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Axonic-Revive Spasticity Reset and Regeneration Multi-Phasic Integrative Strategy for Functional Neural Repair after Complete Traumatic Spinal Cord Transection

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Abstract. Chronic complete spinal cord injury remains a major challenge in regenerative neurobiology due to the formation of an inhibitory microenvironment that limits spontaneous axonal regeneration. This study aimed to evaluate the effectiveness of a sequential three-phase therapeutic strategy combining modulation of glial scarring, neurotrophic stimulation of axonal growth, and brain-computer interface-controlled neuromodulation for motor recovery after chronic spinal cord injury. The study involved 69 male Sprague-Dawley rats with complete spinal cord transection at the T9–T10 thoracic level. After an eight-week period for the development of chronic spasticity, the animals were allocated to standard rehabilitation (n=13), Phase I (n=14), Phase I+II (n=15), Phase I+II+III (n=14), or Phase III only (n=13). Functional, electrophysiological, molecular, and histological outcomes were assessed over 24 weeks. The complete protocol led to a 50.2% reduction in pathological reflex responses and an improved locomotor function to 13.7 points, representing a 15.2-fold increase compared with controls. The regenerating axon density increased 7.8 times, the synaptic density 5.8 times, and reinnervated motor units 17.9 times. Neurotrophic factors increased 3.3–3.7 times, while pro-inflammatory cytokines decreased by 52%. The full protocol was also associated with ectopic sprouting (2.4±1.1 points) and a lower mechanical withdrawal threshold (2.1±0.5 g), indicating greater mechanical hypersensitivity. Regression analysis showed that 91% of the functional recovery variability was explained by structural and molecular predictors. The findings suggest an improved locomotor recovery with sequential multimodal treatment in this rat model, while the safety findings and lack of large-animal validation limit direct clinical extrapolation.

Keywords: microenvironment, glial scar, motor units, proinflammatory cytokines, excitatory-inhibitory balance, synaptic density.

Daugiafazė integruota spastikos atsigavimo ir regeneracijos strategija, siekiant funkcinio nervinių audinių atkūrimo po visiškos trauminės nugaros smegenų transsekcijos

Santrauka. Lėtinis visiškas nugaros smegenų pažeidimas tebėra didelis iššūkis regeneracinėje neurobiologijoje dėl slopinančios mikroaplinkos, kuri riboja savaiminę aksonų regeneraciją, susidarymo. Šio tyrimo tikslas buvo įvertinti nuoseklios trijų etapų terapinės strategijos, apimančios glijos randų moduliavimą, neurotro-

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finę aksonų augimo stimuliaciją bei smegenų ir kompiuterio sąsaja valdomą neuromoduliaciją, motorinių funkcijų veiksmingumui atkurti po lėtinio nugaros smegenų pažeidimo. Tyrime dalyvavo 69 *Sprague-Dawley* žiurkių patinai, kuriems buvo visiškai perkirptas nugaros smegenų stiebas T9–T10 krūtinės lygmeniu. Po aštuonių savaičių, per kurias išsivystė lėtinis spastiškumas, gyvūnai buvo suskirstyti į standartinės reabilitacijos (n = 13), I fazės (n = 14), I + II fazės (n = 15), I + II + III fazės (n = 14) arba tik III fazės (n = 13) grupes. Funkciniai, elektrofiziologiniai, molekuliniai ir histologiniai rezultatai buvo vertinami 24 savaičių laikotarpiu. Įgyvendinus visą protokolą, patologiniai refleksiniai atsakai sumažėjo 50,2 %, o judėjimo funkcija pagerėjo iki 13,7 balo, tai reiškia 15,2 karto didesnį rodiklį, palyginti su kontroline grupe. Regeneruojančių aksonų tankis padidėjo 7,8 karto, sinapsių tankis – 5,8 karto, o reinervuotų motorinių vienetų skaičius – 17,9 karto. Neurotrofiniai faktoriai padidėjo 3,3–3,7 karto, o proinflamaciniai citokinai sumažėjo 52 %. Visas gydymo protokolas taip pat buvo susijęs su ektopiniu ataugimu (2,4±1,1 balo) ir žemesne mechanine atsitraukimo riba (2,1±0,5 g), o tai rodo didesnį mechaninį jautrumą. Regresinė analizė parodė, kad 91 % funkcinio atsigavimo variabilumo paaiškino struktūriniai ir molekuliniai prognozės rodikliai. Šie rezultatai rodo, kad šioje žiurkių modelio studijoje nuoseklusis daugiamodalinis gydymas pagerina jų judėjimo funkcijos atsigavimą, tačiau saugumo duomenys ir patvirtinimo didelių gyvūnų modeliuose trūkumas riboja tiesioginį klinikinį ekstrapoliavimą.

Raktažodžiai: mikroaplinka, glijos randas, motoriniai vienetai, prouždegiminiai citokinai, sužadinančių ir slopinančių impulsų pusiausvyra, sinapsių tankis.

Introduction

Complete traumatic spinal cord injury remains one of the most disabling conditions in neurotraumatology, as existing therapeutic approaches do not provide functional recovery of the pathways below the level of injury. Pathological spasticity forms a competing interneuronal network that hinders axonal regeneration, while incomplete integration of sensory feedback circuits limits the sustainability of motor recovery, justifying the need for multimodal strategies with sequential reprogramming of spinal networks and restoration of sensory-motor coupling before initiating regenerative processes.

Recent evidence indicates that, even after complete spinal cord injury, latent central pattern generator (CPG) networks in the lumbar enlargement (L2–S1), comprising genetically defined ventral interneuron populations (V0–V3) interneuron populations, retain the capacity for rhythmic locomotor output when appropriately modulated (Grillner & Manira [1]), K. Minassian et al. [2] provided direct human evidence by demonstrating epidural stimulation-induced rhythmic motor patterns with characteristic motor deletions in an individual with complete thoracic SCI, thus confirming the two-layer CPG architecture in the human lumbar cord. The phenomenon of 'spinal walking' highlights the therapeutic potential of awakening these dormant networks rather than relying solely on axonal regeneration across the lesion.

Molecular mechanisms of post-traumatic degeneration below the level of injury have been intensively studied. A. Głowacka et al. [3] established partial protection of neuromuscular junctions of the soleus muscle by intraspinal administration of adeno-associated virus (AAV) brain-derived neurotrophic factor (BDNF) after complete transection in rats with selective elevation of vesicular acetylcholine transporter (VAChT) and acetylcholinesterase (AChE) transcripts in the corresponding motor neurons. K. Grycz et al. [4] demonstrated prolonged destabilisation of components of perineuronal networks (aggrecan, brevican, tenascin-R) in suburbarised segments of the rat spinal cord by using quantitative polymerase chain reaction (qPCR), immunohistochemistry, and Wisteria floribunda agglutinin (WFA) labelling.

The role of excitatory-inhibitory imbalance in the pathogenesis of spasticity has been confirmed by studies at various levels of the nervous system. G. L. Mazzone et al. [5], based on data from

organotypic spinal cord sections and electrophysiological recordings, found that thoracic transection reduces potassium-chloride cotransporter 2 (KCC2) and gamma-aminobutyric acid type A (GABAa) receptor signalling from inhibitory to excitatory through a chloride-dependent mechanism. H. Beverungen et al. [6] used a model of complete spinal cord transection in rats to reveal the causal chain of the antispastic effect of physical rehabilitation: blocking KCC2 or chelating BDNF during cyclic exercises completely negated the recovery of frequency-dependent H-reflex depression, thereby establishing the chain BDNF → tropomyosin receptor kinase B (TrkB) → KCC2 → normalisation of chloride homeostasis → restoration of GABAergic inhibition. Clinical confirmation of the possibility of modulating spasticity was provided by A. Wincek et al. [7], where the use of bilateral repetitive transcranial magnetic stimulation (rTMS) of the motor cortex at a frequency of 20–25 Hz in 26 patients with incomplete spinal cord injury (C2-Th12) led to a statistically significant reduction in electromyography (EMG) amplitude at rest and improved motor unit recruitment.

Therapeutic strategies for modification of inhibitory microenvironment include enzymatic degradation of chondroitin sulphate proteoglycans and neutralisation of myelin-associated inhibitors. M. H. Hettiaratchi et al. [8] used consensus design and Rosetta optimisation methods to create a mutant variant of chondroitinase ABC (ChABC)-37 with a 6.5-fold increase in half-life and increased catalytic activity. E. Giraldo et al. [9] developed a polymeric conjugate of the Rho-associated protein kinase (ROCK) inhibitor fasudil with a self-immolative linker, which provided microenvironment-dependent release of the active substance and a synergistic effect with neural progenitor cells. A randomised clinical trial of NISCI intrathecal anti-Nogo-A antibody (NG101) involving 129 patients in 13 centres in Europe did not meet the primary endpoint for the overall population, although a *post-hoc* analysis revealed a benefit in the motor-incomplete injury subgroup and an approximately twofold reduction in the incidence of spasticity (Weidner et al. [10]). M. Zawadzka et al. [11], based on a systematic review of the evidence, found that no single cell type – mesenchymal stem cells, olfactory ensheathing cells, Schwann cells, or neural progenitors – is capable of simultaneously addressing all pathological cascades following injury.

Neuromodulation approaches show significant potential for restoring motor function. A. Rowald et al. [12] demonstrated in the Spinal Cord Epidural Stimulation in Motor-complete patients (STIMO) clinical study that targeted epidural stimulation of the dorsal roots in three patients with chronic complete paralysis American Spinal Injury Association Impairment Scale (AIS-A) restored the ability to stand, walk and control the trunk within the first 24 hours after parameter calibration. H. Lorach et al. [13] implemented the concept of a digital bridge between cortical intention and spinal motor centres via a fully implanted brain-spine interface, providing voluntary walking, stair climbing and movement over complex terrain.

Regenerative strategies based on biomaterials and pharmacological modulation complement external approaches. A. Kourgiantaki et al. [14] demonstrated the restoration of locomotion to intact control levels upon implantation of a collagen-glycosaminoglycan scaffold with neural stem cells in a dorsal hemisection model in mice, with confirmation of the formation of functional synaptic connections by electrophysiological methods. S. Bhowmick and P. M. Abdul Muneer [15] showed that systemic administration of the phosphatase and tensin homolog (PTEN) antagonist peptide stimulates the regeneration of corticospinal and raphe spinal axons through activation of the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mTOR cascade with a reduction in cystic cavity. The timing of combinatorial interventions is critical: J. M. Griffin and F. Bradke [16] found that co-administration of anti-Nogo antibodies with rehabilitation provokes chaotic hyperinnervation and aberrant connections, whereas a sequential regimen ensures functionally adequate recovery.

A common limitation of the analysed studies is their predominant focus on isolated therapeutic modalities without integrating molecular modulation of the microenvironment, neurotrophic

stimulation and activity-dependent neuromodulation into a single consistent protocol, as well as insufficient validation on models of chronic complete spinal cord injury with verified spasticity. Moreover, the majority of preclinical evidence derives exclusively from rodent models, whereas no existing combinatorial protocol has incorporated induced pluripotent stem cells (iPSC)-derived interneuron transplantation to directly supplement damaged spinal networks, and the potential for reactivation and stabilisation of dormant lumbar CPG networks as a complementary mechanism of functional recovery remains largely unexplored. Given these gaps, there was a need for the current work. The study aimed to substantiate the effectiveness of the AXONIC-REVIVE™ multiphase integrative strategy for functional neurorehabilitation after complete traumatic spinal cord injury, including the reactivation of dormant spinal locomotor circuits. The objectives of the study were to establish the effect of sequential application of the therapeutic protocol phases on the suppression of spinal hyperexcitability, restoration of locomotor function, and structural regeneration of conduction pathways; to characterise the dynamics of neurotrophic, neuroinflammatory and electrophysiological parameters according to the phase of intervention and to determine the safety profile of the multimodal protocol; to establish correlational relationships between molecular, structural and functional parameters of regeneration so that to identify predictors of recovery.

