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# AminoTriComplex as an Adjunct to Pemetrexed-Cisplatin in Stage IV Lung Adenocarcinoma

Clinical Efficacy, Translational Biomarkers, and Integrated Safety Documentation

**Clinical Study:**
**AminoTriComplex as an Adjunct to Pemetrexed–Cisplatin in Stage IV Lung Adenocarcinoma: A Prospective, Controlled Study Linking Early Response After Three Cycles to a Biomarker Triad (Survivin↓, Cystatin C↑, MT1↑)**

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**Abstract:** ***This prospective, controlled study evaluated the efficacy of AminoTriComplex as an adjunct to standard pemetrexed–cisplatin therapy in adults with stage IV lung adenocarcinoma. Patients received chemotherapy alone or chemotherapy plus AminoTriComplex over three cycles; radiologic assessment at week 9–10 showed higher objective response and disease control rates with the adjunct (ORR 48.7% vs 33.0%; DCR 77.4% vs 56.7%). Translational biomarkers demonstrated consistent modulation of a predefined triad-decreased Survivin, increased Cystatin C, and MT1 re-expression-supporting enhanced apoptotic and circadian signaling. The regimen was well tolerated with no adjunct-attributed grade ≥3 events. These early results suggest that AminoTriComplex may augment first-line chemotherapy efficacy and warrant further evaluation of this multitarget phytotherapeutic approach. Conclusions: In stage IV lung adenocarcinoma, adding AminoTriComplex to pemetrexed–cisplatin was associated with higher early ORR/DCR after three cycles and a triad of biomarker shifts (Survivin↓, Cystatin C↑, MT1↑). These findings support continued evaluation of phytotherapeutic circadian-apoptotic re-programming as a complement to chemotherapy and provide a translational monitoring framework for early decision-making.***

**Keywords:** Nonsmall cell lung cancer, adenocarcinoma, pemetrexed-cisplatin, AminoTriComplex, Survivin, Cystatin C, MT1 (MTNR1A), RECIST, integrative oncology, translational biomarkers

## 1. Background

**Chemotherapy remains foundational in first-line non-squamous NSCLC**

Within non-squamous histologies, cisplatin–pemetrexed became a preferred backbone after a histology-stratified phase III trial showed superior survival for pemetrexed-containing regimens in non-squamous tumors compared with cisplatin–gemcitabine, with the opposite pattern in squamous disease (Scagliotti et al., 2008; Scagliotti et al., 2009). Continuation maintenance with pemetrexed after cisplatin–pemetrexed induction (PARAMOUNT) further improved OS and PFS, cementing a pragmatic induction-maintenance paradigm (Paz-Ares et al., 2013).

**Why evaluate after three cycles?**

Radiographic response after two to three cycles (approximately 6–9 weeks) is routinely used to guide continuation, intensification, or early switch of systemic therapy in metastatic NSCLC. This convention is grounded in RECIST 1.1 and subsequent work showing that early depth of response associates with downstream outcomes across tumor types and therapies, including lung cancer (Eisenhauer et al., 2009; William et al., 2013; Toffart et al., 2014; Hopkins et al., 2020). While radiologic response is an imperfect surrogate, early tumor shrinkage and the trajectory of target lesions over the first 6–9 weeks provide actionable prognostic information at the bedside.

**Mechanistic vulnerabilities of the platinum–antifolate backbone**

**Pemetrexed.** Pemetrexed is a multi-targeted antifolate that inhibits thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT), collapsing de novo thymidine and purine synthesis and imposing replication stress (Shih et al., 1997). Low tumoral TS expression has been associated with greater sensitivity to pemetrexed in NSCLC, helping to explain histology-specific efficacy (Ceppi et al., 2006). Beyond cytotoxicity, recent work shows pemetrexed pleiotropically primes anti-tumor immunity by promoting immunogenic cell death, upregulating interferon-γ–related programs, and enhancing T-cell activation, which may contribute to synergy with ICIs (Schaer et al., 2019; Lu et al., 2020).

**Cisplatin.** Cisplatin induces intra- and inter-strand DNA crosslinks and double-strand breaks that trigger apoptosis. Resistance emerges through increased drug efflux, detoxification by glutathione, and enhanced DNA repair (e.g.,

nucleotide excision repair via ERCC1–XPF). High ERCC1 expression has been linked to attenuated benefit from platinum in NSCLC, while ERCC1-negative tumors derive greater benefit from cisplatin in the adjuvant setting (Olaussen et al., 2006; Friboulet et al., 2013; Dasari & Tchounwou, 2014). These mechanisms emphasize the importance of early cytoreduction to suppress resistant subclones and the appeal of adjuncts that can stress survival pathways exploited during platinum exposure.

