

SEPTEMBER

2026

Vol. 2 No.3

Zebrafish Models

**Cancer Clonal Variant Screening
System**

**New Approaches in Wilson's
Disease Modelling**

**New Insights into Mitochondrial
Biology - Lazarus Biosciences**

**Selective Serotonin-Dopamine
Reuptake Inhibitors Screening
System in Zebrafish**

Systemic Lupus Erythematosus

Dravet Syndrome

Guillan-Barre Syndrome

Diabetic Macular Edema

PENTAGRIT

www.pentagrit.com

AGGREGATES OF ABETA IN NEOCORTEX

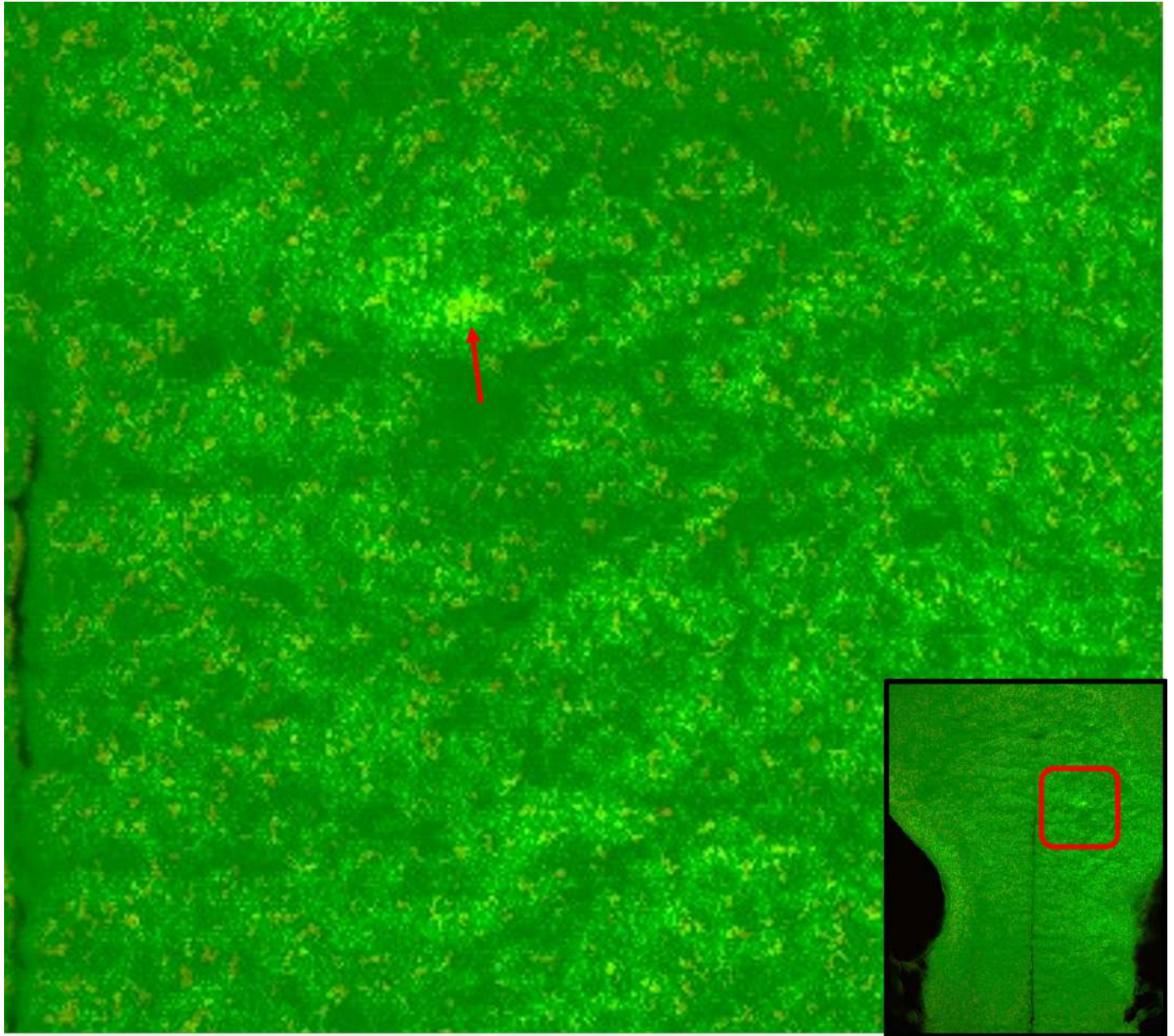


Figure showing brain slice of zebrafish in Alzheimer's model $WT\text{-}app^{pgt115(p.Val717Leu)};WT\text{-}ps^{pgt117/pgt117(p.Leu435His)}$ with aggregates of Abeta initiating in the neocortex region called pallium in zebrafish.

Why Cancer Drugs Fail ?



Mr. Benin Joseph, Director – Innovations at Pentagrit

Top 3 Reasons

Lack of efficacy
Drug Resistance and
Tumor Heterogeneity

Distribution of Cancer Drug Failures (2006-2026)

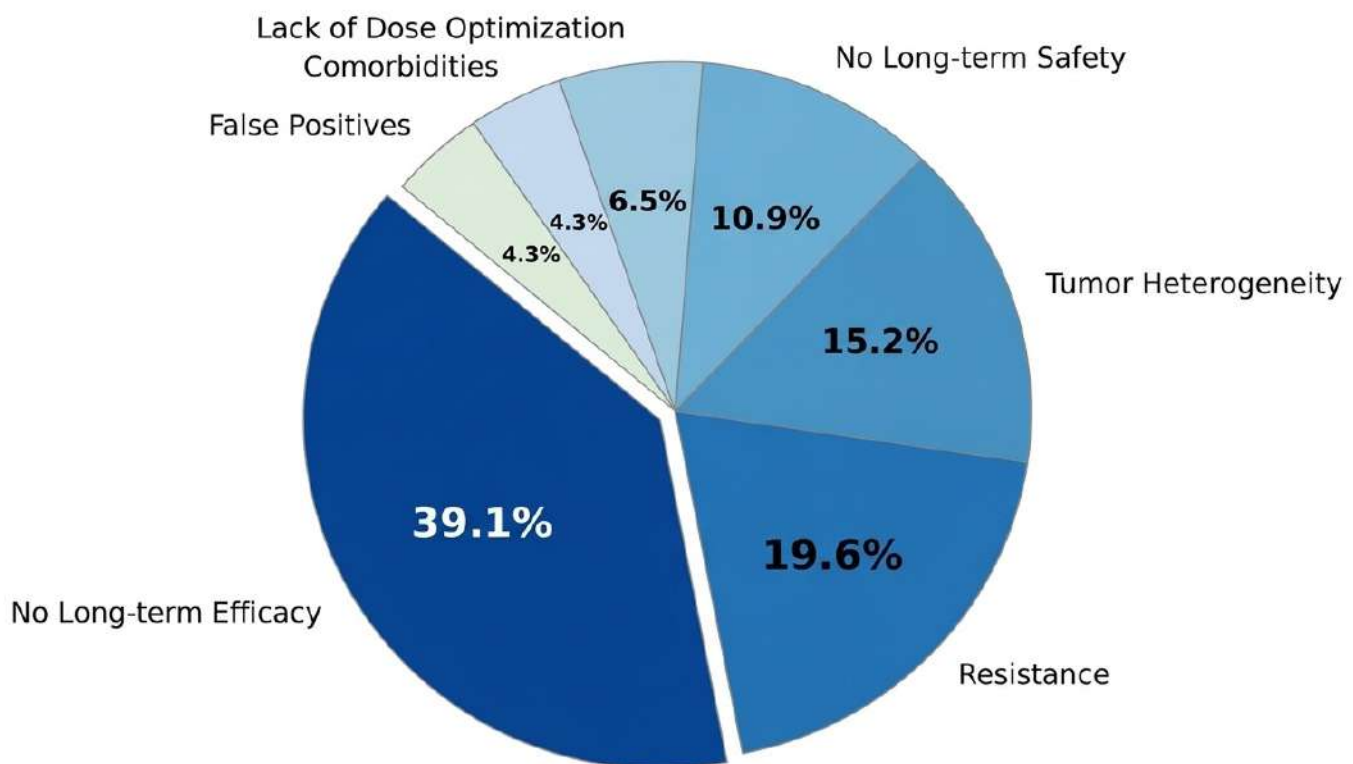


Figure 1 A pie chart representation of distribution of cancer drug failures from 2006 to 2026, Clinicaltrials.gov

All the top 3 reasons are interlinked and are synergetic contributors to drug failure. To overcome this challenge, a number of new methods of selecting compounds have been employed such as tissue variants that are clonally diverse from each other.