Materials and Methods

The study was conducted as a prospective experimental controlled study using a model of complete traumatic spinal cord transection in laboratory rodents. After induction of the injury, the animals were kept for 8 weeks to develop a chronic condition with verified spasticity, after which, a 24-week period of therapeutic intervention began. The phase-day designations used below refer to the start of therapy after the eight-week post-injury period. The experiment was conducted at the vivarium of Kharkiv National Medical University from December 2024 to August 2025.

The study involved 69 male Sprague-Dawley rats aged 10–12 weeks at the time of spinal cord injury induction, weighing 280–320 g. The inclusion criteria were age 8–14 weeks, no previous neurotrauma or systemic diseases, successful modelling of complete transection at the ninth-tenth thoracic (T9–T10) level with confirmation of complete functional deficit Basso, Beattie, Bresnahan (BBB) locomotor rating scale (score 0–1) and development of chronic spasticity for at least 8 weeks after injury induction. Exclusion criteria were incomplete transection according to intraoperative verification, mortality within the first 72 hours, development of infectious complications of the postoperative wound, or spontaneous locomotor recovery of more than 2 points on the BBB scale within the first 8 weeks after injury. Complete transection of the spinal cord was performed under inhalation anaesthesia with isoflurane (induction 4%, maintenance 1.5–2%) with body temperature maintained at 37.0±0.5C by using a thermostatic pad. After a laminectomy at the T9–T10 level, the spinal cord was transected with microsurgical scissors with visual confirmation of complete rupture. Postoperative analgesia was provided with buprenorphine (0.05 mg/kg subcutaneously every 12 hours, 5 days) and meloxicam (1 mg/kg/day, 3 days) and antibiotic prophylaxis with enrofloxacin (10 mg/kg/day, 7 days). The animals received subcutaneous infusion of saline to maintain hydration and manual bladder emptying twice daily until reflex urination was restored. Criteria for humane euthanasia were loss of more than 20% of body weight, signs of uncontrolled pain, sepsis, or extensive pressure sores.

The animals were randomised by using random numbers into five experimental groups: control with standard rehabilitation (n=13), Phase I with microenvironment modulation (n=14), Phase I+II with the addition of neurotrophic stimulation (n=15), Phase I+II+III with a complete multimodal protocol (n=14) and Phase III only with isolated neuromodulation (n=13). This grouping permitted a comparison of microenvironment modulation alone, its combination with neurotrophic stimula-

tion, the complete sequential protocol, and neuromodulation without preceding molecular phases. All animals, regardless of their group affiliation, received the same basic care, which included daily passive joint mobilisation of the hind limbs, hydration support and weight control. The control group received only the specified standard rehabilitation without pharmacological or neuromodulatory interventions.

The AXONIC-REVIVE™ therapeutic protocol was applied in stages following the phases of regenerative processes. Phase I (days 0–14) included intraspinal implantation of collagen-laminin hydrogel with polyethene glycol (PEG) ChABC (0.1 U/mL), ROCK inhibitor (0.5 mg/kg) and AAV-glutamic acid decarboxylase 65/67(GAD65/67) (1×10^{11} IU/mL) at 10–20 μL per centimetre of the lesion length. Phase II (days 14–60) involved a single intrathecal administration of humanised anti-Nogo-A monoclonal antibodies (5 mg/kg, PBS 0.01 M, pH 7.4) and an intraspinal injection of adeno-associated virus serotype 9 (AAV9)-BDNF/neurotrophin-3 (NT-3) (1×10^{11} IU/mL) into the rostral and caudal margins of the lesion zone. Phase III (days 60–180) included epidural electrical stimulation at the L2–S1 segments (40 Hz, 2–4 V), brain-computer interface (BCI)-controlled neuromodulation with recording of motor cortex signals via a 16-channel electrocorticographic array (45-minute sessions, 5 times a week, decoding by linear discriminant analysis, classification threshold ≥75%) and task-specific rehabilitation on a motorised treadmill (20 minutes, 5–12 cm/s with partial body weight support). All experimental procedures were performed following the principles of the Law of Ukraine "*On the Protection of Animals from Cruel Treatment*" and in compliance with the relevant bioethical standards.

Spasticity was assessed by the H/M ratio of the soleus muscle by using the *Neuropack X1* electromyography system (*Nihon Kohden*, Japan). The locomotor function was assessed on a 21-point BBB scale by two independent investigators in a blinded manner with video recording. H/M ratio and BBB assessments were reported at baseline and at weeks 2, 6, 12 and 24 (see Tables 1 and 2). EMG analysis included counting recruited motor units, measuring the amplitude of the compound muscle potential, and measuring the conduction velocity along motor fibres. The structural integrity of the conduction pathways was assessed by diffusion tensor magnetic resonance imaging (MRI) on a *BioSpec 94/30 USR 9.4T* tomograph (*Bruker*, Germany) with calculation of the fractional anisotropy coefficient. The concentrations of neurotrophic factors (BDNF, NT-3), markers of axonal growth-associated protein 43 (GAP-43) and synaptogenesis microtubule-associated protein 2 (MAP-2), as well as pro-inflammatory cytokines (TNF-α, IL-1β) in the cerebrospinal fluid were determined by enzyme-linked immunosorbent assay (ELISA) (*R and D Systems*, USA). The safety profile was assessed by the histological score of ectopic sprouting (scale 0–4), the threshold of mechanical allodynia using von Frey filaments and the titre of antibodies to AAV vectors and ChABC by serological analysis. Neurotrophic, axonal growth, neuroinflammatory and glial markers were reported at week 12 (see Tables 3 and 5); structural, electrophysiological, safety, myelination and vascularisation outcomes were reported at week 24 (see Tables 4 and 6–8). Histological analysis was performed on serial 10 μm thick sections with immunohistochemical staining with antibodies to NF200, synaptophysin, MBP, GFAP and CSPG, visualisation on a *LSM 880* confocal microscope (*Carl Zeiss*, Germany) and quantitative morphometry using ImageJ software.

Statistical data processing was performed in the Statistical Package for the Social Sciences (SPSS) Statistics 26.0 software environment by using one-way analysis of variance with repeated measurements and multivariate analysis of variance to compare intergroup differences. *Post-hoc* analysis was performed by using the Bonferroni method with correction for multiple comparisons and correlation analysis was performed using the Pearson method. The level of statistical significance was set at $p<0.05$, and the data are presented in the format M±SD. The study design did not include translational verification in large animal models or human cell-based systems, iPSC-derived interneu-

ron transplantation, or extended longitudinal follow-up beyond the 24-week observation period to assess delayed adverse events, which constitute acknowledged design limitations addressed in the Discussion.

Results

Spasticity after complete spinal cord injury is characterised by hyperexcitability of segmental reflex arcs, manifested by an increased ratio of the H-reflex amplitude to M-response (H/M ratio). Normalisation of this indicator is a critical marker of successful modulation of pathological interneuronal networks and a necessary condition for further functional regeneration of axons. The dynamics of H-reflex suppression in different experimental groups are presented in Table 1.

Table 1. H/M ratio (%) in experimental groups at different time points of the study (M±SD)

Group	Baseline	Week 2	Week 6	Week 12	Week 24
Control (n=13)	82.3±6.7	81.1±7.2	79.8±8.1	78.5±7.9	77.2±8.3
Phase I (n=14)	83.1±5.9	68.4±6.3*	61.2±7.1*	58.7±6.8*	56.3±7.2*
Phase I+II (n=15)	81.8±6.4	66.9±5.8*	57.3±6.4*#	52.1±5.9*#	48.6±6.1*#
Phase I+II+III (n=14)	82.7±6.1	65.2±6.7*	53.8±5.2*#	45.3±5.6*#†	41.2±5.8*#†
Phase III only (n=13)	81.5±7.3	79.3±7.8	74.6±8.4	71.2±7.6	68.9±8.1

Note: *p<0.05 compared to Control; #p<0.05 compared to Phase I; †p<0.05 compared to Phase I+II.
Source: author's experimentally obtained data.

In the control group, during the 24-week observation period, no statistically significant dynamics of the H/M ratio were found, which remained at 77.2–82.3%, corresponding to a persistent pathological state of spasticity. The Phase III only group, which received only epidural stimulation and BCI rehabilitation without prior molecular modulation of the microenvironment, showed a moderate decrease in the H/M ratio to 68.9±8.1% at 24 weeks, but these changes were not statistically significant compared to the control group.

The Phase I group, which received a combination of ChABC, ROCK inhibitor and AAV-GAD65/67, showed significant suppression of the H-reflex as early as the second week (68.4±6.3%, $p<0.05$), with a further progressive decrease to 56.3±7.2% at week 24. The addition of Phase II components (AXON-ANTI-NOGO and BDNF/NT-3) in the Phase I+II group led to a statistically significant enhancement of the effect starting from week 6 (57.3±6.4% vs. 61.2±7.1% in Phase I, $p<0.05$), reaching 48.6±6.1% at the final stage of observation. The most pronounced dynamics were observed in the full Phase I+II+III protocol group, where the inclusion of epidural stimulation and BCI-controlled neuromodulation provided an additional reduction in the H/M ratio to 41.2±5.8% ($p<0.05$ compared to all other groups).

Overall, the results demonstrate a dose-dependent effect of multimodal intervention on the suppression of pathological spinal hyperexcitability. The most effective was a sequential combination of all three phases of therapy, which reduced the H/M ratio by 50.2% relative to baseline, while isolated use of neuromodulation without prior molecular reprogramming of networks did not result in clinically significant changes. The restoration of voluntary locomotor activity is a key indicator of the functional effectiveness of regenerative interventions after complete thoracic spinal cord transection. The BBB scale provides a standardised assessment of hind limb motor function in rodents, with a gradation from '0' (complete paralysis) to '21' (normal walking). The dynamics of locomotor function recovery in the experimental groups are shown in Table 2.

Table 2. Assessment of locomotor function using the BBB scale (points) in experimental groups at different time points (M±SD)

Group	Baseline	Week 2	Week 6	Week 12	Week 24
Control (n=13)	0.5±0.6	0.6±0.7	0.7±0.8	0.8±0.9	0.9±1.0
Phase I (n=14)	0.6±0.7	1.5±0.9	2.7±1.3*	3.9±1.7*	4.8±1.9*
Phase I+II (n=15)	0.4±0.5	1.7±1.0	4.2±1.5*#	7.1±2.2*#	9.3±2.4*#
Phase I+II+III (n=14)	0.5±0.6	1.9±1.2	5.6±1.8*#	10.3±2.5*#†	13.7±2.9*#†
Phase III only (n=13)	0.6±0.7	1.0±0.8	1.6±1.1	2.2±1.4	2.7±1.6

Note: *p<0.05 compared to Control; #p<0.05 compared to Phase I; †p<0.05 compared to Phase I+II.
Source: author's experimentally obtained data.

At the initial stage of the study, all groups demonstrated comparable baseline locomotor function scores in the range of 0.4–0.6 points, which corresponded to minimal reflex activity of the hind limbs without coordinated movements in animals with chronic complete spinal cord injury. Throughout the 24-week observation period, the control group demonstrated minimal spontaneous dynamics with an increase of only 0.4 points, reaching 0.9±1.0 points at the final stage, which corresponds to the absence of functionally significant recovery of coordinated movements of the hind limbs.

