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Comprehensive Multitarget Strategy for Type 1 Diabetes-Associated Neurological and Sleep Disorders (DANS): Integrating Stem-Cell-Derived Islet Replacement, Immune Modulation, and Neuro-Circadian Therapeutics Preclinical Insights and Strategic Path Forward

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REFERENCES SCAN ME

Overview

Diabetes affects over 537 million adults worldwide, projected to reach 783 million by 2045. Type 1 diabetes (T1D) manifests as a systemic autoimmune disorder, with hyperglycemia driving interconnected pathways of neuroinflammation, oxidative stress, β-cell loss, orexin deficits, and circadian disruption. These mechanisms underpin **Diabetes-Associated Neurological and Sleep Disorders (DANS)**, exacerbating metabolic instability alongside neuropathy, cognitive decline, neurodegeneration (including Type 3 Diabetes), and sleep disorders such as obstructive sleep apnea (~40–50% of T2D) and hypersomnolence syndromes linked to orexin dysregulation.
Current standard-of-care therapies primarily target glycemic control but have limited impact on the neurological, cognitive, and sleep-wake components that define **DANS**. In contrast, the most successful neuropsychopharmacological agents achieve durable outcomes through multimodal mechanisms of action, reflecting the complexity of neuro-metabolic regulation.

To address this holistically, **NewCelX** and its **NCEL-101/IsletRx** stem-cell-derived islet platform, in strategic collaboration with **Eledon Pharmaceuticals** (anti-CD40L mAb, formerly AT-1501)-mediated immune protection. This enables durable graft survival and potential T1D functional cure, as evidenced by clinical insulin independence without calcineurin inhibitor toxicities, thereby restoring glycemic control to mitigate DANS progression.
Synergizing with this regenerative core, **Aexon Labs and its multitarget dihydro-quinazoline, dihydro-benzothiazine, and dihydro-benzoxazine** (featuring DOXA compounds for orexin restoration, Sigma-1 receptor (Sig-1R) modulation, CTSS/CTSL inhibition, and SGLT2/adiponectin targeting) directly combats DANS through anti-inflammatory, neuroprotective, and metabolic actions — reducing neuroinflammation/oxidative stress, preserving β-cell/neural integrity, enhancing glucose control/insulin sensitivity, and normalizing circadian/energy/cognitive functions.

DOXA Combination with β-Cell Therapy

Principle: Dual orexin receptor agonists (DOXA) and immune-enabled islet replacement therapy (**NCEL-101/IsletRx**) act synergistically to restore both systemic neuro-metabolic balance and physiological insulin production. DOXA (OX1R/OX2R agonist) restores orexin signaling in the lateral hypothalamus, improving wakefulness, energy expenditure, insulin sensitivity, and β-cell resilience. When applied as Orexin-A (OXA) preconditioning, it protects transplanted islets from apoptosis and inflammatory stress, enhancing engraftment and long-term function. In parallel, IsletRx leverages allogeneic islet transplantation combined with targeted CD40L pathway inhibition to promote graft survival without broad immunosuppression.

Integration & Synergistic Benefits: Systemic DOXA corrects circadian and sleep–wake disruption while supporting endogenous and transplanted β-cell function via **central orexin/ Sig-1R/TRPC5 pathways**. **NCEL-101/IsletRx** restores β-cell mass and glucose-responsive insulin secretion, while CD40L-mediated immunomodulation enables durable graft tolerance. The combined approach simultaneously targets central neuro-metabolic dysregulation and peripheral β-cell deficiency, effectively breaking the **DANS vicious cycle** at both levels.

Biomarker Monitoring in Preclinical DANS Studies

Rationale
Rigorous, multi-timepoint biomarker monitoring is essential to evaluate target engagement, mechanistic impact, and therapeutic efficacy of the Aexon Labs multitarget platform in diabetic and neuro-metabolic animal models.