The Character of Cancer Clones

1. Every cancer therapy is a selection of resistant cells

Clonal diversity is the primary reason why highly advanced targeted cancer therapies often fail. Every cancer tissue carries a wide range of cells that are highly heterogenous. In principle, for any drug combination provided there would be a number of cells that are highly sensitive, a number of cells that are poorly sensitive and a number of cells that are highly resistant. The resistant cells now form a new clone after treatment. Hence almost all cancer treatments are eventually a clonal selection process that leads to a therapeutic relapse.

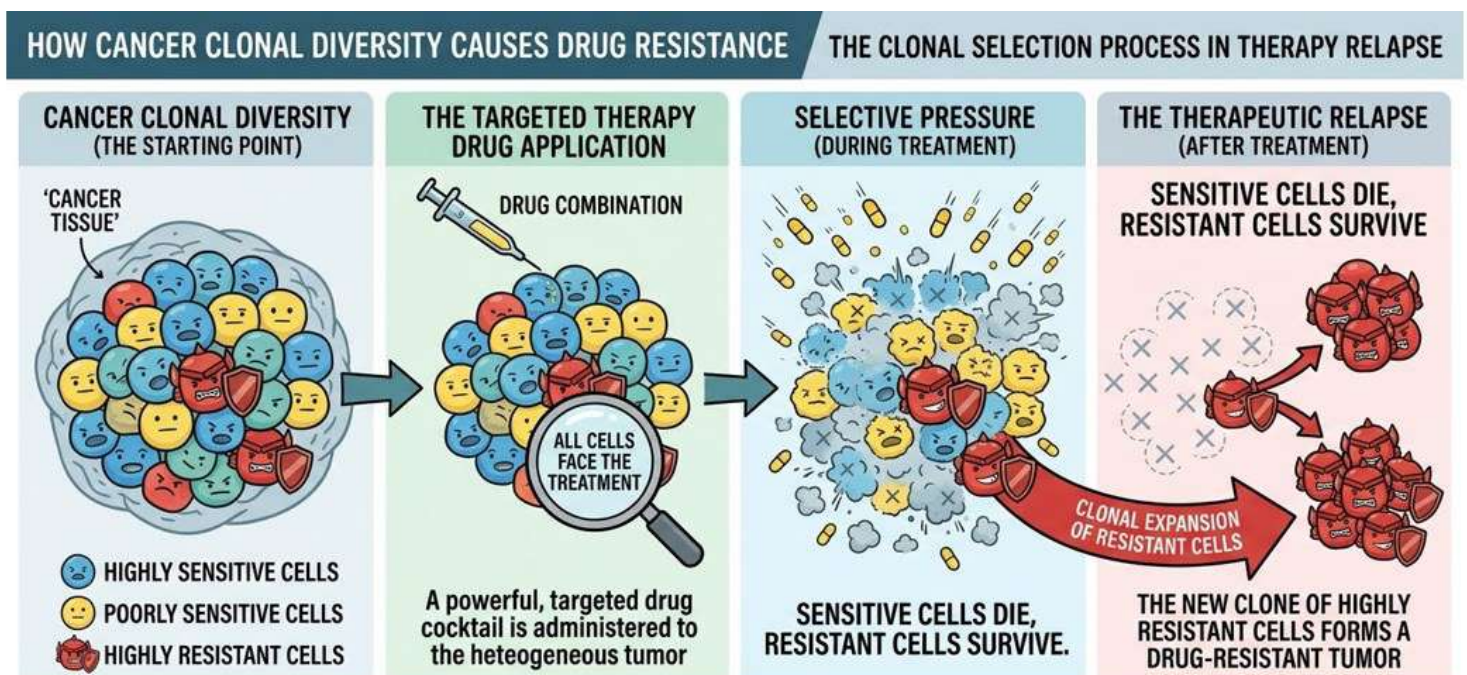


Figure 2 Schematic representation of cancer clonal diversity causing drug resistance

2. Clonal cooperation is a bigger target than kinases

Various type of clones cooperate deeply among themselves since they each bear specific properties. Initially clones regulate their local microenvironment by first secreting amphiregulin either by themselves or by inducing healthy helper cells to secrete. Once secreted, amphiregulin further promotes division and proliferation of clones. At this point, clones mobilize and travel together in clusters by secreting CD44 and E-cadherin that keeps them anchored to each other. Certain clones that secrete matrix metalloproteinases (MMPs) expressing markers such as Keratin

able to penetrate much more deeper through the extracellular matrix into various body tissues. Other clones that do not secrete MMPs also follow this path thereby building a logistics pathway. During the active process of proliferation and transport of clones, they continually suppress NK cells and T cells to evade detection. Eventually a large multiclonal community forms through constant cross talk and exchange of soluble metabolites and vesicles containing miRNAs and other messenger that fortify each other.

Summary of Inter-Clonal Roles: The dynamic can be thought of as a specialized workforce:

Clone Type	Primary Contribution to Metastasis	Molecular/Cellular Mechanism
Invasive Clone	Clears physical tissue barriers	Secretes MMP proteases; expresses Keratin 14.
Producer Clone	Provides temporary signalling boosts	Secretes growth factors like amphiregulin.
Adhesive/Chaperone Clone	Drives aggregation and transit safety	Expresses high levels of CD44 or E-cadherin.
Proliferative/Responder Clone	Populates and expands the new colony	Possesses high intrinsic growth drive (e.g., ERBB2 amplification).

Table 1 Represents summary of clonal type, primary contribution and its mechanism