The Phase III only group, despite the use of epidural stimulation and BCI-controlled rehabilitation, showed limited improvement from a baseline of 0.6±0.7 to 2.7±1.6 points at 24 weeks (an increase of 2.1 points), which did not reach statistical significance compared to the control group and corresponded only to minor movements of individual joints without the body weight load, thus confirming the insufficiency of isolated neuromodulation in the absence of prior molecular reprogramming of the inhibitory microenvironment.

In the Phase I group, there was a progressive increase in the locomotor function from 0.6±0.7 points at baseline to 4.8±1.9 points at week 24 (an increase of 4.2 points, $p<0.05$ compared to the control), characterised by the appearance of limited movement in two to three joints of the hind limbs with episodic loading. The addition of neurotrophic support and neutralisation of myelin-associated inhibitors in the Phase I+II group led to a statistically significant enhancement of locomotor recovery, which was already evident at week 6 (4.2±1.5 vs. 2.7±1.3 points in Phase I, $p<0.05$), with a subsequent achievement of 9.3±2.4 points at week 24 (an absolute increase of 8.9 points from the baseline). This level corresponded to the appearance of coordinated movements of the hind limbs with periodic plantar loading of the body weight and partial coordination of the front and hind limbs.

The most pronounced dynamics were observed in the full Phase I+II+III protocol group, where the BBB index increased from 0.5±0.6 to 13.7±2.9 points (an absolute increase of 13.2 points, $p<0.05$ compared to all other groups). This corresponded to the ability to continuously bear the body weight on the hind limbs with partial coordination of the front and hind paws when stepping, frequent plantar paw placement and initial coordination of the toes, although without complete parallel limb placement. The pace of recovery was most intense between the 6th and 12th weeks, when the increase was 4.7 points (from 5.6 to 10.3), which coincided with the period of active synaptic integration of regenerating axons under the influence of BCI-controlled neuromodulation and activity-dependent synaptic remodelling. Overall, the results confirmed the critical role of sequential multiphase intervention for functional recovery of locomotion. The Phase I+II+III group demonstrated a 15.2-fold increase in the locomotor performance compared to the control group, while the isolated application of any single phase of therapy was insufficient to achieve clinically significant motor improvement.

The molecular profile of cerebrospinal fluid reflects the neurobiological processes occurring in the microenvironment of the damaged spinal cord and can be used to assess therapy effectiveness in

terms of neurotrophic support and axonal growth. BDNF and NT-3 levels characterise neurotrophic activity, while GAP-43 and MAP-2 are specific markers of axonal sprouting and synaptogenesis, respectively. The dynamics of neurotrophic and regenerative biomarkers in cerebrospinal fluid (CSF) are presented in Table 3.

Table 3. Concentrations of neurotrophic factors and axonal growth markers in CSF (pg/mL) at week 12 of the study (M±SD)

Group	BDNF	NT-3	GAP-43	MAP-2
Control (n=13)	142.3±18.7	87.4±12.3	1240±156	2180±284
Phase I (n=14)	187.6±22.4*	118.9±15.8*	1680±198*	2650±312
Phase I+II (n=15)	412.8±38.6*#	287.3±31.2*#	3420±387*#	4870±456*#
Phase I+II+III (n=14)	468.5±42.1*#	325.7±36.4*#	4150±428*#†	6240±518*#†
Phase III only (n=13)	158.4±20.1	93.2±14.6	1390±172	2340±298

Note: *p<0.05 compared to Control; #p<0.05 compared to Phase I; †p<0.05 compared to Phase I+II.
Source: author's experimentally obtained data.

The control group demonstrated baseline levels of neurotrophic factors characteristic of chronic spinal cord injury: BDNF was 142.3±18.7 pg/mL, NT-3 was 87.4±12.3 pg/mL, which corresponds to minimal endogenous neurotrophic support in the absence of active regenerative processes. The markers of axonal growth GAP-43 (1240±156 pg/mL) and synaptogenesis MAP-2 (2180±284 pg/mL) also remained at low levels, thus confirming the absence of spontaneous regenerative activity. The Phase III only group did not show statistically significant differences from the control in any of the biomarkers presented in Table 3, thereby indicating the ineffectiveness of isolated neuromodulation without prior molecular preparation of the microenvironment.

In the Phase I group, there was a moderate but statistically significant increase in the BDNF levels to 187.6±22.4 pg/mL (31.8% higher than the control, $p<0.05$) and NT-3 to 118.9±15.8 pg/mL (36.0% higher than the control, $p<0.05$), reflecting the initial activation of the neurotrophic response due to the modulation of the microenvironment and normalisation of the excitatory-inhibitory balance. The concentration of GAP-43 also increased to 1680±198 pg/mL (which is an increase of 35.5%, $p<0.05$), while MAP-2 showed a tendency to increase without reaching statistical significance (2650±312 pg/mL, $p=0.067$). The addition of Phase II components in the Phase I+II group led to a marked increase in all biomarkers studied. The BDNF level reached 412.8±38.6 pg/mL, which was 2.9 times higher than the control values and 2.2 times higher than the Phase I group ($p<0.05$). The concentration of NT-3 increased to 287.3±31.2 pg/mL (standing at 3.3 times higher than the control), which is consistent with the direct administration of AAV9-BDNF/NT-3 vectors. The axonal growth marker GAP-43 showed marked activation to 3420±387 pg/mL (thus showing an increase of 176% relative to the control, $p<0.05$) and MAP-2 increased to 4870±456 pg/mL (being 123% higher than the control, $p<0.05$), thus indicating active synaptogenesis processes.

The Phase I+II+III group showed the highest concentrations of all biomarkers: BDNF – 468.5±42.1 pg/mL (3.3 times higher than the control), NT-3 – 325.7±36.4 pg/mL (3.7 times higher than the control), GAP-43 – 4150±428 pg/mL (3.3 times higher than the control) and MAP-2 – 6240±518 pg/mL (2.9 times higher than the control). The additional increase in indicators compared to the Phase I+II group was statistically significant for GAP-43 (by 21.3%, $p<0.05$) and MAP-2 (by 28.1%, $p<0.05$), thus reflecting the synergistic effect of BCI-controlled activity-dependent plasticity on the consolidation of newly formed synaptic connections. Overall, the results demonstrated consistent activation of neurotrophic support and regenerative mechanisms following the phasing of the therapeutic protocol. The most pronounced neurotrophic response was observed in groups with direct

administration of BDNF/NT-3 vectors, while the maximum activation of axonal growth and synaptogenesis markers was achieved when the molecular therapy was integrated with activity-dependent neuromodulation.

Restoration of the structural integrity of the spinal cord pathways is a fundamental prerequisite for the functional reintegration of supraspinal and spinal networks [17–19]. Diffusion tensor imaging provides a non-invasive assessment of the microstructural organisation of white matter through the fractional anisotropy (FA) index, which reflects the directionality of water diffusion along axonal tracts, while the histological density of NF200-positive axons and synaptophysin-positive synaptic terminals characterises the morphological substrate of regeneration. Structural indicators of regeneration at week 24 of the study are presented in Table 4.

Table 4. Indicators of structural integrity of conduction pathways and synaptic density at week 24 of the study (M±SD)

Group	FA coefficient (DTI*)	NF200+ axons (×10³/mm²)	Synaptophysin (% area)	CSPG (% from control)
Control (n=13)	0.18±0.03	2.4±0.6	4.2±0.8	100.0±12.3
Phase I (n=14)	0.24±0.04*	4.8±0.9*	7.6±1.2*	62.4±8.7*
Phase I+II (n=15)	0.38±0.05*#	12.3±1.8*#	15.8±2.1*#	58.1±9.2*
Phase I+II+III (n=14)	0.47±0.06*#†	18.7±2.4*#†	24.3±2.8*#†	55.3±8.4*
Phase III only (n=13)	0.21±0.04	3.1±0.7	5.3±1.0	96.8±11.7

Note: *p<0.05 compared to Control; #p<0.05 compared to Phase I; †p<0.05 compared to Phase I+II FA – fractional anisotropy; NF200 – neurofilament 200 kDa; CSPG – chondroitin sulphate proteoglycans; DTI* – diffusion tensor imaging.
Source: author's experimentally obtained data.

The control group showed a low fractional anisotropy coefficient of 0.18±0.03, which corresponds to pronounced disorganisation of white matter in chronic damage with a predominance of chaotic water diffusion due to demyelination and glial scarring. The density of NF200-positive axons was only 2.4±0.6×10³/mm², reflecting the minimal number of preserved or spontaneously regenerating myelinated fibres. Synaptic density, according to the synaptophysin marker, remained at 4.2±0.8% of the area, while CSPG content was taken as a 100% reference value for chronic glial scarring. The Phase III only group showed no statistically significant differences from the control in terms of structural parameters (FA 0.21±0.04, p=0.112), confirming the inability of isolated neuromodulation to stimulate structural regeneration in the absence of molecular modification of the inhibitory microenvironment.

In the Phase I group, there was a statistically significant improvement in all structural parameters: FA increased to 0.24±0.04 (33.3% higher than the control, p<0.05), NF200+ axon density doubled to 4.8±0.9×10³/mm² (p<0.05) and synaptic density increased to 7.6±1.2% (an increase of 81%, p<0.05). The CSPG content decreased to 62.4±8.7% of the control values (p<0.05), reflecting the effectiveness of enzymatic degradation of chondroitin sulphate proteoglycans through the action of ChABC and the creation of permissive corridors for axonal growth. These changes indicate partial restoration of microstructural organisation and initial recolonisation of the lesion area by axonal fibres.

The addition of Phase II components led to a marked increase in structural regeneration in the Phase I+II group. The FA coefficient reached 0.38±0.05, which was 2.1 times higher than the control values and 1.6 times higher than the Phase I group (p<0.05), reflecting the formation of directed axonal tracts with partial myelination. The density of NF200+ axons increased to 12.3±1.8×10³/mm² (5.1 times higher than the control and 2.6 times higher than Phase I, p<0.05), which is consistent with neurotrophic support of axonal sprouting via BDNF/ NT-3 and elimination of myelin-

associated inhibition via anti-Nogo antibodies. Synaptic density showed the largest relative increase to 15.8±2.1% (3.8 times higher than the control, $p<0.05$), indicating active synaptogenesis at the terminal regions of regenerating axons.

The Phase I+II+III full protocol group showed the highest structural reorganisation indices: FA 0.47±0.06 (2.6 times higher than the control, $p<0.05$), axon density $18.7\pm2.4\times10^3/mm^2$ (7.8 times higher than the control, $p<0.05$) and synaptic density 24.3±2.8% (5.8 times higher than the control, $p<0.05$). The additional increase compared to the Phase I+II group was statistically significant for all parameters: FA by 23.7% ($p<0.05$), axon density by 52.0% ($p<0.05$) and synaptic density by 53.8% ($p<0.05$). This reflects the synergistic effect of activity-dependent plasticity, which promotes selective stabilisation of functionally active synaptic connections and the subsequent myelination of axons involved in BCI-controlled motor patterns. The results demonstrated parallelism between molecular modulation of the microenvironment, neurotrophic stimulation and structural regeneration of axonal tracts. The most effective structural reorganisation was achieved when all three phases of therapy were combined, which ensured not only a quantitative increase in the axonal density but also a qualitative improvement in the microstructural organisation with the formation of functionally integrated pathways. Chronic inflammation and reactive gliosis are key components of the pathological microenvironment after traumatic spinal cord injury, creating both molecular and structural barriers to axonal regeneration [20–22]. Proinflammatory cytokines TNF-α and IL-1β maintain a neurotoxic environment and contribute to secondary neuronal damage, while GFAP reflects astrocyte reactivity and the intensity of glial scarring. Levels of KCC2, a potassium-chloride cotransporter, characterise the functional maturity of inhibitory neurotransmission and mTOR signalling activity determines the anabolic capacity of neurons for regenerative growth. The dynamics of neuroinflammatory and glial markers are presented in Table 5.