Analytical Workflow

Phase	Timing	Action Sampling	Key Biomarkers Sample Types	Methods	Expected Outcome	Statistical Data Analysis
Baseline	Pre-induction	Blood CSF collection	TNF-α, IL-6, MDA, cathepsins, glucose, insulin (plasma/CSF)	ELISA, multiplex, chromatography	Establish reference state; confirm group comparability	Descriptive stats, t-test / ANOVA
Early Phase	Week 4–6	Midpoint sampling	Cytokines, orexin (CSF), cathepsins, early metabolic markers	ELISA, PCR	Assess target engagement (DOXA, anti-inflammatory, circadian effects)	Repeated measures ANOVA with mixed models
Mid–Late Monitoring	During therapy	Expanded pathway profiling	SGLT2, DPP-4, adiponectin, leptin receptor and Sig-1R	PCR, immunoassays	Track metabolic and receptor-level modulation	Longitudinal trend analysis
Endpoint	Week 8–12	Final sampling and tissue collection	PPARγ, SIRT1, TRPC5 (brain/kidney), orexin (CSF), NSE, cathepsins and Sig-1R	ELISA, PCR, Western blot, activity assays	Confirm efficacy: ↓ inflammation, ↓ neuronal injury, ↑ metabolic control, TRPC5 and Sig-1R engagement	ANOVA with post hoc, effect size
Histology	Endpoint	Tissue validation	β-cell mass, neuronal integrity, podocyte structure	Immuno histochemistry	Structural confirmation of therapeutic benefit	Quantitative image analysis
Data Integration	Post-analysis	Multimodal correlation	All biomarkers with behavioral readouts	Statistical modeling, ML	Link molecular effects to outcomes (cognition, sleep, neuropathy)	ROC curves, regression, ML pattern recognition

Mechanistic Pathways in DANS: Neuroinflammation, Circadian Disruption, Oxidative Stress, and Metabolic Dysfunction

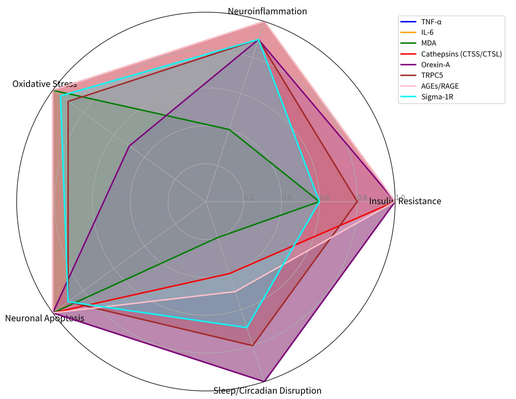
DANS is driven by a tightly interconnected network of pathophysiological pathways.
Chronic hyperglycemia and insulin resistance trigger **neuroinflammation** through microglial activation and elevated pro-inflammatory cytokines (**TNF-α** and **IL-6**), while simultaneously promoting **oxidative stress** (elevated **MDA**) and accumulation of advanced glycation end-products (**AGEs/RAGE**).
These processes converge with **circadian and sleep disruption** via orexin deficiency and **TRPC5 dysregulation**, impairing wakefulness, energy expenditure, and glucose homeostasis. **Cathepsins (CTSS/CTSL)** and impaired **Sigma-1 receptor (Sig-1R)** signaling further exacerbate neuronal and β-cell apoptosis, closing a self-reinforcing vicious cycle that accelerates cognitive decline, diabetic neuropathy, neurodegeneration (including Type 3 Diabetes), and sleep disorders (OSA, narcolepsy, hypersomnia).

The **Comprehensive Biomarker Table** below summarizes the physiological functions, roles in **DANS**, and specific interventions via **DOXA multitarget platform**.

Biomarker	Physiological Function	Role in DANS	Platform Target Intervention
TNF-α	Pro-inflammatory cytokine	Insulin resistance, microglial activation, neuronal apoptosis	Indirect suppression via DOXA and cathepsin inhibition
IL-6	Inflammatory and metabolic regulator	Neuroinflammation and insulin resistance	Indirect anti-inflammatory effect of DOXA
MDA	Marker of lipid peroxidation and oxidative stress	Neuronal membrane damage and cognitive/neuropathy progression	Indirect reduction via TRPC5 modulation and metabolic improvement
Cathepsins (CTSS/CTSL)	Lysosomal proteases	Neuronal/β-cell apoptosis, myelin degradation	Direct inhibition
Orexin-A	Wakefulness, energy balance, glucose homeostasis	Sleep-wake disruption, metabolic instability	Direct agonist (OX1R/OX2R)
TRPC5	Neuronal excitability (orexin neurons) and podocyte function	Impaired arousal, metabolic imbalance, DKD	Chemotype-based modulation via quinazolinone scaffolds
AGEs/RAGE	Glycation products driving inflammation and oxidative stress	Vascular/neuron damage and chronic inflammation	Indirectly improved by glycemic and anti-inflammatory actions
Sig-1R	ER mitochondria chaperone protein	Neuroprotection, oxidative stress regulation, NLRP3 modulation	Direct agonist and modulation activity

This table highlights the interconnected role of key biomarkers (**TNF-α, IL-6, cathepsins (CTSS/CTSL), orexin-A, TRPC5, and Sig-1R**) in sustaining the **DANS vicious cycle**.
The **DOXA multitarget platform** directly targets these critical nodes through orexin receptor agonist activity, **Sig-1R** modulation, cathepsin inhibition, and **TRPC5** modulation. Combined with **NCEL-101/IsletRx** stem-cell islet replacement and CD40L-mediated immunomodulation, it effectively breaks the pathological cycle and offers true disease-modifying potential in T1D and **DANS**.