INSIDE THE MULTICLONAL COMMUNITY: HOW CANCER CELLS WORK TOGETHER

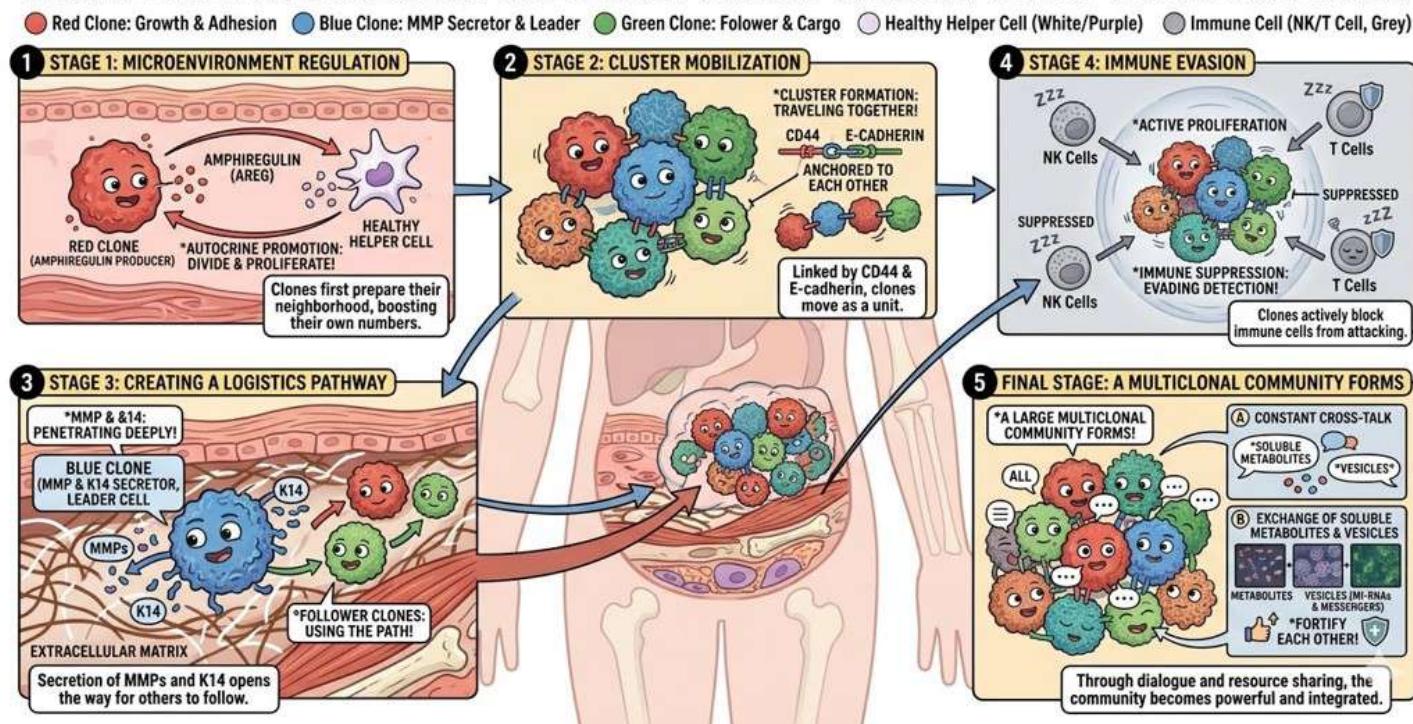


Figure 3 Schematic representation of formation of multiclonal cancer cell community

3. Clones become dormant when conditions are not favourable but remain fully alive

Under conditions where there is a lack of nutrients and an increased immune surveillance, clones go into a quiescent stage. They arrest their cell division cycle and stay at either G0/G1 stage. All active growth pathways such as Ras/MAPK and PI3K/Akt are shut down and the ratio of p38 MAPK to ERK is increased to pause cell division. Within certain organs, clones send sleep signals such as TGF- β 2,

BMP4 or type III collagen secretion around themselves to stay functional. They also balance the rate of destruction of old cells versus the formation of new clone cells thereby surviving any stress without nutrient depletion. A classical pathway is to downregulate cell surface MHC class I to become invisible to immune cells and if detected, display PD-L1 and paralyze the immune cell.

THE COOPERATIVE CELLULAR SLEEP PROTOCOL: SURVIVING HIGH-STRESS MICROENVIRONMENTS

HOSTILE ENVIRONMENT: LACK OF NUTRIENTS + INCREASED IMMUNE SURVEILLANCE.

CLONES SECRETE TGF- β 2, BMP4, OR TYPE III COLLAGEN TO STAY FUNCTIONAL.

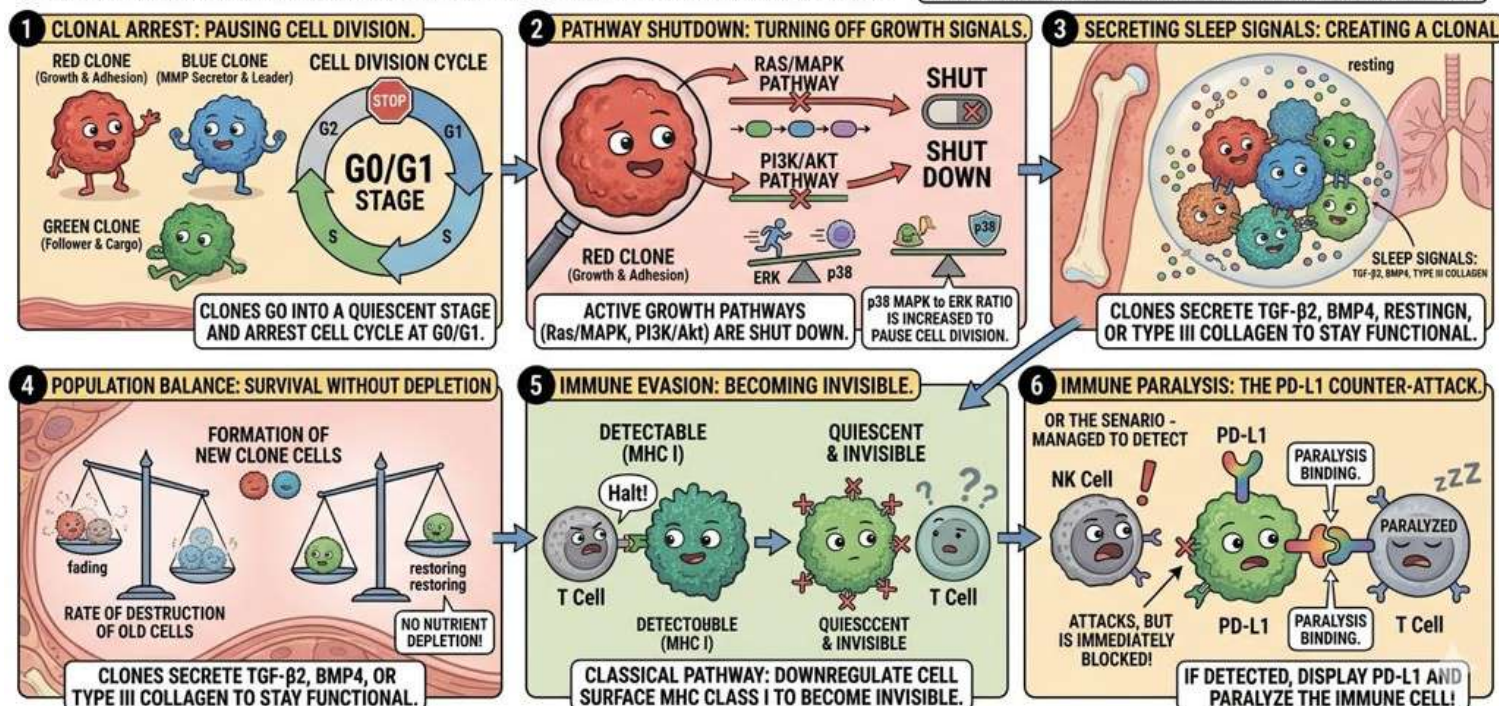


Figure 4 Schematic representation of how cancer cells survive high stress microenvironments

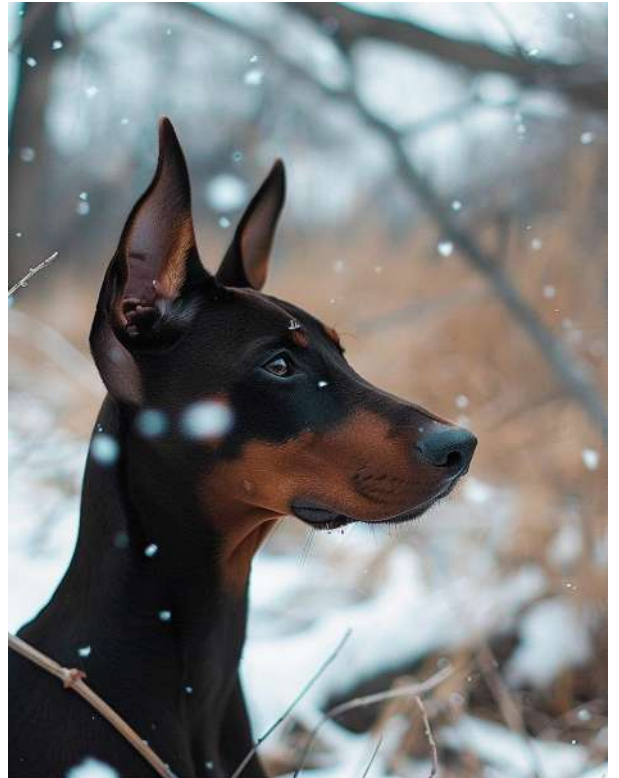
A drug may suppress tumor growth today, but what happens when resistant clones emerge?