Table 5. Concentrations of neuroinflammatory markers in CSF and glial proteins in spinal cord tissue at week 12 of the study (M±SD)

Group	TNF-α (pg/mL)	IL-1β (pg/mL)	GFAP (ng/mL)	KCC2 (% from norm)	p-mTOR (% from norm)
Control (n=13)	186.4±24.3	142.8±18.6	487.3±52.4	38.2±6.4	42.7±7.8
Phase I (n=14)	124.7±16.8*	96.3±14.2*	328.6±41.7*	64.8±8.9*	58.3±9.2*
Phase I+II (n=15)	98.2±13.5*#	74.6±11.8*#	284.1±38.3*	72.4±9.6*	86.7±11.4*#
Phase I+II+III (n=14)	89.3±12.1*#	68.2±10.7*#	256.8±35.9*#	78.9±10.3*#	112.6±13.8*#†
Phase III only (n=13)	178.2±22.9	138.4±17.3	469.1±49.6	41.6±7.1	46.8±8.4

Note: *p<0.05 compared to Control; #p<0.05 compared to Phase I; †p<0.05 compared to Phase I+II; TNF-α – tumour necrosis factor alpha; IL-1β – interleukin-1 beta; GFAP – glial fibrillary acidic protein; KCC2 – potassium-chloride cotransporter 2; p-mTOR – phosphorylated mammalian target of rapamycin.
Source: author's experimentally obtained data.

The control group was characterised by high levels of pro-inflammatory cytokines in the cerebrospinal fluid: TNF-α was 186.4±24.3 pg/mL and IL-1β was 142.8±18.6 pg/mL, which corresponds to a state of chronic neuroinflammation with persistent activation of microglia and astrocytes. The concentration of GFAP reached 487.3±52.4 ng/mL, reflecting pronounced reactive gliosis with the formation of dense astrocytic scarring. KCC2 expression was reduced to 38.2±6.4% of the normal values, thus indicating impaired maturation of inhibitory neurotransmission and contributing to the development of spasticity. mTOR signalling activity also remained suppressed at 42.7±7.8% of the normal, indicating limited anabolic capacity of neurons. The Phase III only group did not show statistically significant differences from the control group in any of the parameters studied.

In the Phase I group, a statistically significant decrease in both pro-inflammatory cytokines was observed: TNF-α to 124.7±16.8 pg/mL (which is a reduction of 33.1%, $p<0.05$) and IL-1β to 96.3±14.2 pg/mL (representing a reduction of 32.6%, $p<0.05$), reflecting the anti-inflammatory effect of the combined use of ChABC, ROCK inhibitor and AAV-GAD65/67. The concentration of GFAP decreased to 328.6±41.7 ng/mL (at 32.6% lower than the control, $p<0.05$), thus indicating a partial reduction in reactive gliosis due to CSPG degradation and modulation of astrocytic activity. KCC2 expression showed a significant recovery to 64.8±8.9% of the normal (an increase of 69.6% relative to the control, $p<0.05$), which is consistent with the restoration of excitatory-inhibitory balance through the introduction of GAD65/67 vectors and an enhanced GABAergic inhibition. p-mTOR activity increased to 58.3±9.2% (36.5% higher than the control, $p<0.05$), reflecting the initial metabolic activation of neurons.

The Phase I+II group demonstrated a further reduction in pro-inflammatory markers: TNF-α to 98.2±13.5 pg/mL (21.3% lower than Phase I, $p<0.05$) and IL-1β to 74.6±11.8 pg/mL (22.5% lower than Phase I, $p<0.05$). The GFAP level decreased to 284.1±38.3 ng/mL, although the difference with the Phase I group was not statistically significant ($p=0.089$). KCC2 expression was 72.4±9.6% of the normal with no significant increase compared to Phase I ($p=0.156$), while p-mTOR activity increased to 86.7±11.4% (which is 48.8% higher than Phase I, $p<0.05$), reflecting the potent anabolic effect of neurotrophic stimulation via BDNF/NT-3 and the removal of myelin-associated inhibition via anti-Nogo antibodies.

The full Phase I+II+III protocol group showed the lowest levels of pro-inflammatory cytokines: TNF-α – 89.3±12.1 pg/mL (52.1% reduction relative to the control, $p<0.05$) and IL-1β – 68.2±10.7 pg/mL (52.2% reduction relative to the control, $p<0.05$). The GFAP concentration decreased to 256.8±35.9 ng/mL (47.3% lower than the control and 21.8% lower than Phase I, $p<0.05$), thus indicating the most pronounced reduction in glial scarring. KCC2 expression reached 78.9±10.3% of the normal (21.8% higher than Phase I, $p<0.05$), reflecting the additional effect of activity-dependent consolidation of inhibitory synapses under the influence of BCI-controlled stimulation. The highest p-mTOR activity was observed in the Phase I+II+III group, reaching 112.6±13.8% of normal values (which is 29.9% higher than Phase I+II, $p<0.05$), which exceeded physiological levels and was consistent with increased neuronal metabolic activation under the influence of combined neurotrophic stimulation and activity-dependent plasticity. Thus, the results demonstrated the cascading anti-inflammatory and neuroprotective effects of the multiphase protocol. Phase I provides initial modulation of neuroinflammation and restoration of inhibitory balance, Phase II adds powerful metabolic activation through mTOR signalling, while Phase III consolidates the changes achieved through activity-dependent stabilisation of functional neuronal networks. The restoration of mTOR activity above the normal values in the Phase I+II+III group may reflect compensatory hyperactivation of anabolic processes in response to prolonged regenerative stimulation.

Electromyographic assessment of voluntary and reflex muscle activity provides an objective characterisation of functional reinnervation of muscles below the level of injury and restoration of supraspinal control over segmental motor neurons. The number of recruited motor units reflects the degree of reinnervation of muscle fibres, the amplitude of the compound muscle action potential (CMAP) characterises the synchrony and power of neuromuscular transmission, and the conduction velocity along motor fibres indicates the quality of myelinisation of regenerating axons. Electromyographic parameters at week 24 of the study are presented in Table 6.

The control group demonstrated critically low electromyographic activity with a minimum number of recruited motor units of 0.8±0.6, which corresponds to a complete absence of voluntary motor control below the level of damage. The CMAP amplitude was only 0.3±0.2 mV, which is a subcritical value and reflects the denervation of most muscle fibres. The conduction velocity in the preserved

motor fibres was reduced to 12.4±2.8 m/s, indicating demyelination and degenerative changes in the axons. The F-wave, which characterises conduction along long motor pathways with impulse return to spinal motor neurons, was absent in all animals in the control group, thus confirming the complete loss of the functional integrity of the descending and ascending pathways. The Phase III only group showed minimal changes compared to the control without reaching statistical significance for any parameter.

Table 6. Electromyographic parameters of hind limb muscles at week 24 of the study (M±SD)

Group	Motor units (n)	CMAP amplitude (mV)	Speed (m/s)	F-wave latency (ms)
Control (n=13)	0.8±0.6	0.3±0.2	12.4±2.8	none
Phase I (n=14)	3.2±1.1*	1.2±0.4*	18.6±3.4*	42.8±8.6
Phase I+II (n=15)	8.7±1.8*#	3.8±0.7*#	28.4±4.2*#	34.2±6.4*
Phase I+II+III (n=14)	14.3±2.4*#†	6.2±1.1*#†	36.7±4.8*#†	28.6±5.2*#
Phase III only (n=13)	1.4±0.8	0.5±0.3	14.2±3.1	none

Note: *p<0.05 compared to Control; #p<0.05 compared to Phase I; †p<0.05 compared to Phase I+II; CMAP – compound muscle action potential; F-wave – late response reflecting conduction along motor axons to spinal motor neurons and back.
Source: author's experimentally obtained data.

In the Phase I group, there was a statistically significant increase in the number of recruited motor units to 3.2±1.1 (a 4-fold increase relative to the control, $p<0.05$), reflecting the initial reinnervation of muscle fibres due to local axonal sprouting. The CMAP amplitude increased to 1.2±0.4 mV (a 4-fold increase, $p<0.05$), and the conduction velocity improved to 18.6±3.4 m/s (a 50% increase, $p<0.05$), indicating partial restoration of myelination of regenerating axons. The F-wave was recorded in 71% of animals in this group with an average latency of 42.8±8.6 ms, indicating the formation of initial, albeit slow, conduction along long motor pathways. The addition of Phase II components in the Phase I+II group led to a marked improvement in all electromyographic parameters. The number of motor units increased to 8.7±1.8 (2.7 times higher than Phase I, $p<0.05$), the CMAP amplitude reached 3.8±0.7 mV (3.2 times higher than Phase I, $p<0.05$), and the conduction velocity increased to 28.4±4.2 m/s (52.7% higher than in Phase I, $p<0.05$). F-wave latency decreased to 34.2±6.4 ms ($p<0.05$ compared to the control), reflecting improved conduction along regenerating pathways under the influence of neurotrophic stimulation and elimination of myelin-associated inhibition. The F-wave was recorded in 93% of animals in this group.

The Phase I+II+III full protocol group demonstrated the highest neuromuscular function indicators: the number of motor units reached 14.3±2.4 (17.9 times higher than the control and 1.6 times higher than Phase I+II, $p<0.05$), CMAP amplitude – 6.2±1.1 mV (20.7 times higher than the control and 1.6 times higher than Phase I+II, $p<0.05$), conduction velocity – 36.7±4.8 m/s (3.0 times higher than the control and 29.2% higher than Phase I+II, $p<0.05$). F-wave latency decreased to 28.6±5.2 ms (16.4% lower than Phase I+II, $p<0.05$), which is close to normal values for rats (25–30 ms), and which reflects a significant recovery of conduction velocity along long motor tracts. The F-wave was recorded in 100% of animals in the Phase I+II+III group with the least latency variability, indicating stable and uniform quality of conduction pathway regeneration under the influence of activity-dependent selective stabilisation of functional synaptic connections. Electromyographic data confirmed the progressive restoration of functional muscle reinnervation based on the phasing of the therapeutic protocol. The most pronounced improvement was observed with the integration of molecular modulation of the microenvironment, neurotrophic stimulation and BCI-controlled activity-dependent plasticity, which ensured not only a quantitative increase in recruited motor units

but also a qualitative restoration of neuromuscular transmission parameters and conductivity along long motor pathways. The restoration of the F-wave in the Phase I+II+III group with latency close to physiological is particularly significant, as it indicates the formation of functionally integrated supraspinal-spinal neuronal circuits.

Safety profile assessment is a critical component of translational research, especially when applying multimodal interventions using viral vectors, biologically active molecules and invasive neuromodulation. Aberrant axonal sprouting can lead to the formation of ectopic non-functional synaptic connections and exacerbate neuropathic pain, while immune responses to viral vectors and foreign proteins can limit therapeutic efficacy and pose safety risks [23–25]. Histological assessment of dysraphic sprouting, behavioural assessment of mechanical allodynia and serological analysis of antibodies at week 24 of the study are presented in Table 7.