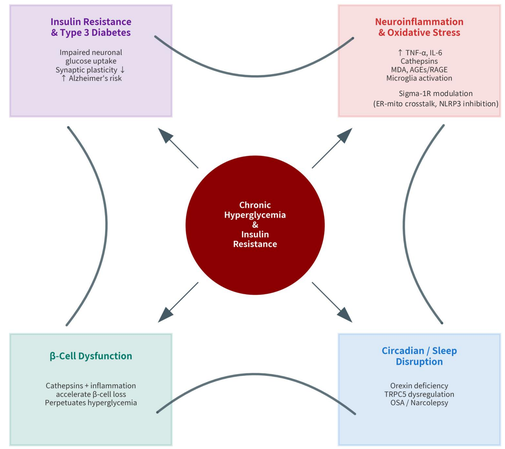
The radar chart visualizes the degree of involvement (0 = no direct link to 1 = strong involvement) of each major biomarker across the five core pathophyxsiological mechanisms of DANS.



Biomarkers such as **TNF-α, IL-6, and SIRT1** demonstrate broad, near-maximal involvement across all five core **DANS** mechanisms, underscoring their central role in linking metabolic, inflammatory, and neurological damage.
Orexin-A, Cathepsins (CTSS/CTSL), and Sigma-1 Receptor (Sig-1R) stand out as particularly high-impact targets. While Orexin-A and Cathepsins strongly connect circadian/sleep disruption with neuroinflammation and β-cell dysfunction, **Sig-1R** provides critical neuroprotection through ER-mitochondrial crosstalk, NLRP3 inflammasome inhibition, and modulation of oxidative stress and ion channels.

These interconnected nodes represent ideal therapeutic targets for the **DOXA multitarget platform**.

DANS arises from a self-reinforcing vicious cycle triggered by chronic hyperglycemia and insulin resistance.



These central drivers simultaneously activate neuroinflammation (↑ TNF-α, IL-6, cathepsins), oxidative stress (↑ MDA, AGEs/RAGE), circadian/sleep disruption (orexin deficiency + TRPC5 dysregulation), and β-cell dysfunction. Each pathway feeds into and amplifies the others, progressively leading to cognitive decline, diabetic neuropathy, neurodegeneration (including Type 3 Diabetes), and sleep disorders (OSA, narcolepsy, hypersomnia).

Breaking this vicious cycle is the core rationale for the integrated therapeutic strategy with its **DOXA multitarget platform** (dual orexin receptors agonist activty, **Sig-1R modulation**, cathepsin inhibition, and TRPC5 modulation) combined with **NewCelX NCEL-101/IsletRx** stem-cell-derived islet replacement and **tegoprubart** (anti-CD40L) immune protection

Preclinical Methods in Animal Studies

To evaluate the efficacy and mechanisms of the Aexon Labs multitarget platform, robust and reproducible preclinical studies were conducted in validated rodent models of DANS.

Animal Models
Type 2 diabetes DANS model: High-fat diet (HFD) for 8 weeks followed by low-dose streptozotocin (STZ, 40 mg/kg i.p.) in C57BL/6 mice (most translational model). **Genetic models**: db/db mice (leptin receptor mutation) and orexin neuron-specific TRPC5 conditional knockout mice. **Age**: 8–12 weeks at study start; chronic treatment duration 8–12 weeks.

Treatment Protocols
DOXA compounds: Oral gavage once daily (doses 10–30 mg/kg, selected from pharmacokinetic and pharmacodynamic studies). **DOXA preconditioning + IsletRx**: Human iPSC-derived islets preconditioned ex vivo with DOXA (1–10 μM, 24–48 h) prior to transplantation under the kidney capsule. **Controls**: Vehicle, single-target comparators (GLP-1R agonist, SGLT2 inhibitor), and sham transplantation. **Group size**: n = 10–15 per group (powered for behavioral and metabolic endpoints).