Tumor response is shaped not only by the cancer cell itself, but by the tumor microenvironment and evolving resistant clones.

Our screening strategy integrates tumor biology, microenvironmental factors, and resistance-associated responses to help build a more comprehensive picture of inhibitor performance.

Reach out to us for a comprehensive study plan and to explore the scope of our screening platform designed to evaluate tumor inhibitors in the context of microenvironmental interactions, treatment response, and mechanisms driving clonal resistance for tumor inhibitors.

What hunting dogs have in common with fish and humans?



If you own a Bedlington or Labrador Retriever you would want to keep copper out of their bowls since they are prone to copper accumulation in their liver. A fish also does something similar - absorb copper from the pond water into their liver

Fascinatingly enough, the exact phenomenon happens in humans., about one in 100 people carry a defective gene *atp7b* whose responsibility is to flush out excess copper leading to copper accumulation in the liver called Wilson's disease.



Ms. Kalaichitra Periyasamy, Founder and Scientific Director, Pentagrit

“Routine chelation therapies which are the best way to treat copper accumulation are ineffective in epileptics or autistic children”

Approaching Wilson’s Disease

In Wilson’s disease, copper accumulates in the liver, kidney and brain and those organs are primarily affected. The protein machinery that is responsible to remove copper is compromised.

The brighter side is that the key regions of the protein responsible for copper absorption and detoxification are preserved between humans, dogs and zebrafish. Therefore, new drugs can be screened in epileptic fish carrying defective *atp7b* protein to identify safe drugs that work in humans.

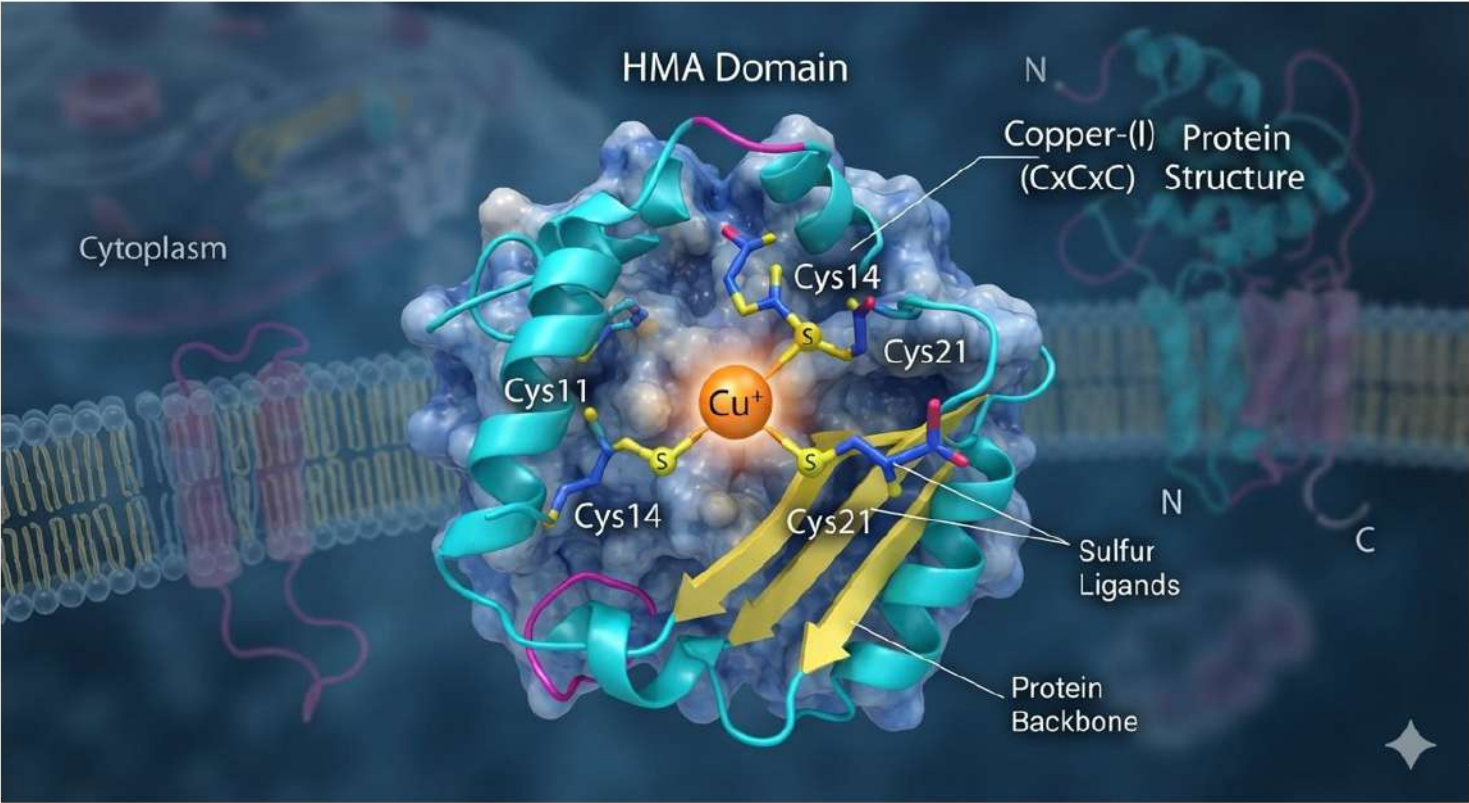


Figure 5 Schema of the protein region called the Heavy Metal-Associated (HMA) domain that identifies and transfer copper into the ATP7B protein.

Structural Domain	Conserved Sequence Motif	Functional Significance	Conservation Status between humans, dogs and zebrafish
Actuator (A-domain)	TGES	Mediates the dephosphorylation step of the catalytic cycle.	100% Conserved across all three species.
Phosphorylation (P-domain)	DKTGTLT	Contains the invariant Aspartate (D) residue that accepts the phosphate group from ATP.	100% Conserved across all three species.
Nucleotide-binding (N-domain)	GDGVND / KGAPE	Forms the core binding pocket for adenosine triphosphate (ATP) molecules.	Highly Conserved; identical pocket geometry in zebrafish.

Table 2 showing : Conservation Status between humans, dogs and zebrafish

Pentagrit's Core Expertise

Through the use of gene editing tools, a zebrafish mutant was created that lacked ATP7b function which developed Wilson's disease pathology. Offsprings were employed for drug discovery and screening for new investigative compounds.

WT ATP7b vs Mutant ATP7b

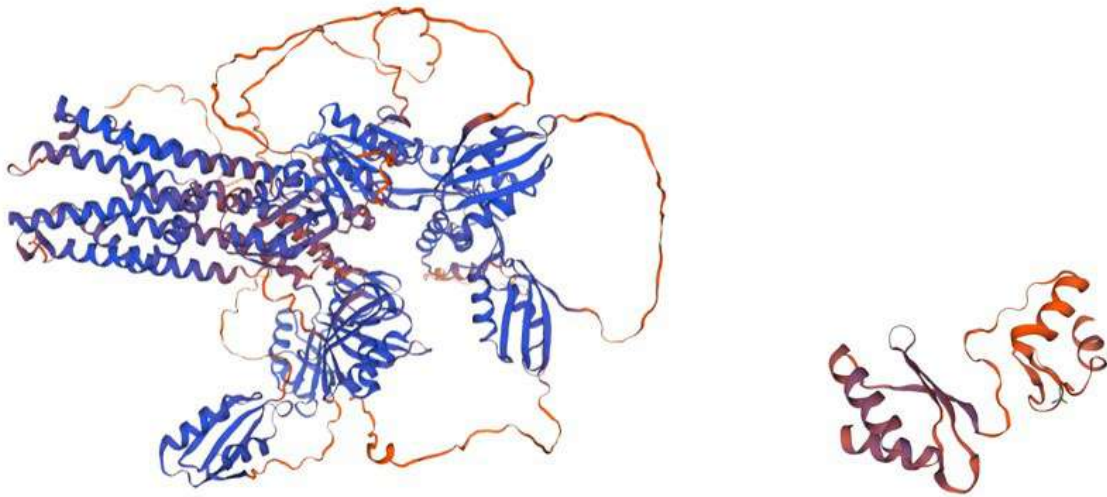


Figure 6 Human ATP7b full length protein (left side) vs mutated protein (right side) in zebrafish Wilson's disease model