Table 7. Safety and adverse event rates at week 24 of the study (M±SD)

Group	Ectopic sprouting (0-4 points)	Mechanical allodynia (g)	Anti-AAV antibodies (titre)	Anti-ChABC antibodies (titre)	Spinal haematoma (%)
Control (n=13)	0.3±0.5	4.2±0.8	none	none	0
Phase I (n=14)	0.9±0.7	3.6±0.7	1:128±1:64	1:256±1:128	0
Phase I+II (n=15)	1.8±0.9*	2.8±0.6*	1:512±1:256*	1:384±1:192	0
Phase I+II+III (n=14)	2.4±1.1*#	2.1±0.5*#	1:640±1:320*	1:448±1:224	7.1
Phase III only (n=13)	0.4±0.5	4.0±0.9	none	none	7.7

Note: *p<0.05 compared to Control; #p<0.05 compared to Phase I. Ectopic sprouting: 0 – none, 1 – minimal, 2 – noticeable, 3 – expressed, 4 – massive; Mechanical allodynia: Paw withdrawal threshold during stimulation with von Frey filaments (lower values=greater pain sensitivity); Spinal haematoma: proportion of animals with intraspinal haemorrhages >2 mm^3, verified on serial histological sections stained with haematoxylin and eosin.
Source: author's experimentally obtained data.

The control group demonstrated a minimal level of ectopic axonal sprouting of 0.3±0.5 points, which corresponds to spontaneous degenerative-regenerative processes without clinically significant formation of aberrant synaptic connections. The mechanical allodynia threshold was 4.2±0.8 g, reflecting physiological pain sensitivity without signs of neuropathic pain. Antibodies to AAV vectors and ChABC were absent in all animals, as these components were not used. No spinal haematomas were observed in any animal in the control group. The Phase III only group showed comparable safety profiles to the control, but the incidence of spinal haematomas was 7.7% (1 out of 13 animals), which is associated with the invasive procedure of epidural electrode implantation.

In the Phase I group, a moderate level of ectopic sprouting of 0.9±0.7 points was observed, which did not reach statistical significance compared to the control (p=0.063), and which reflects controlled axonal growth within the created permissive corridors after CSPG degradation. The mechanical allodynia threshold decreased to 3.6±0.7 g without statistical significance (p=0.124), which may reflect transient hypersensitivity during the period of active regeneration. Low titres of antibodies to AAV (1:128±1:64) and ChABC (1:256±1:128) were detected, indicating a minimal humoral immune response without clinical manifestations. No spinal haematomas were recorded.

The Phase I+II group demonstrated a statistically significant increase in ectopic sprouting to 1.8±0.9 points (p<0.05 compared to the control), which is consistent with enhanced neurotrophic stimulation of axonal growth via BDNF/NT-3 and the elimination of myelin-associated inhibition. Despite this, the level of sprouting remained moderate and was not accompanied by functional impairments. The mechanical allodynia threshold decreased to 2.8±0.6 g (p<0.05), reflecting the

development of mild neuropathic hypersensitivity as a potential side effect of intensive regenerative stimulation. AAV antibody titres increased to 1:512±1:256 (p<0.05), while anti-ChABC antibody levels remained stable (1:384±1:192, p=0.187). No spinal haematomas were detected.

The full protocol Phase I+II+III group showed the highest level of ectopic sprouting, 2.4±1.1 points (p<0.05 compared to Phase I), which corresponds to moderately pronounced aberrant axonal growth. This effect may be related to the synergistic action of neurotrophic stimulation and electrical neuromodulation, which can non-selectively activate both functional and non-functional neuronal circuits. The mechanical allodynia threshold in the Phase I+II+III group was the lowest among the active treatment groups (2.1±0.5 g; p<0.05 compared to Phase I), consistent with greater mechanical hypersensitivity rather than an analgesic effect. Anti-AAV antibody titres reached maximum values of 1:640±1:320 (p<0.05), while anti-ChABC antibody levels remained moderate (1:448±1:224). Spinal haematomas were recorded in 7.1% of animals (1 out of 14), which is comparable to the Phase III only group, and which is associated with the invasive procedure of epidural implantation.

Overall, the safety profile of the multimodal protocol was acceptable with controllable side effects. Ectopic axonal sprouting, although increasing proportionally to the intensity of regenerative stimulation, remained within moderate levels without the formation of massive dysraphic connections. Mechanical hypersensitivity was greater in the groups receiving more intensive regenerative interventions, with the lowest withdrawal threshold observed in the Phase I+II+III group. The humoral immune response to viral vectors was moderate and was not accompanied by clinical manifestations of immune rejection or systemic reactions. The incidence of spinal haematomas associated with epidural implantation was 7.1–7.7% and is consistent with the expected risks of invasive neurosurgical procedures in small laboratory animals. No mortality associated with therapeutic interventions was reported in any of the groups. It should be noted that the 24-week observation period may be insufficient for the detection of delayed adverse events, including potential neoplastic changes associated with sustained mTOR hyperactivation above physiological levels, progression of neuropathic pain beyond the allodynia thresholds recorded at the final time point, or chronic immunological consequences of repeated AAV vector administration. Extended longitudinal follow-up was not conducted within the scope of the present study.

Functional recovery of the spinal cord's conduction pathways depends not only on the number of regenerating axons but also on the quality of their myelination and adequate blood supply to the regeneration zone. Myelin basic protein (MBP) characterises the maturity of myelin sheaths, the ratio of myelinated to unmyelinated axons reflects the effectiveness of oligodendroglial support for regeneration, while capillary density and VEGF expression determine the angiogenic activity and metabolic supply of newly formed nerve tissue. Histological indicators of myelination and vascularisation at week 24 of the study are presented in Table 8.

The control group was characterised by minimal immunoreactivity to MBP at a level of 8.4±1.6% of the section area, reflecting predominant demyelination in the area of chronic damage with limited remnants of preserved myelin. The ratio of myelinated to unmyelinated axons was only 0.12±0.03, thus indicating that there are approximately 8 demyelinated fibres for each myelinated axon. Capillary density was reduced to 3.2±0.7×10²/mm², corresponding to hypovascularisation of the glial scar, and VEGF expression remained at a minimum level of 6.8±1.4%, reflecting the absence of active angiogenesis. The thickness of the myelin sheaths on the preserved fibres was 0.18±0.04 μm, which is a suboptimal indicator. The Phase III only group did not show statistically significant differences from the control.

In the Phase I group, there was a statistically significant increase in MBP immunoreactivity to 14.2±2.3% (an increase of 69%, p<0.05), the ratio of myelinated to unmyelinated axons increased to 0.24±0.06 (doubling relative to the control, p<0.05), indicating initial remyelination of regenerating

axons. Capillary density increased to 5.8±1.1×10²/mm² (81% higher than the control, $p<0.05$) and VEGF expression increased to 11.3±2.1% (an increase of 66%, $p<0.05$), reflecting angiogenic activity stimulated by the creation of a permissive microenvironment and a reduction in glial inhibition. Myelin thickness improved to 0.28±0.06 μm (56% higher than the control, $p<0.05$).

Table 8. Histological markers of myelination and vascularisation in the spinal cord injury zone at week 24 of the study (M±SD)

Group	MBP (% area)	Myelinated/ unmyelinated axons	Capillary density (×10²/mm²)	VEGF expression (% area)	Myelin thickness (μm)
Control (n=13)	8.4±1.6	0.12±0.03	3.2±0.7	6.8±1.4	0.18±0.04
Phase I (n=14)	14.2±2.3*	0.24±0.06*	5.8±1.1*	11.3±2.1*	0.28±0.06*
Phase I+II (n=15)	26.7±3.4*#	0.52±0.09*#	10.4±1.8*#	18.6±2.8*#	0.46±0.08*#
Phase I+II+III (n=14)	34.8±4.1*#†	0.68±0.11*#†	14.2±2.3*#†	22.4±3.2*#	0.58±0.09*#†
Phase III only (n=13)	9.6±1.8	0.14±0.04	3.8±0.9	7.4±1.6	0.20±0.05

Note: *p<0.05 compared to Control; #p<0.05 compared to Phase I; †p<0.05 compared to Phase I+II; MBP – myelin basic protein; VEGF – vascular endothelial growth factor.
Source: author's experimentally obtained data.

The Phase I+II group demonstrated a marked increase in all myelination and vascularisation parameters. MBP immunoreactivity reached 26.7±3.4% (3.2 times higher than the control and 1.9 times higher than Phase I, $p<0.05$), the ratio of myelinated to unmyelinated axons increased to 0.52±0.09 (4.3 times higher than the control and 2.2 times higher than Phase I, $p<0.05$), thus indicating effective support for oligodendroglial maturation under the influence of BDNF/NT-3. Capillary density increased to 10.4±1.8×10²/mm² (3.3 times higher than the control, $p<0.05$), and VEGF expression increased to 18.6±2.8% (2.7 times higher than the control, $p<0.05$), reflecting the potent angiogenic effect of neurotrophic factors and the possible paracrine influence of mesenchymal stem cells (MSC). Myelin thickness reached 0.46±0.08 μm (2.6 times higher than the control and 64% higher than Phase I, $p<0.05$), thus approaching normal values for small myelinated fibres in rats (0.5–0.8 μm).

The full Phase I+II+III protocol group showed the highest myelination and vascularisation rates: MBP immunoreactivity 34.8±4.1% (4.1 times higher than the control and 30% higher than Phase I+II, $p<0.05$), the ratio of myelinated to unmyelinated axons 0.68±0.11 (5.7 times higher than the control and 31% higher than Phase I+II, $p<0.05$), which corresponds to the presence of two myelinated axons for every three unmyelinated ones. Capillary density reached 14.2±2.3×10²/mm² (4.4 times higher than the control and 37% higher than Phase I+II, $p<0.05$), approaching the density in the intact grey matter of the spinal cord (15–18×10²/mm²). VEGF expression was 22.4±3.2% (3.3 times higher than the control, $p<0.05$ compared to the control, but without statistical significance relative to Phase I+II, p=0.078), which may reflect the stabilisation of angiogenic activity in the context of the formed vascular network. Myelin thickness reached 0.58±0.09 μm (3.2 times higher than the control and 26% higher than Phase I+II, $p<0.05$), which is within the normal range. Thus, parallel activation of myelination and angiogenesis was demonstrated in accordance with the intensity of axonal sprouting. The most effective remyelination was observed with a combination of neurotrophic support and activity-dependent plasticity, which may reflect selective myelination of functionally active axons under the influence of BCI-controlled stimulation. Restoration of vascular density to levels close to intact tissue in the Phase I+II+III group is critical for the metabolic support of energy-intensive synaptic transmission processes and the maintenance of regenerated neuronal circuits.

An integrative analysis of the relationships between functional, electrophysiological, molecular and structural parameters established key predictors of successful regeneration and reveals causal relationships between molecular modulation of the microenvironment, axonal growth and functional recovery. Pearson's correlation analysis was performed on a combined sample of all experimental groups (n=69) with the objective to assess universal biological patterns regardless of the therapeutic protocol. The correlation coefficient matrix is presented in Table 9.

Table 9. Pearson correlation matrix (r) between key regeneration indicators (combined sample n=69)

Parameter	BBB score	H/M ratio	FA	NF200+	BDNF	GAP-43	Motor units	CMAP	MBP
BBB score	1.00	-0.89***	0.92***	0.88***	0.74***	0.81***	0.94***	0.91***	0.86***
H/M ratio	-0.89***	1.00	-0.83***	-0.79***	-0.68***	-0.72***	-0.85***	-0.82***	-0.76***
FA	0.92***	-0.83***	1.00	0.95***	0.71***	0.84***	0.89***	0.93***	0.91***
NF200+	0.88***	-0.79***	0.95***	1.00	0.76***	0.87***	0.86***	0.90***	0.94***
BDNF	0.74***	-0.68***	0.71***	0.76***	1.00	0.82***	0.72***	0.75***	0.73***
GAP-43	0.81***	-0.72***	0.84***	0.87***	0.82***	1.00	0.79***	0.83***	0.85***
Motor units	0.94***	-0.85***	0.89***	0.86***	0.72***	0.79***	1.00	0.96***	0.84***
CMAP	0.91***	-0.82***	0.93***	0.90***	0.75***	0.83***	0.96***	1.00	0.88***
MBP	0.86***	-0.76***	0.91***	0.94***	0.73***	0.85***	0.84***	0.88***	1.00
Capillary density	0.79***	-0.71***	0.81***	0.83***	0.69***	0.77***	0.76***	0.80***	0.82***

Note: ***p<0.001; FA – fractional anisotropy; NF200+ – density of NF200-positive axons; BDNF – concentration in CSF; Motor units – number of motor units recruited; CMAP – amplitude of the compound muscle potential; MBP – immunoreactivity of myelin basic protein.
Source: author's experimentally obtained data.

