-Sulfanilamide-Disaster.pdf

Thalidomide tragedy

- Thalidomide, anti-flu and sedative drug identified in 1950
- First entered German market in 1957 as an over-the-counter remedy, based on the maker's safety claims.
- Advertised as "completely safe" for everyone, including mother and child, "even during pregnancy,"
- By 1960, thalidomide was marketed in 46 countries, with sales nearly matching those of aspirin.

thalidomide.ca

Thalidomide tragedy

- Thousands of samples were distributed to doctors, who were encouraged to prescribe it to pregnant women in order to alleviate pregnancy nausea.
- Thousands of pregant mothers had been prescribed thalidomide..
- Problem started….
- biggest man-made medical disaster ever

thalidomide.ca

Thalidomide tragedy

https://helix.northwestern.edu/article/thalidomide-tragedy-lessons-drug-safety-and-regulation

Thalidomide tragedy

https://theecologist.org/

Thalidomide tragedy

https://www.liverpoolecho.co.uk/

Thalidomide tragedy

- Thalidomide has teratogenic property
- Babies born to these mothers developed phocomelia,
  - side effect of the drug thalidomide,
  - resulting in the shortening or absence of limbs.
- Biggest man-made medical disaster ever

thalidomide.ca

Aftermath

- In 1962, all new drugs had to be approved by the Food and Drug Administration (FDA) to gain market authorization.
- Approval could be gained based on proven efficacy and safety
- In 1965, Directive 65/65/EEC in the European Economic Community was passed

Aftermath

- animal models became the gold standard to demonstrate safety and efficacy of drug for humans
- Drug manufacturers must prove drugs are safe and effective before they are marketed.
- Now, drug approval can take between eight and twelve years, involving animal testing and tightly regulated human clinical trials

Fda.gov

Aftermath

Before starting clinical studies in humans, testing in animals is still the only way to fully evaluate

- the efficacy,
- pharmacology,
- pharmacokinetic and
- safety of a potential drug candidate

Fda.gov

Why drugs fail in clinical trials

- Drugs fails due to
  - Lack of efficacy, the capacity to produce an effect (eg, lower blood pressure or cholesterol).
  - Toxic issues

Fda.gov

Problem of drug toxicity in humans

- In 1993, Fialuridine , Hepatitis B virus therapy was considered safe after rigorous animals studies
- But in a small scale clinical study, 15 patients with chronic hepatitis B infections were administered Fialuridine
- The drug caused lactic acidosis in seven patients
  - Two patients died,
  - five received liver transplantation.
  - Overall, 5 out of 15 patients died
- One of the first cases of human-specific, drug-induced toxicity