Wilson's disease zebrafish model was employed in multiple screening studies such as

1. Validating drugs that showed results in rodents or in vitro
2. Generating toxicity profile under chronic exposure
3. Validating juvenile response during epileptic and autistic comorbidity
4. Biomarker rescue
5. Demonstration of mechanism during the presence of drug activity pathways



Unlocking New Insights into Mitochondrial Biology

Lazarus Biosciences is at the forefront of mitochondrial biology, developing advanced sensing, imaging and data technologies to investigate metabolic and mitochondrial function with greater sensitivity and resolution

<https://www.lazarusbiosciences.com>

Measuring Highly Accurate Mitochondrial response with Lazarus Biosciences Platform

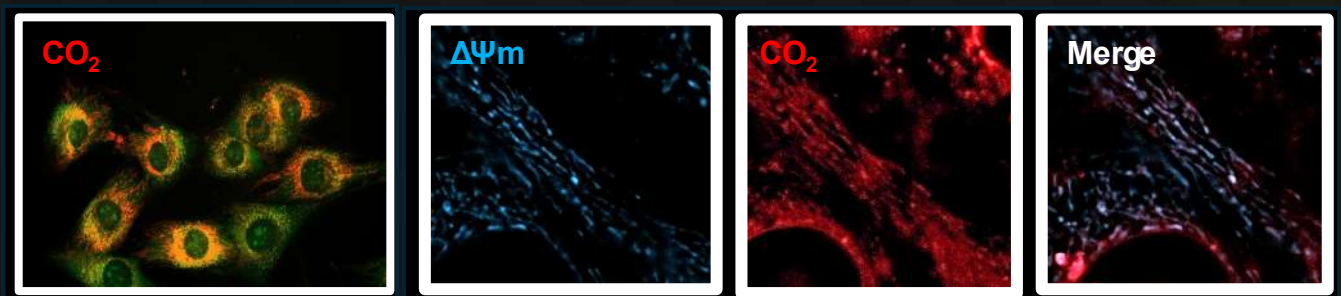
- Breakthrough proprietary chemistry
- 1 hour mitochondrial assays
- Measures made in vitro and in zebrafish

Human Gene	Human Disease	Zebrafish Ortholog	Zebrafish Model / Line
TP53	Li-Fraumeni Syndrome / Cancer	tp53	tp53 ^{M214K} mutant
DMD	Duchenne Muscular Dystrophy	dmd	dmd ^{ta222a} (sapje) mutant
SHANK3	Phelan-McDermid Syndrome / Autism	shank3a / shank3b	shank3a/b CRISPR mutants
HTT	Huntington's Disease	htt	htt exon 1 Tg / knock-in
CFTR	Cystic Fibrosis	cftr	cftr ^{pd1048} mutant
GBA1	Gaucher Disease	gba1	gba1 ^{sa16214} mutant

The Lazarus Assay Platform

The assay platform provides multimodal analysis of intracellular CO₂ production and mitochondrial activity, enabling both quantitative live-cell measurements and spatiotemporal visualisation. The platform incorporates interchangeable, highly photostable fluorophores, providing flexibility across experimental configurations and imaging applications.

Carbon dioxide (CO₂) production is closely linked to mitochondrial activity, as CO₂ is generated during the tricarboxylic acid (TCA) cycle. Measuring intracellular CO₂ production therefore provides a functional readout of mitochondrial catabolic flux. Changes in CO₂ generation reflect alterations in mitochondrial activity.



Our assay technologies are compatible with multiplexed analysis, as demonstrated in the figure above. CO₂ detection can be combined with ΔΨ_m/MMP-sensitive dyes to simultaneously assess mitochondrial membrane potential and intracellular CO₂ production, enabling the identification of distinct cellular populations with different metabolic profiles.



Office:
NovaUCD, Belfield, Dublin 4, Ireland



Measuring mitochondrial myopathies and off target toxicities with Lazarus Biosciences Platform

- **Mitochondrial Myopathies:** CRISPR-engineered nuclear gene mutants (like *dnajb6a/b* or *coa6*) exhibit late-onset progressive muscle weakness, structurally disorganized mitochondrial cristae, and severe cardiomyopathy.

Screening for off-target mitochondrial toxicity for New Drug Development

Oxygen Consumption Rate (OCR): Respiration is the most direct indicator of mitochondrial health. During oxygen consumption, an abrupt drop or an unnatural spike flags off-target interference.

Mitochondrial Membrane Potential: Mitochondria must maintain an electrical gradient across their inner membrane to produce ATP. High-throughput screening programs, from Lazarus Biosciences screen large libraries using fluorescent, voltage-sensitive dyes.

Reactive Oxygen Species (ROS) Generation: Off-target disruption of the Electron Transport Chain (ETC) frequently causes electrons to leak, creating highly destructive free radicals. Lazarus Bioscience's luminescent or fluorescent assays screen for sudden spikes in mtROS accumulation.

Why SDRIs Go Haywire: The Challenge of Dual Neurotransmitter Modulation

by Keerthana Rajendran

Selective Serotonin-Dopamine Reuptake Inhibitors (SDRIs) simultaneously modulate serotonergic and dopaminergic neurotransmission through inhibition of SERT and DAT. This dual mechanism offers a potential strategy for neuropsychiatric disorders involving mood, motivation, reward, cognition and affective regulation.

However, serotonin and dopamine also participate in the regulation of locomotion, arousal, exploration, motivation and motor function. This broad physiological involvement makes phenotypic interpretation a critical challenge in SDRI development.

The objective is therefore not simply to identify a treatment associated change, but to establish whether that response represents disease relevant phenotypic normalization.

The Risk of False Positives in Behavioral Assessment

False positive efficacy can arise when a pharmacological effect produces a phenotype that resembles therapeutic improvement.

For example, increased locomotor activity in a disease model may appear to represent recovery from hypolocomotion,

but could instead result from enhanced dopaminergic activity. Increased exploration may be consistent with reduced anxiety like behavior, yet may also reflect altered arousal. Conversely, reduced activity may be interpreted as normalization of hyperactivity when it is actually associated with sedation or impaired motor function.

These possibilities make behavioral endpoints particularly vulnerable to pharmacological confounding.

A robust assessment should therefore establish whether the observed phenotype is:

- **Disease relevant** – reflects correction of a defined pathological deficit rather than an unrelated change.
- **Dose dependent** – shows a consistent relationship with drug exposure within the therapeutic range.
- **Reproducible** – remains consistent across biological replicates and independent experimental studies.
- **Mechanistically consistent** – aligns with the expected consequences of SERT/DAT modulation and supporting molecular evidence.
- **Independent of major alterations in motor function or arousal** – persists without generalized hyperactivity, sedation or impaired motor capacity.