Analysis of the correlation matrix revealed a highly integrated system of relationships between all studied parameters, confirming the coordination of regenerative processes at the molecular, structural and functional levels. The H/M ratio showed a strong negative correlation with the BBB score (r=-0.89, $p<0.001$), reflecting the fundamental antagonism between pathological spasticity and functional locomotor recovery. This is consistent with the conceptual basis of the AXONIC-REVIVE™ protocol, according to which, suppression of spastic networks is a prerequisite for the formation of functional regenerative circuits. The strongest positive correlation was observed between the number of recruited motor units and the CMAP amplitude (r=0.96, $p<0.001$), which is expected given the direct dependence of compound potential amplitude on the synchronous activation of multiple motor units. The locomotor function (BBB score) showed a strong correlation with the number of motor units (r=0.94, $p<0.001$) and the CMAP amplitude (r=0.91, $p<0.001$), thus confirming that functional recovery directly depends on the effectiveness of muscle reinnervation.

Structural parameters showed pronounced consistency: NF200+ axon density correlated with the fractional anisotropy coefficient (r=0.95, $p<0.001$) and MBP immunoreactivity (r=0.94, $p<0.001$), reflecting the parallelism of axonal growth, structural organisation of tracts and their myelination. Notably, both structural indicators showed almost equally high correlation with functional parameters: NF200+ with BBB score (r=0.88, $p<0.001$) and FA with BBB score (r=0.92, $p<0.001$), thus indicating that both the quantity and quality of regenerating axon organisation are critical determinants of the functional outcome. The level of BDNF in CSF correlated with all studied parameters of regeneration with coefficients r=0.68–0.76 ($p<0.001$), thereby demonstrating the universal role of neurotrophic support in regenerative processes. The strongest association of BDNF was observed with the axonal sprouting marker GAP-43 (r=0.82, $p<0.001$) and NF200+ axon density (r=0.76, $p<0.001$),

confirming the central role of neurotrophins in stimulating axonal growth. GAP-43, as a marker of active axonal sprouting, showed a strong correlation with all structural parameters (r=0.84–0.87, $p<0.001$), validating it as a prognostic biomarker of structural regeneration.

Capillary density correlated with all other parameters with coefficients r=0.69–0.83 ($p<0.001$), with the strongest correlation observed with NF200+ axon density (r=0.83, $p<0.001$) and MBP immunoreactivity (r=0.82, $p<0.001$). This confirms the critical role of adequate vascularisation for the metabolic support of energy-intensive processes of axonal growth and myelination. A moderate but statistically significant correlation between vascularisation and BDNF (r=0.69, $p<0.001$) reflects the angiogenic properties of neurotrophic factors and their role in the formation of neurovascular niches.

Multiple regression analysis with BBB score as the dependent variable and structural-molecular parameters as predictors revealed that 91% of the variability in the locomotor recovery ($R^2=0.91$, $p<0.001$) is explained by a combination of five independent factors: the number of motor units (β=0.38), FA coefficient (β=0.24), H/M ratio suppression (β=-0.18), MBP immunoreactivity (β=0.16) and BDNF concentration (β=0.12). This indicates the multifactorial nature of functional recovery, where effective muscle reinnervation, structural organisation of conduction pathways, spasticity control, quality myelination and neurotrophic support make independent contributions to the outcome. Correlation analysis confirmed the systemic nature of neuroregeneration, where molecular, structural and functional processes interact in a coordinated manner at all levels of the nervous system organisation. The strong correlations identified validate the selected biomarkers as relevant predictors of functional recovery and justify the AXONIC-REVIVE™ multimodal approach, which simultaneously targets multiple interrelated targets of the regenerative process.

Discussion

In the study, the Phase III only group, which received only epidural stimulation and BCI-controlled neuromodulation, demonstrated a BBB score of 2.7±1.6 at week 24 without achieving statistical significance compared to the control group, which was interpreted as evidence of the ineffectiveness of isolated neuromodulation in the absence of prior molecular preparation of the microenvironment. This conclusion partially contradicts the data of M. Boakye et al. [26], where a series of 25 patients with motor-complete chronic traumatic spinal cord injury (TBI) achieved voluntary movements after implantation of epidural stimulation combined with intensive task-specific training without prior pharmacological or gene therapeutic interventions. At the same time, a systematic review by J. T. Hachmann et al. [27] found that the completeness of the injury is the main determinant of the response to epidural stimulation: patients with AIS-B demonstrated independent walking on the surface, while patients with AIS-A were limited to partially assisted locomotion on a treadmill. The discrepancy may be explained by the fact that the study used a complete transection model (equivalent to AIS-A), where the response to isolated stimulation is expected to be minimal, as well as differences in stimulation parameters and the intensity of rehabilitation training. Furthermore, J. T. Hachmann et al. [27] noted that, by 2020, the total number of patients included in relevant publications was only 24, indicating a limited evidence base, even for positive results of epidural stimulation.

Phase III of the AXONIC-REVIVE™ protocol involved the use of a closed-loop BCI interface as a means of activity-dependent consolidation of regenerated neural circuits, which resulted in an additional increase in the BBB score from 9.3 (Phase I+II) to 13.7 (Phase I+II+III). A systematic review by J. J. Levett et al. [28], covering 19 studies and 21 patients with cervical TBI, found that no existing BCI technology provides complete functional restoration of autonomy. The results were heterogeneous, and intracortical microelectrode arrays (85.7% of cases) showed signal degradation

over time. All patients had cervical injuries, which limits generalisation to thoracic and lumbar levels. BCI, as an isolated tool, is not capable of bypassing interrupted spinal conduction and requires a combination with functional electrical stimulation or epidural neuromodulation. In the study, the BCI component was applied after prior axonal regeneration (Phases I and II), which created a neuronal substrate for decoding BCI signals and may explain its greater effectiveness compared to isolated BCI application.

In the study, chondroitinase ABC (ChABC) was used as a key component of Phase I for the degradation of chondroitin sulphate proteoglycans (CSG), which led to a decrease in CSG content to 62.4±8.7% of the control values in the Phase I group. A meta-analysis by A. Khanteymoori et al. [29], which covered 1066 animals, found only a 15.9% improvement in locomotor performance (95% confidence interval (CI)=11.3–20.6%) when ChABC was used as monotherapy, which is a moderate effect with significant heterogeneity. Trim and Fill analysis identified 19 hypothetical missing experiments, indicating a systematic error in publications in the field of ChABC research. The study noted that systematic reporting bias in experimental studies of TBI is common and is not limited to a specific intervention. In the study, ChABC was used in combination with a ROCK inhibitor and AAV-GAD65/67, which may provide a synergistic effect not achievable with monotherapy, but the results of the meta-analysis indicate the need for cautious interpretation of the contribution of ChABC to the overall therapeutic outcome.

In the AXONIC-REVIVE™ protocol, anti-Nogo antibodies were included in Phase II, where their combination with BDNF/NT-3 neurotrophic factors resulted in an increase in NF200+ axon density to $12.3\pm1.8\times10^3/mm^2$ in the Phase I+II group compared to $4.8\pm1.8\times10^3/mm^2$ in the Phase I group. NT-3 resulted in an increase in NF200+ axon density to $12.3\pm1.8\times10^3/mm^2$ in the Phase I+II group compared to $4.8\pm0.9\times10^3/mm^2$ in the Phase I group. A randomised double-blind clinical trial by G. Maynard et al. [30] using soluble Nogo receptor-Fc decoy (AXER-204) in 27 randomised patients with chronic cervical TBI did not show clinically significant improvements in any of the planned efficacy analyses. Post-hoc subgroup analysis showed a trend toward an effect exclusively in patients with incomplete TBI, suggesting the need for preserved neural pathways for Nogo-dependent sprouting and plasticity. These results indicate that Nogo pathway blockade as monotherapy is not capable of overcoming a complete lesion gap. In the study, anti-Nogo antibodies were applied after prior creation of axonal substrate via ChABC and ROCK inhibition in Phase I, which may explain the difference in results. It should also be noted that the mechanism of action of AXER-204 (soluble Nogo receptor-Fc decoy) differs from that of the direct anti-Nogo antibodies used in the AXONIC-REVIVE™ protocol.

In the study, a 47.3% reduction in the GFAP concentration in the Phase I+II+III group (from 487.3±52.4 to 256.8±35.9 ng/ml) was interpreted as a positive result, indicating a reduction in reactive gliosis and glial scarring. Data from T. M. O'Shea et al. [31], obtained by using transgenic linear tracing, single-nucleus RNA sequencing (snRNA-seq) and immunohistochemistry, demonstrated that more than 90% of astrocytes forming the border around the injury zone originate from local Aldh1l1+ astrocytes and perform a neuroprotective function in wound repair. These cells re-establish the integrity of the central nervous system (CNS) by separating the neuronal parenchyma from stromal and immune cells and the authors reframe them not as 'scar tissue' but as essential cells for wound healing. Additional confirmation is provided by J. E. Burda et al. [32], who identified more than 12,000 differentially expressed genes of astrocytic reactivity with marked heterogeneity between different diseases, demonstrating that astrocytic reactivity and GFAP elevation are not uniformly harmful processes. GFAP is a marker of reactive astrocytes in general and does not differentiate between scar-forming neuroprotective and neurotoxic subpopulations, making it difficult to interpret its reduction as a clearly positive result in the study.

In the Phase I+II+III group, a 3.3-fold increase in the BDNF concentration in CSF (468.5±42.1 pg/ml versus 142.3±18.7 pg/ml in the control) was recorded, with a simultaneous decrease in the mechanical allodynia threshold to 2.1±0.5 g along with an increase in the ectopic sprouting score to 2.4±1.1. K. K. Martin et al. [33], by using transgenic TrkB616A mice, demonstrated that the pharmacogenetic inhibition of the TrkB receptor (BDNF receptor) after contusion TBI (T10) blocked the development of mechanical hypersensitivity ($p<0.05$) and simultaneously improved the locomotor performance on the Basso Mouse Scale (BMS). These data indicate a causal relationship between BDNF-TrkB signalling and the development of neuropathic pain after TBI. A comprehensive review by J. Rosner et al. [34] documents that central neuropathic pain affects approximately 60% of patients with TBI, and regenerative therapies, including neurotrophic delivery, may exacerbate or fail to prevent neuropathic pain syndrome through mechanisms of maladaptive plasticity and aberrant sprouting of nociceptive afferents. In the study, the reduction in the allodynia threshold to 2.1 g in the full protocol group corresponds to clinically significant hypersensitivity, which may limit the translational potential of the protocol, although the GAD65/67 component of Phase I is theoretically capable of partially compensating for BDNF-induced hyperexcitability through enhanced GABAergic inhibition.

p-mTOR activity in the Phase I+II+III group reached 112.6±13.8% of normal values, exceeding physiological levels, which was interpreted as compensatory hyperactivation of anabolic processes in response to prolonged regenerative stimulation. M. Metcalfe and O. Steward [35] demonstrated that PTEN deletion via AAV-rg in mice with cervical TBI initially improved motor recovery, but, at 5–7 weeks post-injection, some animals developed progressive late pathophysiologies, including excessive scratching and rigid limb extension, thus indicating long-term risks of mTOR hyperactivation. The study also revealed sex differences in response to treatment. N. K. Dhaliwal et al. [36] showed in human iPSC-derived neurons and cortical organoids that PTEN loss causes synergistic hyperactivation of both mammalian target of rapamycin complex 1 (mTORC1) and mammalian target of rapamycin complex 2 (mTORC2), leading to neuronal hypertrophy, electrical hyperactivity and enhanced proliferation, highlighting the oncogenic potential of mTOR hyperactivation through the role of PTEN as a tumour suppressor. Although the study reports a moderate excess of mTOR activity (112.6%) achieved indirectly through neurotrophin stimulation rather than through genetic PTEN deletion, which may have a different safety profile, long-term monitoring for neoplastic and neuropathological changes remains necessary.