Cohen J *Science* 2014

Liver - a major drug metabolism organ

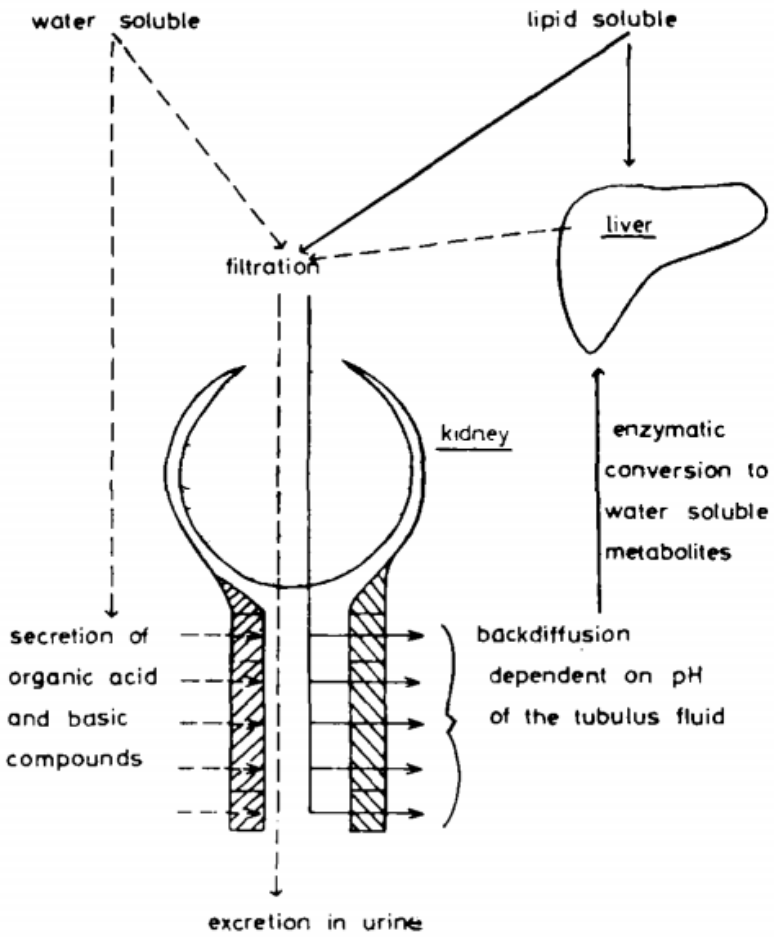


Figure 1. Elimination of drugs.

TABLE I Metabolism of Drugs by Enzymes Located in the Endoplasmic reticulum

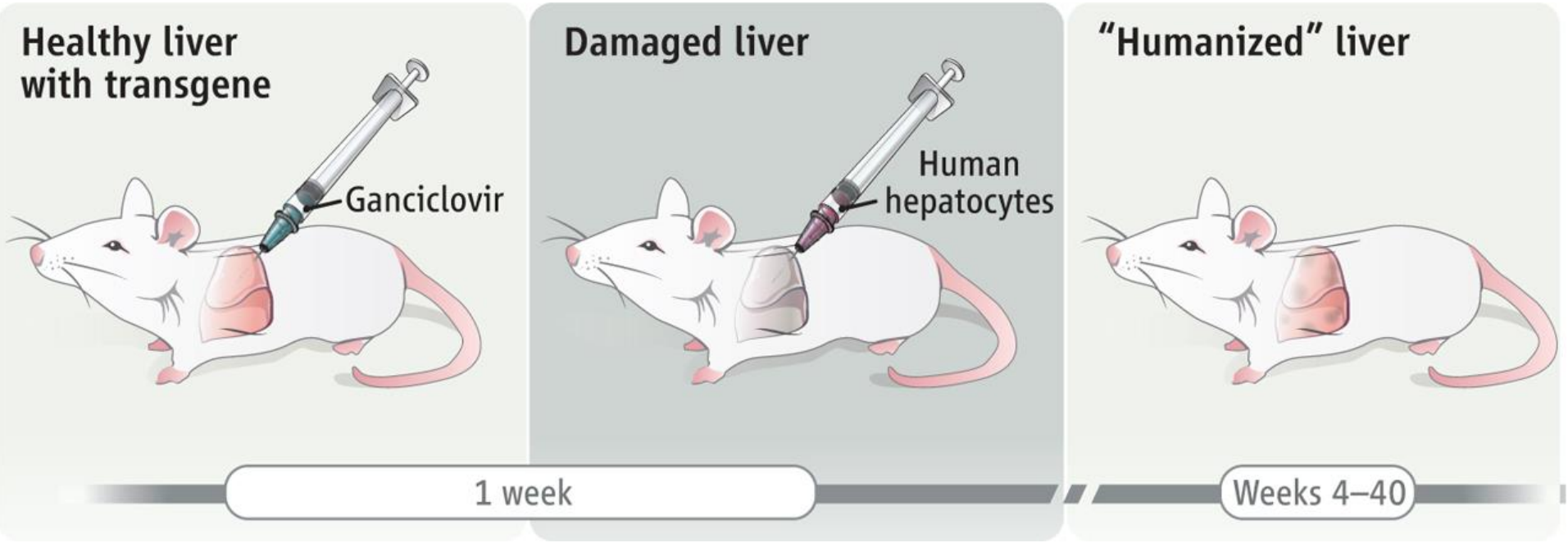
Metabolism	Enzyme
Oxidations of aliphatic and aromatic groups: barbiturates, diazepoxides, phenothiazines, meprobamate, phenytoine, etc. antihistamics, phenacetin, aminopyrine, antipyrine and congeners synthetic steroids, contraceptives, anabolic androgens (digitoxin)	Cytochrome P_{450}
Reductions of azo- and nitro-groups: azo-dyes, (chloramphenicol), etc.	Flavin enzymes
Hydrolyses of esters and acidamides: procaine, lidocaine, meperidine, atropine	Esterases
Conjugations of glucuronic acid with following groups: (a) alcoholic and phenolic (b) (carboxyl) (c) (amine)	Transferases

NOTE: Parentheses indicate that the metabolic pathway mentioned plays a minor role in conversion.