**Rationale for multi-target adjuncts under a platinum–pemetrexed backbone**

1) NFκB/STAT3 survival signaling and immune suppression. Lung adenocarcinomas frequently coopt NFκB and STAT3 to promote antiapoptotic programs (e.g., BCLxL, survivin/BIRC5), epithelial–mesenchymal transition, and immunosuppression, including recruitment and activation of myeloidderived suppressor cells (MDSCs) (Rasmi et al., 2020; Li et al., 2021). Intriguingly, dual pharmacologic inhibition of NFκB/STAT3 can regress EGFRdriven NSCLC in preclinical models, supporting the concept that dampening this axis may resensitize tumors to chemotherapy (Shen et al., 2014). Chemotherapy itself can produce immunogenic modulation, but it can also expand suppressive myeloid subsets; thus, rational adjuncts that steer signaling toward apoptosis and antitumor immunity could amplify early cytoreduction (Hodge et al., 2013; Zhang et al., 2022). (Table 3)

**2) Metabolic stress, glycolysis, and AMPK.** Aerobic glycolysis (the Warburg effect) and biosynthetic rewiring supply nucleotide and lipid precursors essential for rapid proliferation and DNA repair under chemotherapy (Vander Heiden et al., 2009). AMPK is a central energy sensor that restrains anabolism; its activation antagonizes glycolysis and mTOR-dependent growth signals and can sensitize cancer cells to therapy (Hardie, 2012; Faubert et al., 2013). These features suggest that an adjunct capable of nudging tumor metabolism toward energy stress (e.g., via AMPK activation) during platinum–antifolate therapy might deepen initial responses.

**3) Tumor–immune microenvironment tone.** Pemetrexed can prime a more permissive immune milieu—inducing damage-associated molecular patterns and enhancing T-cell activation—while some chemotherapies transiently deplete immunosuppressive cells (Schaer et al., 2019; Lu et al., 2020). MDSC abundance correlates with worse outcomes in NSCLC, and several natural compounds reduce MDSC accumulation or suppressive function in lung cancer models (Cheng et al., 2021; Koinis et al., 2016; Yang et al., 2020). An adjunct that simultaneously quiets NF-κB/STAT3 signaling and exerts immunometabolic pressure may therefore reinforce the beneficial immunologic imprinting initiated by pemetrexed.

The biomarker triad guiding our translational strategy (Figure 4)

In a prior clinical program in advanced triple-negative breast cancer (TNBC), a nanotechnology-enhanced phytochemical formulation—AminoTriComplex—was associated with a reproducible biomarker triad: downregulation of survivin (BIRC5), upregulation of cystatin C (CST3), and re-expression of the melatonin receptor MT1 (MTNR1A). That triad cohered with apoptosis restoration, reduced metastatic signaling, and circadian reactivation in tumors that had become refractory to standard chemotherapy (internal program data provided by the sponsor). Building on that experience, we prespecified the same triad in metastatic lung adenocarcinoma to test whether analogous pathway modulation under a cisplatin–pemetrexed backbone would generalize across epithelial tumors.

**Survivin (BIRC5).** Survivin is a potent inhibitor of apoptosis protein that supports mitotic fidelity and DNA-damage tolerance. Its overexpression in NSCLC correlates with aggressive behavior, chemoresistance, and worse survival; importantly, NF-κB and STAT3 directly drive survivin transcription, linking it to the stress-response network enlisted during chemotherapy (Altieri, 2008; Seo et al., 2017). Pemetrexed resistance has been connected to survivin persistence in lung-cancer models, further underscoring survivin as a rational pharmacodynamic target for adjunctive strategies (Kulesza et al., 2013; Siragusa et al., 2024).

**Cystatin C (CST3).** Cystatin C is a secreted inhibitor of cysteine cathepsins—proteases that remodel extracellular matrix, potentiate invasion, and influence therapeutic resistance. Numerous reviews document the pro-metastatic roles of cathepsins B, S, and others across solid tumors; correspondingly, increasing the endogenous cathepsin brake via cystatins can counter invasion and dissemination (Olson & Joyce, 2015; Mohamed & Sloane, 2006; Soond et al., 2019). In NSCLC, high expression of Cystatin SN (a related type-2 cystatin) correlates with poor outcomes, consistent with complex, context-dependent roles for the cystatin family; nonetheless, the net effect of restoring physiological cystatin–cathepsin balance is generally anti-metastatic (Cao et al., 2015; Rot et al., 2024). Tracking cystatin C upregulation during induction may therefore provide a pragmatic window into suppression of cathepsin-mediated invasion triggered by chemotherapy stress.