Several translational limitations of the present study warrant explicit consideration. All results were obtained exclusively in rodent models, where the distance of axonal regeneration required to bridge the lesion gap is substantially shorter than in large animals or humans, and the spinal cord anatomy, immune response dynamics and biomechanical demands differ considerably from the clinical setting. No verification was performed in large animal models such as non-human primates or porcine preparations, which are generally regarded as a prerequisite for translational confidence in spinal cord injury research. The pharmacokinetic profiles of the therapeutic agents, particularly the biodistribution and transduction efficiency of AAV9 vectors in the human spinal cord, the half-life of PEGylated ChABC in a larger tissue volume and the dose-response relationship of anti-Nogo-A antibodies at clinically relevant scales, require independent validation. The discrepancies between preclinical efficacy in rodent complete transection models and the negative outcomes of recent clinical trials targeting individual components of the regenerative cascade reinforce the necessity of intermediate large-animal validation before human application. Additionally, the exclusive reliance on endogenous circuit remodelling without iPSC-derived interneuron transplantation restricts the ability to determine whether exogenous neuronal populations would enhance or compete with the regenerative processes initiated by the protocol. The 24-week observation period, while sufficient to

demonstrate functional improvement, may fail to capture delayed complications including potential neoplastic transformation associated with sustained mTOR activity exceeding physiological levels (112.6%), progressive neuropathic pain driven by chronic BDNF-TrkB signalling, or cumulative immunological consequences of sequential AAV vector administration with antibody titres reaching 1:640. The clinical translation pathway therefore requires not only large-animal validation but also extended longitudinal monitoring protocols designed to detect these specific delayed adverse events.

The AXONIC-REVIVE™ protocol involves two-stage administration of AAV vectors: AAV-GAD65/67 in Phase I and AAV-BDNF/NT-3 in Phase II, with anti-AAV antibody titres in the Phase I+II+III group reaching 1:640±1:320. A review by H. C. Verdera et al. [37] showed that 40–80% of the human population has pre-existing neutralising antibodies (NAT) against various AAV serotypes and after the first administration of an AAV vector, patients universally develop high-titre multiserotype cross-reactive NATs that persist for up to 15 years and preclude the effectiveness of re-administration. For the AXONIC-REVIVE™ protocol, this means that AAV-GAD65/67 in Phase I may induce NATs capable of reducing the efficacy of AAV-BDNF/NT-3 in Phase II. Clinical confirmation of this problem is provided by D. X. Bharucha-Goebel et al. [38] where a Phase I trial of intrathecal self-complementary adeno-associated virus serotype 9 (scAAV9) therapy in 14 patients with giant axonal neuropathy recorded the development of an AbT titre greater than 1:150,000 in one seronegative patient after 8 months, while seropositive patients showed accelerated vector elimination. The use of different AAV serotypes in Phases I and II may partially circumvent this problem and intrathecal administration into the CNS provides relative immune privilege, but complete isolation from the systemic immune response is not achieved.

In the study conducted, AAV-GAD65/67-mediated overexpression of glutamate decarboxylase was used in Phase I to enhance GABAergic inhibition and suppress spasticity, which contributed to a decrease in the H/M ratio from 83.1% to 56.3% in the Phase I group at week 24. J. N. Bilchak et al. [39] demonstrated that pharmacological enhancement of KCC2 with CLP257 in rats with complete thoracic transection (T12) reduced hyperreflexia and decreased the phasic EMG response by approximately 50% ($t(8)=3.89$, $p=0.0046$) without the use of gene therapy. The study argues that the root cause of spasticity is KCC2 downregulation, which makes GABA/glycine receptor activation depolarising rather than inhibitory. This mechanism calls into question the feasibility of the GAD65/67 approach: increasing GABA synthesis in the presence of an impaired chloride gradient due to KCC2 loss may paradoxically increase excitability. The study recorded a restoration of KCC2 to 78.9% of normal (from 38.2% in the control group), which may restore the inhibitory function of GABA and make GAD65/67 overexpression effective, but the mechanism of this restoration and its dependence on the GAD65/67 component still need to be clarified.

The conceptual basis of the AXONIC-REVIVE™ protocol is the sequential (multiphase) application of the therapeutic components, which is justified by the need to pre-reprogram the microenvironment before initiating regenerative processes and the subsequent consolidation of newly formed contours. Z. Álvarez et al. [40] demonstrated in a publication in *Science* that a single injection of a bioactive scaffold, which simultaneously presented two bioactive signals – β1-integrin-activating (IKVAV-laminin, which promotes neuronal differentiation and reduces glial scarring) and FGF2 receptor-activating (promoting angiogenesis) – resulted in axonal regeneration, functional revascularisation, myelination of regenerated axons and motor neuron survival in a mouse model of contusion TBI. The dual scaffold outperformed single-signal variants, contradicting the thesis of the need for an exclusively sequential approach. The discrepancy may be explained by the difference in models: the contusion model in mice (incomplete injury) differs significantly from the complete transection in the study, where the greater complexity of the regenerative task may indeed require a phased organisation of interventions. The scaffold approach provides localised controlled delivery,

while systemic or intrathecal administration of multiple agents simultaneously can create antagonistic interactions [41,42].

A BBB score of 13.7±2.9 in the Phase I+II+III group, which was 15.2 times higher than the control values and a multiple regression model with R^2=0.91 for predicting locomotor recovery from the basis for conclusions about the effectiveness of the multiphase protocol. N. D. Jeffery et al. [43] analysed BBB scores in 86 rats after standardised contusion TBI without therapeutic intervention and found a 95% reference interval of 2.3 to 15.9 points on day 21 – thus representing a range of 13.6 points. A bootstrap sample of 100 virtual groups of 10 rats showed that the mean scores ranged from 9.5 to 13.5 in a purely random manner (a 4-point difference). The data were left-skewed, violating the assumption of normality for parametric tests, which means that the 2–4-point improvements in BBB, often reported in combinatorial studies, may fall within the range of spontaneous variability. The aforementioned study used a contusion model, whereas the present study used a transection model, where the baseline variability of BBB is significantly lower (most control animals have 0–1 points), which reduces the influence of spontaneous variability. However, the ordinal nature of the BBB scale and the non-linearity of the transition between levels remain a methodological caveat.

In the study, the Phase I+II+III group achieved a BBB score of 13.7 with coordinated hindlimb stepping, which exceeded the level expected from mere axonal regeneration across the lesion gap and may reflect reactivation of dormant lumbar CPG circuits. This interpretation is supported by D. L. Garcia-Ramirez et al. [44], who demonstrated that Shox2-positive CPG neurons in the lumbar cord preserve their intrinsic rhythmogenic properties after complete thoracic transection while receiving enhanced excitatory synaptic input and serotonergic modulation, indicating that these neurons remain available for pharmacological or molecular reactivation. N. J. Stachowski et al. [45] further showed that AAV-delivered BDNF promotes stepping in completely transected mice, and that selective activation of RORβ interneurons reduces hyperreflexia and enhances locomotor performance when combined with epidural stimulation. The parallel between these findings and the AXONIC-REVIVE™ protocol is notable: Phase II of the protocol delivers BDNF/NT-3 via AAV9 vectors, while Phase III applies epidural stimulation at L2–S1, which may together engage the same CPG populations identified by N. J. Stachowski et al. [45]. However, the present study did not include direct electrophysiological recordings from lumbar interneurons, and therefore the contribution of CPG reactivation versus *de novo* circuit formation through axonal regeneration cannot be definitively distinguished.

The Phase III component of the AXONIC-REVIVE™ protocol included task-specific treadmill training with partial body weight support, which was associated with the most intensive period of functional recovery between weeks 6 and 12. The critical role of sensory feedback in driving this recovery is consistent with the findings of J. S. Calvert et al. [46], who demonstrated simultaneous motor restoration and somatosensory feedback via perilesional epidural stimulation in three participants with chronic motor-complete SCI, where supralesional stimulation evoked proprioceptive sensations synchronised with leg movement. Unlike the J.S. Calvert et al. [46] study, where perilesional stimulation was applied as a standalone intervention, the AXONIC-REVIVE™ protocol combines epidural stimulation with prior molecular and neurotrophic preparation of the spinal microenvironment, which may create a more receptive substrate for sensory-driven plasticity. The convergence of these results suggests that proprioceptive and cutaneous afferent input, including feedback from plantar mechanoreceptors during weight-bearing treadmill training, provides the necessary sensory drive for CPG entrainment, task-specific modulation of gait and activity-dependent refinement of newly formed synaptic connections. The closed-loop nature of Phase III – where BCI-decoded cortical intention is synchronised with epidural stimulation and real-time sensory feedback – may selectively strengthen functional synaptic connections while preventing maladaptive hyperexcitability.

The AXONIC-REVIVE™ protocol did not include cell transplantation as a therapeutic component, which may represent a limitation in terms of supplementing damaged interneuronal networks. T. Kitagawa et al. [47], by using inhibitory DREADDs to reversibly silence grafted human iPSC-derived neurons in a mouse SCI model, provided direct causal evidence that transplanted cells form functional relays integrated into host spinal circuits, as CNO-mediated silencing caused a significant reversible decrease in locomotor function only after graft maturation. X. Yao et al. [48] demonstrated that iPSC-derived spinal neural progenitors differentiate *in vivo* into motor neurons and V2/dI4 interneuron subtypes – populations directly implicated in CPG circuits – while simultaneously shifting the injury microenvironment toward neuroprotective phenotypes. In contrast to these cell-based approaches, the present study relied on endogenous circuit remodelling stimulated by molecular and neuromodulatory interventions. The comparison suggests that incorporating iPSC-derived region-specific progenitors as an additional phase of the AXONIC-REVIVE™ protocol could supplement dormant or damaged CPG networks with functionally relevant interneurons, potentially bridging the gap between the partial recovery achieved in the present study and full weight-bearing locomotion.

The mechanistic interpretation of the functional outcomes is further constrained by the absence of direct electrophysiological confirmation of relay circuit formation through the lesion zone and the lack of cell-type-specific ablation or silencing experiments that would definitively attribute locomotor recovery to specific neuronal populations rather than diffuse plasticity. In the study, KCC2 expression recovered to 78.9% of normal in the Phase I+II+III group, which was interpreted as restoration of chloride homeostasis necessary for functional GABAergic inhibition. This finding aligns with Y. Zuo et al. [49], who developed a KCC2 agonist delivery system using ROS-responsive nanoparticles that simultaneously scavenged reactive oxygen species and restored inhibitory GABAergic signalling in a rat SCI model, achieving significant locomotor recovery through a mechanism complementary to the AAV-GAD65/67 approach used in Phase I of the present protocol. Y. Wang et al. [50] extended these findings by demonstrating that pharmacological KCC2 activation combined with neural stem cell transplantation facilitated graft integration into host spinal networks in a complete SCI model, thus establishing KCC2 restoration as a critical enabler of relay circuit formation – a principle that may also underlie the enhanced integration of regenerating axons observed in the Phase I+II+III group. The broader paradigm of combinatorial therapy is supported by B. Chen et al. [51], who, in a randomised clinical trial, showed that combining Hebbian stimulation with 4-aminopyridine and rehabilitation improved walking speed in 60% of chronic incomplete SCI participants versus 10% with single-agent treatment, providing the first clinical evidence of synergistic multimodal therapy for SCI. Although the B. Chen et al.'s [51] study involved incomplete injuries and a different therapeutic combination, the principle of synergistic interaction between molecular, electrophysiological and activity-dependent modalities is consistent with the dose-dependent multimodal effect observed across experimental groups in the present study. The high reproducibility of functional outcomes within the Phase I+II+III group, reflected in the multiple regression model (R^2=0.91), further suggests that the phased integration of therapeutic components creates a predictable synergistic effect that stabilises locomotor outputs across experimental subjects.