Can we replace animal liver cells with human liver cells?

Remmer H *Am. J. Med.* 1970

Mice model with humanized liver

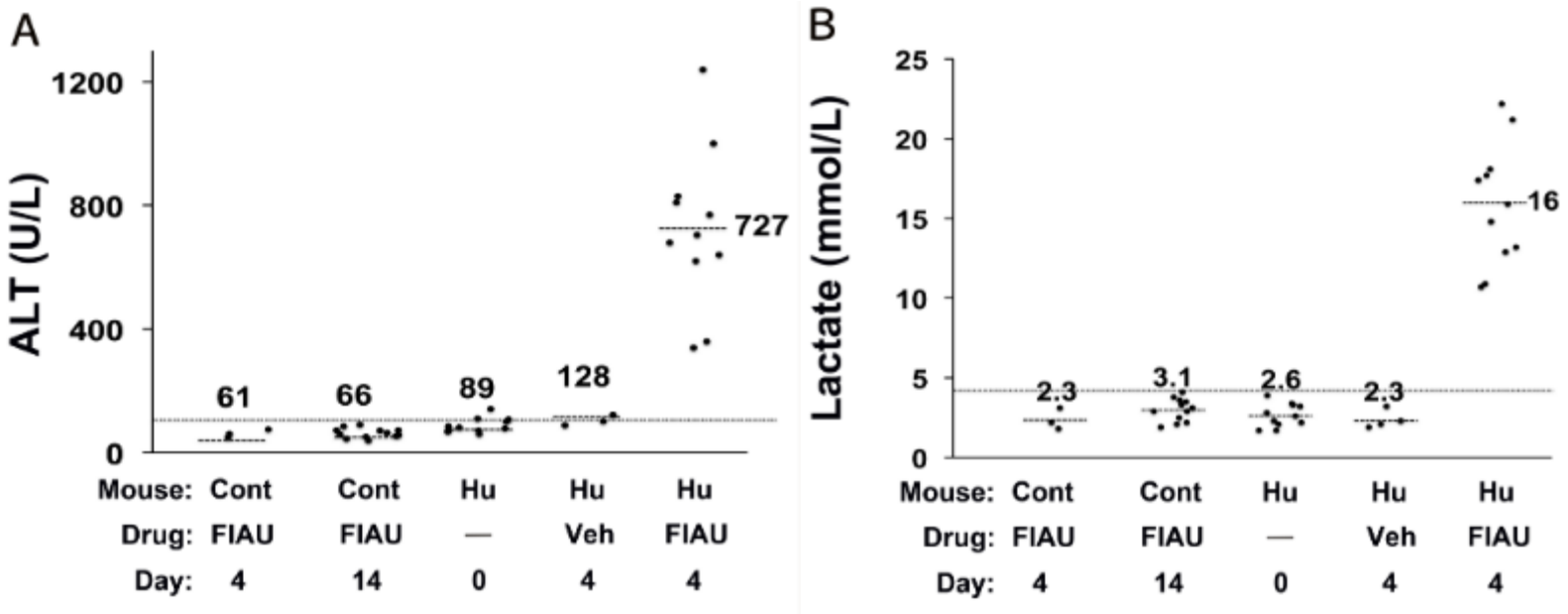


TK-NOG mice

- immunodeficient mice,
- expressing a herpes simplex virus type 1 thymidine kinase (TK) transgene within the liver

Cohen J *Science* 2014

Mice model with humanized liver



FIAU-treated mice with humanized livers

- develop increased levels of serum ALT and lactate,
- indicators of liver injury

Xu D et al., *PLoS Med.* 2014

Limitations of TK-NOG humanized liver model

- TK-NOG mice are highly immunocompromised
- Such mice model cannot be used to analyze immune-mediated drug toxicities.