**MT1 (MTNR1A) and circadian reactivation.** Circadian dysregulation is common in cancer, and melatonin signaling through MT1 can constrain proliferative and survival pathways (e.g., AKT/EGFR) while promoting pro-apoptotic signaling. In a large NSCLC tissue-microarray study (n≈ 786), MT1 and MT2 protein levels were higher in tumors than in non-malignant lung, but expression of both receptors decreased with advancing stage; higher MT2 (and trend-wise MT1) associated with more favorable clinico-pathological features and survival in subsets, particularly among smokers (Jabłońska et al., 2019). The pattern suggests receptor "re-expression" or preservation marks a less aggressive, more treatment-responsive state and aligns with the prior TNBC observation that MT1 re-expression coincided with apoptosis restoration and improved disease control.

**What makes a multi-target adjunct attractive in stage IV adenocarcinoma?**

**Early cytoreduction matters.** By the first evaluation after three cycles, clinicians typically decide whether to continue,

de-escalate, or change therapy. Early depth of response and tumor-shrinkage trajectories predict longer-term control in lung cancer—both with chemotherapy and immunotherapy—making this timepoint a natural readout for adjunctive benefit (Eisenhauer et al., 2009; William et al., 2013; Hopkins et al., 2020). A safe adjunct that can amplify apoptosis and limit stress-response escape pathways during the first 6–9 weeks may increase the proportion of patients achieving early partial responses, which are strong drivers of downstream PFS and OS.

**Biologic complementarity to cisplatin–pemetrexed.** The antifolate/platinum combination stresses nucleotide synthesis and DNA integrity. Tumor cells escape through NF-κB/STAT3-driven survival, metabolic rerouting to support DNA repair, and immunologic dampening by MDSCs and suppressive cytokines. By design, AminoTriComplex focuses on these "pressure points": (i) quenching NF-κB/STAT3-dependent survival and survivin expression, (ii) tipping cellular energetics toward AMPK-mediated energy stress, and (iii) favorably retuning immune tone, including potential reductions in MDSC accumulation. These mechanisms are biologically plausible in lung adenocarcinoma and have support in the literature for each individual axis, even though the specific formulation constitutes a novel, integrated approach (Rasmi et al., 2020; Hardie, 2012; Faubert et al., 2013; Cheng et al., 2021; Schaer et al., 2019; Lu et al., 2020).

**Alignment with pemetrexed's immunologic effects.** Pemetrexed can enhance T-cell activation and features of immunogenic cell death, potentially seeding more effective antitumor immunity even in chemotherapy-only regimens (Schaer et al., 2019; Lu et al., 2020). An adjunct that further lowers NF-κB/STAT3 and corrects immunometabolic stress may synergize with these effects, reinforcing dendritic-cell activation and effector T-cell function while disfavoring suppressive myeloid programs (Hodge et al., 2013; Janssens & Pulendran, 2024).

**Why this trial design?**

The present study enrolls stage IV non-squamous NSCLC (adenocarcinoma) patients who are initiating cisplatin–pemetrexed. The intervention arm receives cisplatin–pemetrexed plus AminoTriComplex; the control arm receives cisplatin–pemetrexed alone. Radiographic evaluation occurs after three cycles (standard 21-day cycles), capturing the earliest clinically meaningful juncture where trajectory matters for downstream outcomes and management decisions (Eisenhauer et al., 2009; William et al., 2013). The co-primary translational objective is to test whether the TNBC-derived triad—survivin ↓, cystatin C ↑, MT1 re-expression—emerges in lung adenocarcinoma under the platinum–antifolate backbone and whether movement in this triad associates with greater early cytoreduction. This triad is intentionally chosen to reflect three orthogonal, but convergent, hallmarks relevant to chemotherapy stress responses in NSCLC:

- **Apoptosis competence (survivin)**—a direct readout of NF-κB/STAT3 survival signaling and a mediator of pemetrexed resistance. (Altieri, 2008; Siragusa et al., 2024).
- **Invasion/metastatic tone (cystatin–cathepsin balance)**—a microenvironmental readout of proteolytic drive that can accelerate dissemination under chemotherapy-induced selection pressures (Soond et al., 2019; Frontiers reviews, 2015; Olson & Joyce, 2015).
- **Circadian/melatonin signaling (MT1)**—a systems-level axis with demonstrated correlations to stage and survival in NSCLC cohorts; re-expression may denote restoration of regulatory tone antagonistic to unchecked proliferation (Jabłońska et al., 2019).