From a systems medicine perspective, the AXONIC-REVIVE™ protocol constitutes a multiscale therapeutic architecture in which functional recovery emerges from multiplicative interactions across molecular, cellular and electrophysiological levels rather than from isolated interventions. The regression model (R^2=0.91) with five independent predictors empirically validates this architecture, demonstrating that any component approaching zero diminishes the overall regenerative output. This multiplicative dynamic accounts for the negligible functional gains observed with monotherapy (BBB 2.7–4.8), a finding consistent with the moderate 15.9% locomotor improvement

reported in the ChABC monotherapy meta-analysis by A. Khanteymoori et al. [29] and the absence of clinically significant effects in the AXER-204 monotherapy trial by G. Maynard et al. [30], compared to the synergistic effect of multimodal integration (BBB 13.7), which aligns with the principle of combinatorial synergy demonstrated clinically by B. Chen et al. [51].

At the electrophysiological level, the restoration of F-wave latency to near-physiological values (28.6±5.2 ms) and the tripling of conduction velocity indicate that functional recovery exceeded predictions based on the structural axonal density alone. This suggests that phase synchronisation between cortical, spinal and sensory oscillatory circuits amplifies the functional efficacy of a limited number of regenerated pathways, which is consistent with the findings of J. S. Calvert et al. [46], who demonstrated that perilesional epidural stimulation restores somatosensory feedback synchronised with motor output. The disproportionate contribution of motor unit recruitment (β=0.38) relative to axonal density in the regression model supports the interpretation that the quality of temporal coupling within the reconstituted oscillatory network is more determinative of locomotor outcome than the absolute number of regenerated fibres, which may also explain the limited response to isolated epidural stimulation in motor-complete injuries reported by J. T. Hachmann et al. [27], where the absence of prior molecular preparation precluded the formation of a sufficient oscillatory substrate.

The biochemical profile reveals a narrow therapeutic window in which regenerative benefit and adverse consequences are simultaneously determined by the same signalling cascades. The 3.3-fold BDNF elevation drives plasticity through TrkB → PI3K/Akt → mTOR but concurrently engages nociceptive circuits (allodynia threshold 2.1±0.5 g), which is consistent with the causal relationship between BDNF-TrkB signalling and mechanical hypersensitivity established by K. K. Martin et al. [33] and with the broader evidence that regenerative therapies may exacerbate neuropathic pain through maladaptive sprouting of nociceptive afferents, as documented by J. Rosner et al. [34]. Supraphysiological mTOR activation (112.6%) provides anabolic drive but carries proliferative risk, as demonstrated by M. Metcalfe and O. Steward [35], who reported progressive late pathophysiologies following PTEN-mediated mTOR hyperactivation and by N. K. Dhaliwal et al. [36], who showed that PTEN loss causes synergistic mTORC1/mTORC2 hyperactivation with neuronal hypertrophy and electrical hyperactivity in human iPSC-derived neurons. The 52% cytokine suppression resolves chronic neuroinflammation, yet the 47.3% GFAP reduction must be interpreted cautiously given the evidence of T. M. O'Shea et al. [31] that border-forming astrocytes perform a neuroprotective wound-repair function and the demonstration by J. E. Burda et al. [32] that astrocytic reactivity encompasses heterogeneous transcriptional programmes not uniformly amenable to interpretation through a single marker. Therapeutic success therefore depends on maintaining a biochemical equilibrium in which disinhibitory and activating signals are temporally coordinated.

The pharmacological rationale for sequential phasing is grounded in the requirement for substrate preparation before growth induction. Phase I establishes permissive corridors through CSPG degradation and restores the chloride gradient via AAV-GAD65/67, converting GABAergic signalling from paradoxically excitatory to inhibitory (KCC2 recovery to 78.9%). This mechanism is complementary to the pharmacological KCC2 restoration achieved by Y. Zuo et al. [49] using ROS-responsive nanoparticles and to the demonstration by Y. Wang et al. [50] that KCC2 activation facilitates relay circuit formation in complete SCI. However, as J. N. Bilchak et al. [39] demonstrated, increasing GABA synthesis in the presence of an impaired chloride gradient may paradoxically enhance excitability, which underscores the importance of concurrent KCC2 restoration as a prerequisite for GAD65/67 efficacy. Phase II exploits the permissive corridors through directed neurotrophic stimulation and Nogo pathway blockade, although the sequential AAV administration raises immunological concerns given the evidence of H. C. Verdera et al. [37] regarding cross-reactive neutralising antibodies persisting for up to 15 years and the accelerated vector elimination in seropositive

patients reported by D. X. Bharucha-Goebel et al. [38]. Phase III consolidates the newly formed circuits through activity-dependent synaptic selection, where BCI-decoded cortical intention selectively stabilises functional connections, a mechanism that may engage the same Shox2-positive and RORβ-positive CPG populations identified by D. L. Garcia-Ramirez et al. [44] and N. J. Stachowski et al. [45], while the closed-loop integration with proprioceptive feedback during treadmill training provides the sensory drive for CPG entrainment.

The pathomorphological substrate confirms that the protocol supports not merely quantitative axonal regrowth but qualitative structural maturation: directed tract organisation (FA 0.47), myelinated-to-unmyelinated ratio of 0.68, myelin thickness within the normal range (0.58±0.09 μm) and capillary density approaching intact tissue values ($14.2\pm2.3\times10^2/mm^2$). The 17.9-fold increase in recruited motor units and the restoration of F-wave conduction provide electrophysiological evidence for partial reconstitution of the cortex-spinal cord-muscle axis, which is consistent with the causal evidence from T. Kitagawa et al. [47] that transplanted neurons form functional relays within host circuits and suggesting that endogenous circuit remodelling may achieve analogous relay formation without exogenous cell transplantation, although the incorporation of iPSC-derived region-specific progenitors, as demonstrated by X. Yao et al. [48], could further supplement the reconstituted circuitry. In summary, the functional restoration observed reflects a hybrid recovery mechanism in which chronic complete spinal cord injury operates as a network disorder and the AXONIC-REVIVE™ protocol achieves its effect through network reactivation and oscillatory synchronisation rather than complete anatomical reconstitution of severed pathways.

A critical unresolved question is whether the functional gains achieved at week 24 would persist after cessation of Phase III neuromodulatory support. Activity-dependent plasticity requires sustained reinforcement to consolidate newly formed synaptic connections and withdrawal of epidural stimulation, and BCI-controlled training may lead to progressive degradation of regenerated circuits through synaptic weakening and disuse-dependent atrophy of reinnervated motor units. B. Chen et al. [51] reported that functional improvements in chronic SCI participants required ongoing combinatorial treatment, thereby suggesting that single-course interventions may be insufficient for permanent recovery. Similarly, the elevated p-mTOR activity (112.6%) and BDNF concentrations (468.5 pg/mL), as recorded in the Phase I+II+III group, represent a state of active regenerative stimulation whose trajectory after protocol completion cannot be extrapolated from the available data.

Conclusions

The study found that the consistent application of a three-phase protocol of multimodal intervention in chronic complete spinal cord injury, bridging molecular, cellular and electrophysiological modalities to awaken dormant spinal networks, provides statistically and clinically significant recovery of motor functions, which is unattainable with the isolated use of individual therapeutic components. The combined use of molecular modulation of the microenvironment, neurotrophic stimulation and BCI-controlled neuromodulation resulted in a 50.2% reduction in pathological spinal hyperexcitability according to the H/M ratio, achieving 13.7 points on the BBB scale, which corresponds to the ability to bear body weight with partial interlimb coordination, as well as a 7.8-fold increase in the density of NF200-positive axons and a 5.8-fold increase in synaptic density compared to the control. The concentrations of neurotrophic factors in the cerebrospinal fluid increased by 3.3–3.7 times, accompanied by a 52% reduction in pro-inflammatory cytokines and restoration of mTOR signalling activity to a level exceeding the physiological norm.

Electrophysiological assessment confirmed the functional reintegration of supraspinal circuits: the number of recruited motor units in the full protocol group increased by 17.9 times, F-wave latency approached physiological values, and the conduction velocity along motor fibres tripled relative

to the control. Histological analysis showed restoration of the ratio of myelinated to unmyelinated axons to 0.68, which suggests an achievement of the myelin sheath thickness within the normal range and approximation of vascular density to intact tissue values. Multiple regression analysis established that 91% of the variability in functional outcome is determined by five independent factors, among which the effectiveness of muscle reinnervation is central. The safety profile remained acceptable, although intensification of regenerative stimulation was associated with moderate ectopic sprouting and a decrease in the mechanical allodynia threshold. The integrative multiscale analysis indicates that functional recovery reflects a hybrid mechanism of structural regeneration and network-level reorganisation, where chronic complete spinal cord injury operates as a network disorder requiring concurrent disinhibition, growth induction and oscillatory synchronisation rather than complete anatomical reconstitution of severed pathways.

The limitations of the study include the exclusive use of a rodent model without translational verification in large animals, where shorter axonal regeneration distances and divergent immune dynamics may not predict clinical efficacy; the absence of iPSC-derived interneuron integration; and the 24-week observation period, insufficient to detect delayed adverse events including neoplastic risk from supraphysiological mTOR activation and chronic immunological sequelae of sequential AAV administration. Promising directions include verification in large animal models, incorporation of iPSC-derived progenitor cells, extended longitudinal follow-up and validation of CPG reactivation through direct interneuronal recordings. The phased protocol design may facilitate scalable clinical translation across varying injury severities.

List of abbreviations

BDNF – brain-derived neurotrophic factor; NT-3 – neurotrophin-3; CSF – cerebrospinal fluid; AAV – adeno-associated virus; AAV9 – adeno-associated virus serotype 9; BCI – brain-computer interface; ChABC – chondroitinase ABC; ROCK – Rho-associated protein kinase; GAD65/67 – glutamic acid decarboxylase 65/67; GAP-43 – growth-associated protein 43; MAP-2 – microtubule-associated protein 2; DTI – diffusion tensor imaging; PEG – polyethene glycol; ELISA – enzyme-linked immunosorbent assay; SPSS – Statistical Package for the Social Sciences; TBI – traumatic brain injury; CNS – central nervous system; CI – confidence interval; mTORC1 – mammalian target of rapamycin complex 1; mTORC2 – mammalian target of rapamycin complex 2; PTEN – phosphatase and tensin homolog; PI3K – phosphoinositide 3-kinase; Akt – protein kinase B; TrkB – tropomyosin receptor kinase B; BMS – Basso Mouse Scale; iPSC – induced pluripotent stem cells; snRNA-seq – single-nucleus RNA sequencing; MSC – mesenchymal stem cells; qPCR – quantitative polymerase chain reaction; WFA – Wisteria floribunda agglutinin; AMPA – α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; UEMS – Upper Extremity Motor Score; AIS – American Spinal Injury Association Impairment Scale; scAAV9 – self-complementary adeno-associated virus serotype 9.

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