Assessments
Metabolic: Body weight, fasting glucose, insulin tolerance test (ITT), glucose tolerance test (GTT), HbA1c. **Neurological/Cognitive**: Morris water maze, Y-maze (spatial memory), novel object recognition. **Neuropathy**: Von Frey filament test (mechanical allodynia), hot-plate test (thermal hyperalgesia). **Sleep/Circadian**: EEG/EMG polysomnography, actigraphy, orexin neuron firing (patch-clamp ex vivo). **Biomarkers**: Serial plasma/CSF sampling (TNF-α, IL-6, MDA, cathepsins CTSS/CTSL, orexin-A, adiponectin). **Histopathology**: β-cell mass (insulin immunostaining), podocyte integrity (kidney), neuronal density and synaptic markers (hippocampus, hypothalamus), microglial activation (Iba1). **Statistical Analysis:** Two-way ANOVA with repeated measures, post-hoc Tukey or Bonferroni tests; correlation analyses between biomarkers and functional outcomes; $p < 0.05$ considered significant. **Expected Outcomes with its Platform** Significant improvement in glycemic control, cognitive performance, sleep architecture, neuropathic pain, and reduction of neuroinflammatory and oxidative stress markers compared to standard-of-care or vehicle.

DOXA Compound Candidates

The DOXA platform is based on privileged **dihydro-quinazoline, dihydro-benzothiazine, and dihydro-benzoxazine** scaffolds (WO2024115797A2, WO2024141660A2), enabling multimodal neuro-metabolic activity including OX1R/OX2R, Sig-1R agonist activty, TRPC5 modulation, and potential cathepsin inhibition.

Patent-Derived Lead Compounds

Compound	Scaffold Class	Primary Activity	Predicted TRPC5 Fit Potency	Notes Profile
194	Dihydro-benzothiazine	Strong dual orexin agonist	Moderate–good (5–25 μM)	Most promising dual agonist; favorable safety (no mutagenicity)
300	Dihydro-quinazoline	OX2R-biased agonist	Weak (>50 μM)	OX2R-preferring
84	Dihydro-quinazoline	OX2R agonist CTSH inhibitor	Moderate–good (10–50 μM)	Compact bicyclic core
90	Dihydro-quinazoline	OX2R/Sig-1R agonist	Moderate–good (5–25 μM)	Compact hydrophobic face
87, 99, 155	Dihydro-quinazoline and benzothiazine	Dual Orexin agonist	Moderate (10–50 μM)	Good chemical tractability
152, 196	Polar nitro-substituted benzothiazine	Dual orexin activity and mGluR2	Weak–moderate (25–100 μM)	More polar analogues

In Silico-Optimized DOXA Series

Compound	Scaffold	Profile	Predicted Potency	Strategic Role
QZ-1	Quinazolinone	TRPC5-selective + Sigma-1R modulation	0.8–3 μM (TRPC5)	Peripheral (DKD, metabolism)
QZ-3	Quinazolinone	TRPC5-biased + Sigma-1R modulation	2–6 μM (TRPC5)	Kidney disease focus
QZ-2	Quinazolinone	Dual TRPC5–Orexin + Sigma-1R modulation	1–10 μM	Neuro-metabolic integration
QZ-4	Quinazolinone	Tunable dual + Sigma-1R modulation	3–15 μM	Optimization flexibility
BT-1	Benzothiazine	Strong OX1R agonist + Sigma-1R modulation	0.5–5 μM (OX1R)	Core DOXA (arousal/metabolism)
BO-1	Benzoxazine	OX2R-biased + Sigma-1R modulation	2–10 μM (OX2R)	Sleep–wake regulation

Conclusion

The integration of the **DOXA multitarget platform** and its metabolic optimization with **NewCelX NCEL-101/IsletRx** stem-cell-derived islet replacement and **tegoprubart** (anti-CD40L) immune protection represents a true **paradigm shift** in Type 1 diabetes and DANS management.
This unified regenerative + immunomodulatory + neuro-circadian strategy simultaneously targets central dysregulation (orexin/TRPC5/Sig-1R), peripheral β-cell loss, and graft rejection, effectively **breaking the vicious cycle** of hyperglycemia, neuroinflammation, oxidative stress, and circadian disruption.
Preclinical data on OXA **preconditioning and Sig-1R/DOXA neuroprotection**, combined with durable islet graft survival, position this approach to deliver **disease modification and long-term functional cure** in T1D and **DANS**.