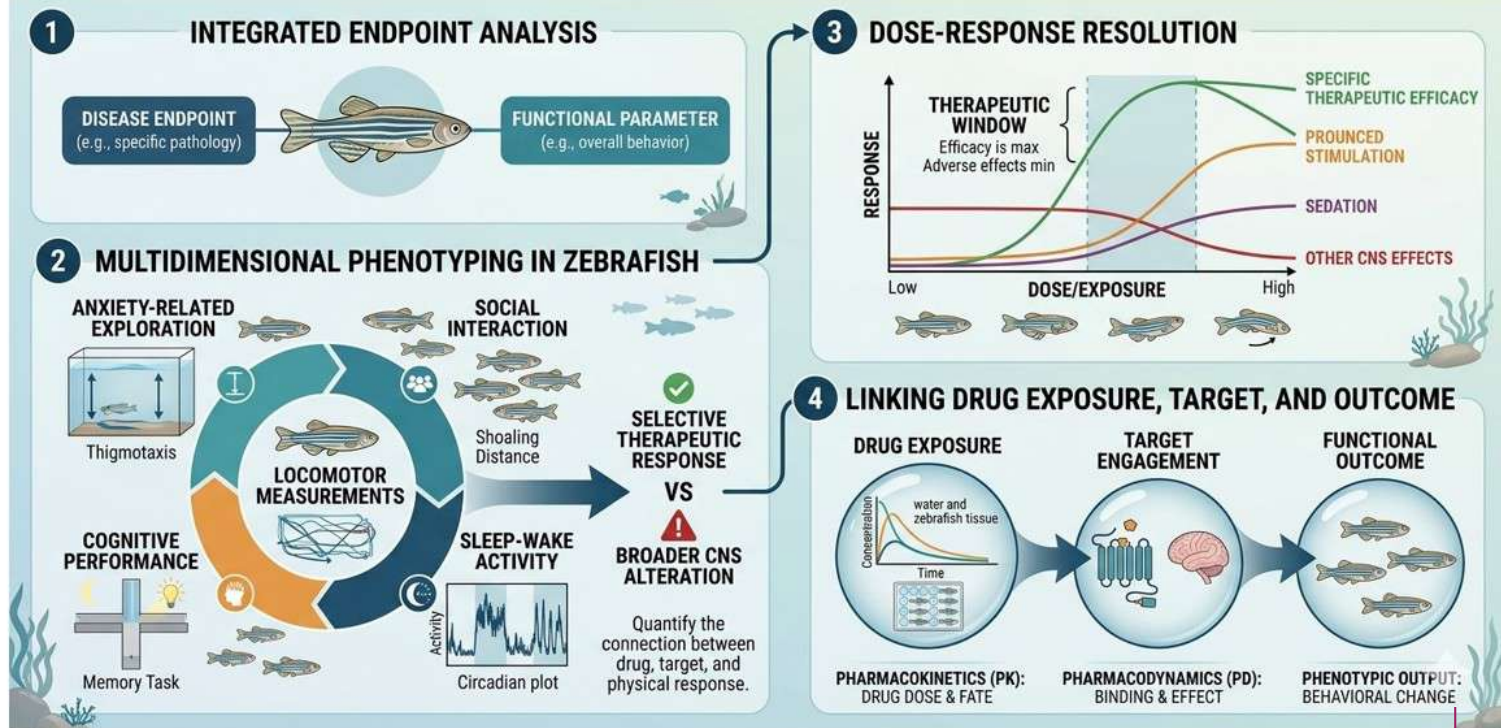


Figure 7 Schematic representation of integrated methodology to measure SDRI response

Can We Really Tell When an SDRI is Working?

SDRI development faces a fundamental challenge: a change in behavior does not always mean therapeutic efficacy.

With serotonin and dopamine pathways being modulated simultaneously, distinguishing genuine pharmacological effects from nonspecific behavioral responses can be difficult.

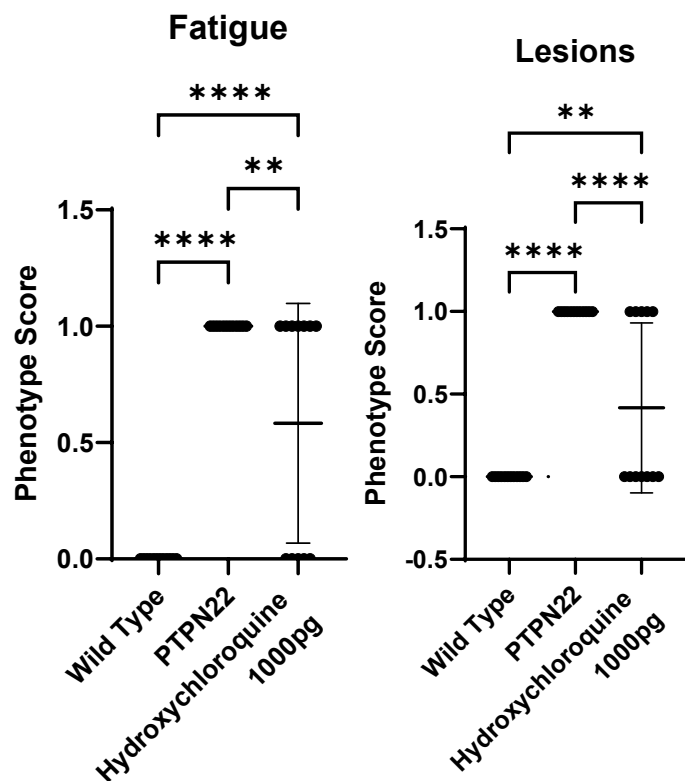
A new zebrafish methodology brings together complementary behavioral and functional readouts to provide a more systematic way to characterize SDRI responses and strengthen preclinical interpretation.

What does this methodology measure? How does it help distinguish true drug response from misleading signals?

Reach out to us for a comprehensive SDRI study plan and screening platform scope, integrating complementary behavioral and functional endpoints to support more reliable pharmacological characterization.

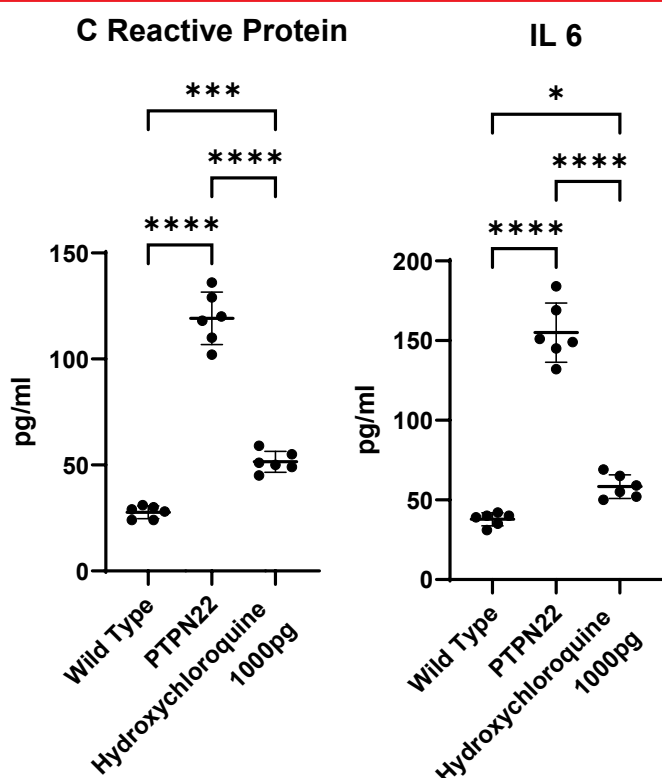
THERAPEUTIC PHENOTYPE & BIOMARKERS In Zebrafish

Autoimmune - Systemic Lupus Erythematosus
Model - Ptpn22 KO



Phenotype:

Ptpn22 KO zebrafish exhibit a SLE phenotype characterized by increased fatigue and development of inflammatory lesions compared to wild type, reflecting dysregulated immune signaling and systemic inflammation. Hydroxychloroquine treatment produces limited attenuation of these phenotypes, with minimal improvement in fatigue and lesion severity.