Cohen J *Science* 2014

Challenge of genetic background

- Opioid addiction is studied using morphine administration in mice
- C57BL/6J mice show addictive behaviour to morphine
- DBA/2J mouse do not show addictive behaviour
- Genetic diversity must be considered at all steps of the drug discovery pipeline

Elmer GI et al., *Physchopharmcology*, 2010

Conclusions

- Animal models can be used in drug discovery
- Unlike other models, animals represent the biological complexity of integrated organ systems
- Success in drug discovery in animals models requires
  - robust, reproducible data,
  - predictive of human biology

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A WOMAN DOCTOR WHO WOULD NOT BE HURRIED

by JOHN MULLIKEN

The application was submitted by the William S. Merrell Co., an old Cincinnati drug firm. When it arrived on Dr. Kelsey's desk in the form of three blue- and black-bound folders, each the size of a telephone book, it seemed routine enough. Merrell was asking the Food and Drug Administration to evaluate and license a drug called thalidomide which they wanted to market, by prescription, under the trade name Kevadon—from all accounts the perfect sleeping pill. The folders contained Merrell's complete laboratory, clinical, pharmaceutical and animal testing data, and because of thalidomide's resounding success in Europe the firm wanted to get it on the U.S. market.

For Frances Kelsey, a pharmacologist, physician and mother of two daughters, the application seemed even easier than routine to process. But she knew her job was to move cautiously and watch for that 10th chance in 10 that the drug might be unsafe. As she began to read the contents of the folders, Dr. Kelsey became uncomfortable. "There was something a little different about this one," she explains, "so it seemed better to be safe and sure." The drug did not, for example, have the same effect on animals: they were not put to sleep by it. Dr. Kelsey knew she had to start from scratch—to treat thalidomide as though it had never been used before.

As part of the application procedure Merrell had already sent the drug out to American doctors—the number eventually reached 1,200—*me you couldn't print,*" she says.

While company men were voicing their opinion of Dr. Kelsey to her superiors, her division boss, Julius Hauser, decided to hold fast. "When they got too thick around Dr. Kelsey," says Hauser, "I would just come in with my little hatchet and clear them out." While this kind of backing was most welcome, it could not prevent Dr. Kelsey from reading editorials like the one that appeared in a bi-weekly magazine sent to doctors, the *Medical World News*, castigating the FDA for ". . . dilatory tactics which certainly cause a loss to the industry of millions of dollars . . . and even loss of life. . . ."

Under this intensive pressure, Dr. Kelsey kept writing letters to Merrell telling them that the information in the application was still incomplete. Then in February 1961 she discovered the first item that confirmed her now 6-month-old suspicions. Buried in the letters column of the *British Medical Journal* was a note from a reader which warned that reports had been drifting in to a company marketing thalidomide in England of "a possible toxic hazard with the sedative drug . . ." and there were ". . . signs suggestive of peripheral neuritis in patients receiving thalidomide for periods of six months or more."

Over the next few months other cases were reported. There were strong indications that the drug could cause numbness, shaking, tingling of hands and other minor and sensory disturbances. Dr. Kelsey was not jubilant, for this was not that kind of

necessary, to keep the drug off market. "Needless to say, now t were many people telling me the 60 days were up," she remem

Then at the end of November e thing fell into place. All her s cions, all her patience turned o have been worthwhile.

From West Germany it was re ed that a Dr. Lenz had made a sp indicating that some drug might causing the phocomelia "epide which had been spreading in his c try for the last three years. In speech Lenz had not named the d but afterward another doctor come to him and asked, "Will you me confidentially, is it the drug C tergan? I ask because we have a child and my wife took Contergan Lenz had to admit that it was."

Other tragic stories began to back to Dr. Kelsey from Austr Scotland, Sweden, Belgium, Swi land, Lebanon, Israel and Pers concerning women with deformed bies who had taken one form o other of thalidomide.

On Nov. 26 Contergan was drawn from the German market. rell, which actually informed Dr. ney of the Lenz discovery, held further consideration of Kevadon the FDA upon hearing of the sp in November and immediately their investigators back to Europe March they formally canceled application.

While Dr. Kelsey's stubborn has prevented uncounted tragedi has also brought her unlooked congratulations. Senator Kefa has proposed that she be giv