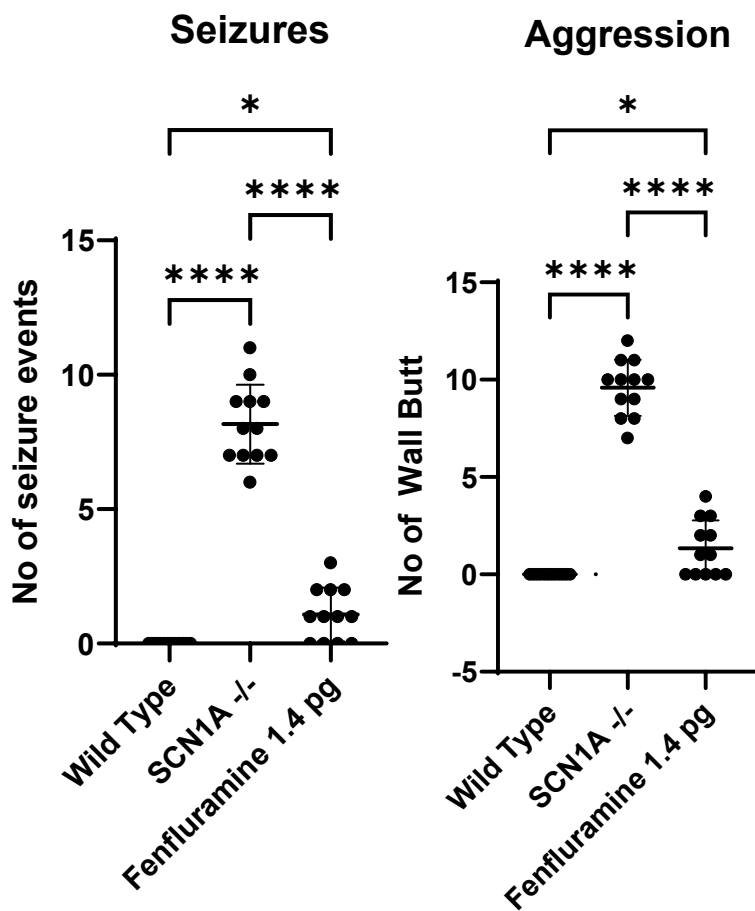


Biomarkers:

Ptpn22 KO zebrafish exhibit elevated CRP and IL6 levels compared to wild type, reflecting immune dysregulation and enhanced systemic inflammation associated with systemic lupus erythematosus. Hydroxychloroquine treatment attenuates these inflammatory responses by modulating immune activation, thereby reducing CRP and IL6 levels.

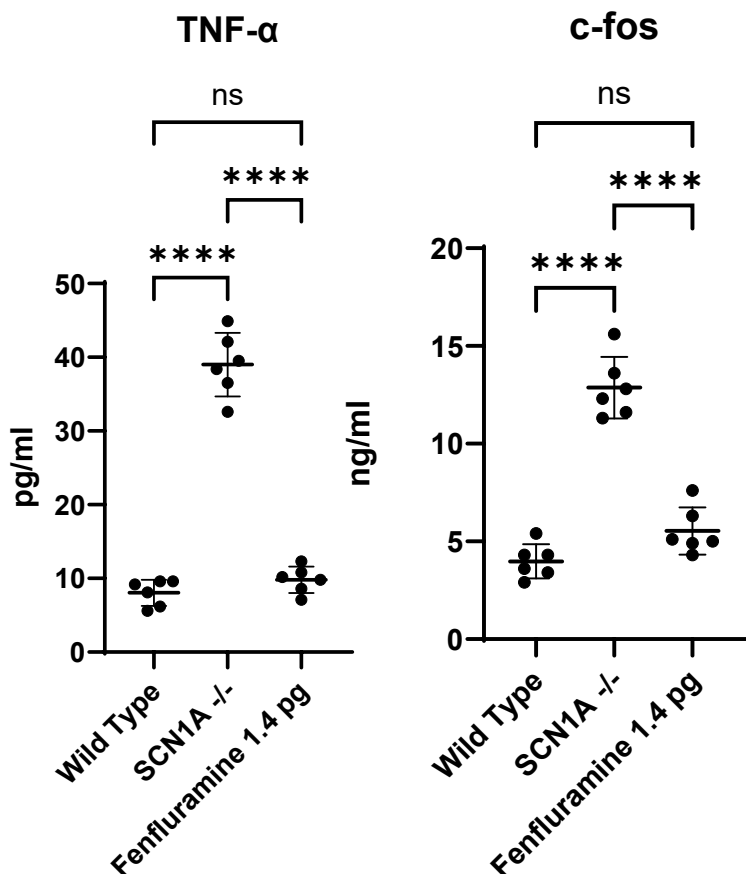
Neuro - Dravet Syndrome

Model - *scn1a* -/-



Phenotype:

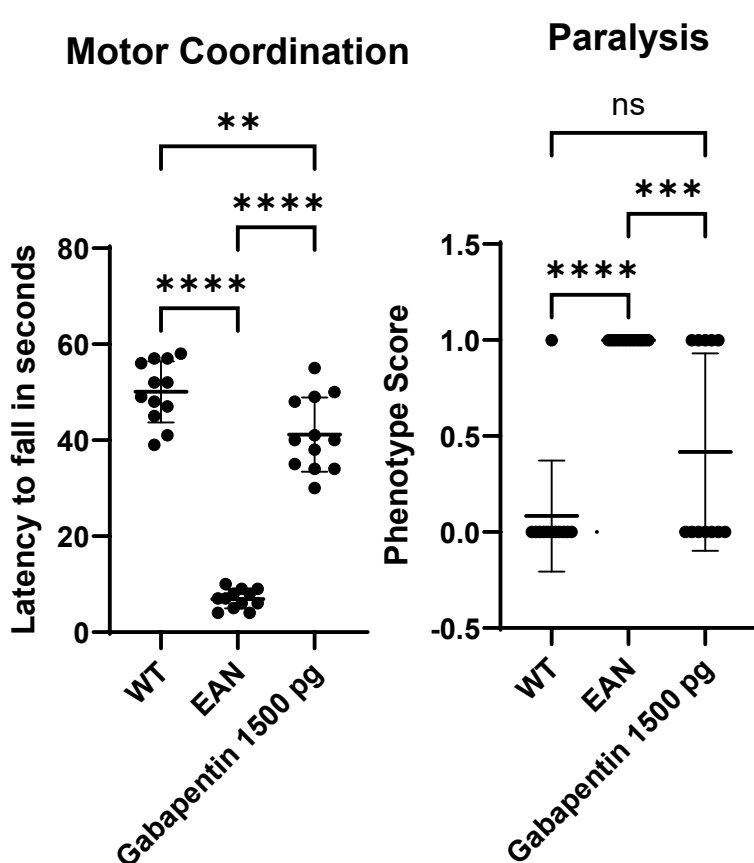
scn1a -/- zebrafish exhibit a Dravet syndrome phenotype characterized by increased seizure activity and aggression compared to wild type, reflecting neuronal hyperexcitability and impaired inhibitory neurotransmission associated with the disorder. Fenfluramine treatment attenuates these deficits by reducing seizure activity and improving behavioral abnormalities.



Biomarkers:

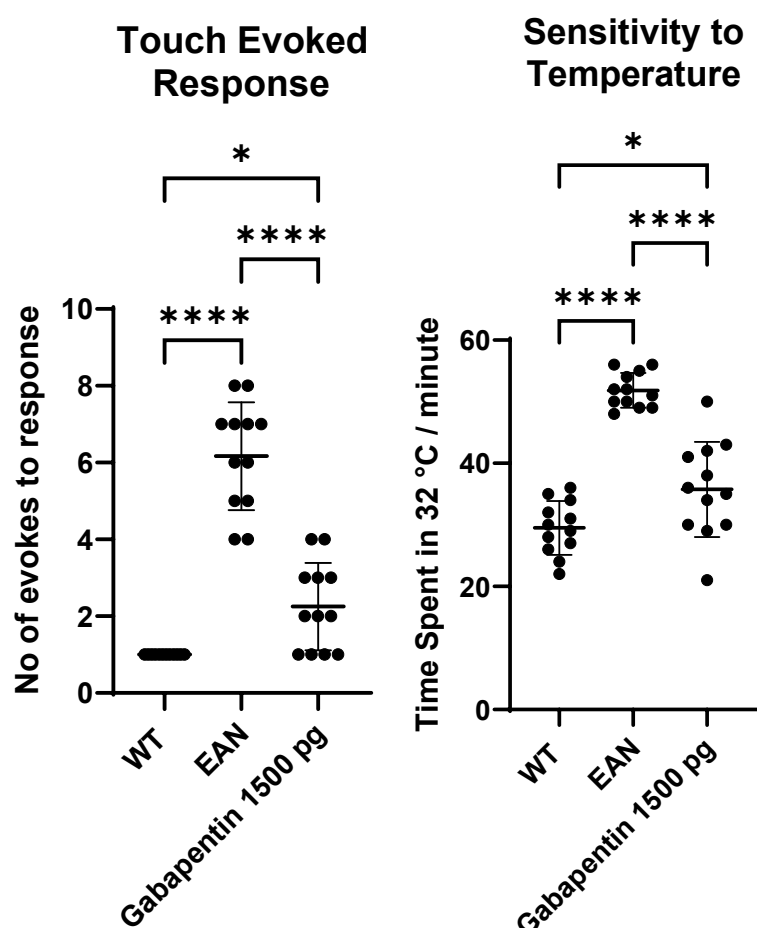
scn1a -/- zebrafish exhibit elevated TNF-α and c-Fos expression compared to wild type, reflecting neuronal hyperexcitability and neuroinflammatory activation associated with Dravet syndrome. Fenfluramine treatment reduces TNF-α and c-Fos expression, attenuating neuroinflammatory signaling and neuronal hyperactivity.

Model - EAN



Phenotype:

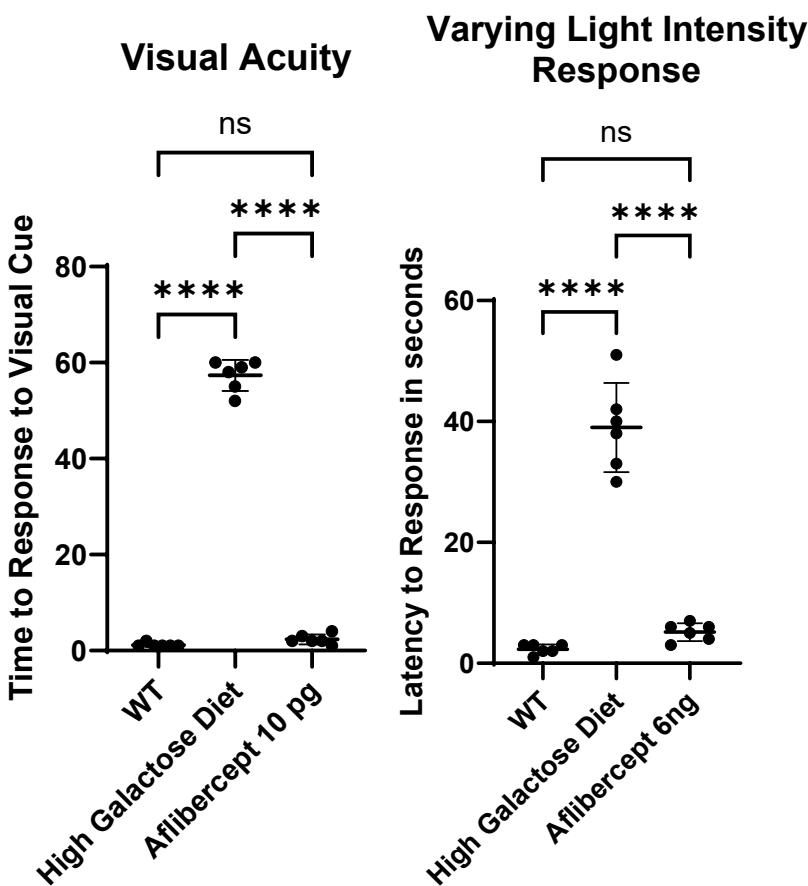
EAN-induced zebrafish model exhibit impaired motor coordination and paralysis compared to wild type, reflecting peripheral nerve dysfunction and motor deficits associated with Guillain-Barre syndrome. Gabapentin treatment attenuates these deficits by reducing neuropathic dysfunction and improving motor coordination and paralysis-associated responses.



Phenotype:

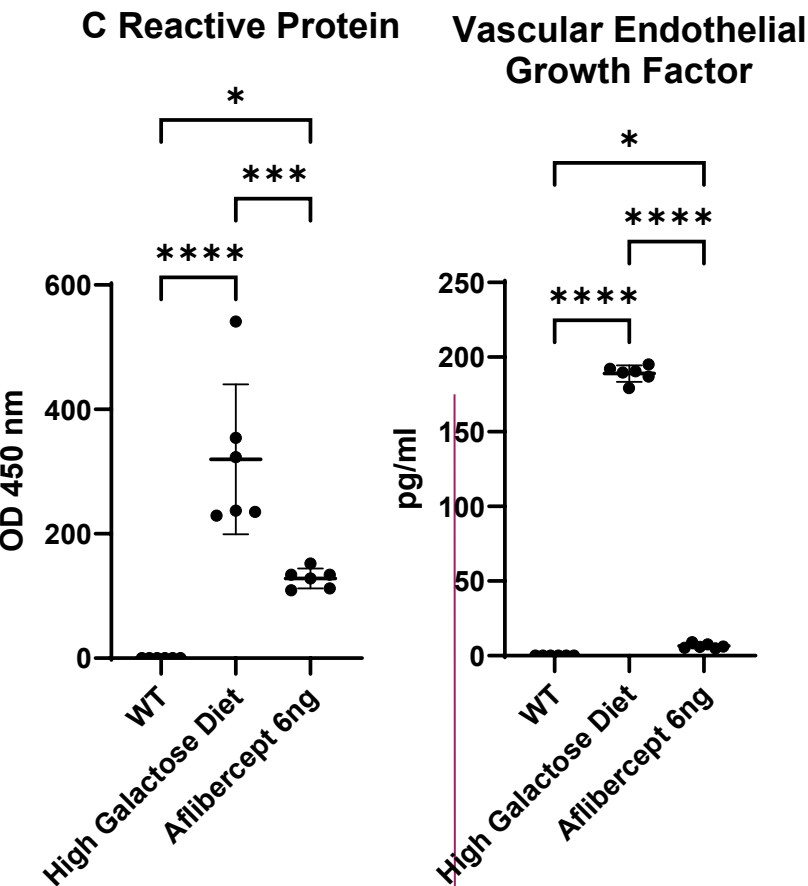
EAN-induced zebrafish model exhibit altered touch-evoked responses and increased sensitivity to temperature compared to wild type, reflecting peripheral nerve dysfunction and sensory abnormalities associated with Guillain-Barre syndrome. Gabapentin treatment attenuates these sensory deficits by reducing abnormal neuronal excitability and improving touch evoked and temperature response behaviors.

Model - High Galactose Diet



Phenotype:

High galactose diet induced zebrafish model exhibit impaired visual acuity and altered responses to varying light intensities compared to wild type, reflecting retinal dysfunction and visual impairment associated with diabetic macular edema. Aflibercept treatment attenuates by improving visual responses and reducing diabetes associated retinal dysfunction.



Biomarkers:

High galactose diet induced zebrafish model exhibit elevated CRP and VEGF levels compared to wild type, reflecting systemic inflammation and pathological angiogenic signaling associated with diabetic macular edema. Aflibercept treatment attenuates these abnormalities by reducing CRP and VEGF expression, thereby mitigating retinal vascular dysfunction.

CONTACT *US*

“

TOP 3 CSO Questions this Quarter!

1. Fast track data sets for PARG inhibitor that got FDA approval - Can the same data endpoints be produced in zebrafish?
2. How do you measure tri-complex inhibitor suppressing RAS signaling, inducing tumor growth inhibition/regression, and prompting anti-tumor immune responses in RAS-dependent pancreatic adenocarcinoma?
3. How do you evaluate gene therapies for tau protein reduction?

Write to us, kal@pentagrit.com