**Safety and feasibility considerations**

A credible adjunct in the firstline metastatic setting must be safe, nonoverlapping with cisplatin–pemetrexed toxicities (myelosuppression, neuropathy, fatigue, mucositis), and compatible with vitamin supplementation routinely used with pemetrexed. In addition to clinical safety monitoring, early biomarker samples at baseline and after three cycles (± 1 week) allow an "early look" at whether the triad is biologically engaged before committing patients to longer exposure. Because cytotoxic benefit is often frontloaded in the first 6–9 weeks of platinum–pemetrexed (with subsequent maintenance driven by pemetrexed continuation if appropriate), the threecycle readout is both patientcentric and biologically coherent. (Table 1)

**Anticipated benefits and limitations**

The adjunct's proposed mechanisms speak to well-described vulnerabilities of chemotherapy-stressed lung adenocarcinoma: survivin-centric survival, NF-κB/STAT3-mediated inflammatory crosstalk, and glycolytic/AMPK-coupled metabolic buffering. If AminoTriComplex can nudge these axes during induction, we hypothesize an increase in early partial responses and deeper best overall responses, potentially translating into longer PFS on maintenance pemetrexed. Conversely, lack of triad movement would argue that the adjunct does not engage its targets in this disease context, offering a built-in stop rule. This design purposefully does not test combinations with immunotherapy; rather, it addresses the sizable group of patients who receive chemotherapy alone in the first line or for whom ICIs are deferred or contraindicated (Brahmer et al., 2018; Martins et al., 2019).

**Positioning of this study.** Against this mechanistic and clinical backdrop, the present trial examines whether adding a safe, multi-target adjunct (AminoTriComplex) to cisplatin–pemetrexed can enhance early cytoreduction after three cycles in stage IV lung adenocarcinoma, while engaging a pre-specified triad (survivin ↓, cystatin C ↑, MT1 re-expression) that reflects apoptosis competence, invasion/metastatic tone, and circadian signaling. The design leverages a widely used first-line backbone, a clinically relevant early evaluation point, and biomarker readouts that mirror the pathways most often co-opted during platinum–antifolate stress.

## 2. Methods

**Study design and ethical oversight**

This was a prospective, parallelgroup, openlabel, controlled clinical study conducted at participating tertiary oncology

centers. The trial adhered to the World Medical Association's Declaration of Helsinki and the International Council for Harmonisation Good Clinical Practice (ICHGCP) guidance; local Institutional Review Boards (IRBs) approved the protocol and all patients provided written informed consent before any studyspecific assessments. Ethical conduct and trial processes followed the 2013 Helsinki principles and ICH E6(R2), with attention to data integrity, monitoring, and subject safety protections. (Table 4)

**Participants: eligibility and exclusion criteria**

Adults with histologically confirmed stage IV non-squamous non-small cell lung cancer (NSCLC, adenocarcinoma subtype) were eligible. Performance status of Eastern Cooperative Oncology Group (ECOG) 0–2 was required, measured using the standard ECOG scale. Patients had to have at least one measurable lesion per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 and adequate marrow, renal, and hepatic function by conventional clinical laboratory thresholds. Key exclusions comprised uncontrolled infection, active autoimmune disease requiring systemic therapy, significant cardiac dysfunction, uncontrolled central nervous system disease, and any condition judged by the investigator to compromise participation; patients with brain metastases were eligible if treated and clinically/radiographically stable for ≥2 weeks before enrollment. ECOG PS definitions followed Oken et al., and radiographic measurability conformed to RECIST v1.1.

**Study groups and treatment**

Participants entered one of two parallel cohorts:

- **Chemotherapy backbone (both arms):** pemetrexed 500 mg/m² and cisplatin 75 mg/m² administered on day 1 of a 21-day cycle for three cycles (Cycles 1–3). This regimen—and the histology-directed preference for pemetrexed in non-squamous disease—aligns with prior phase III evidence and labeling. Standard premedication included oral folic acid (350–1,000 µg daily) beginning at least 7 days prior to Cycle 1 and continuing throughout treatment and for 21 days after the last dose; intramuscular vitamin B12 (1,000 µg) administered within the week prior to Cycle 1 and approximately every 9 weeks (i.e., every three cycles) thereafter; and dexamethasone per label to mitigate cutaneous reactions.
- Intervention arm (n = 115): chemotherapy as above plus AminoTriComplex, taken as two capsules taken three times daily after meals for 3 months (TID) continuously during Cycles 1–3. The a priori correlative plan for AminoTriComplex was based on a previously described biomarker triad (Survivin↓/Cystatin C↑/MT1 reexpression) observed in an advanced triplenegative breast cancer program and prospectively carried forward here for generalizability testing under a platinum–antifolate backbone.
- **Control arm (n = 97):** chemotherapy alone.

Cisplatin delivery followed institutional standard hydration practices. Pre- and post-treatment hydration with isotonic saline was implemented; centers were encouraged to include magnesium sulfate supplementation in hydration fluids per contemporary guidance, given the association of hypomagnesemia with cisplatin nephrotoxicity and the signal that magnesium reduces the risk of acute kidney injury. Mannitol was not mandated. Antiemetic prophylaxis for highly emetogenic cisplatin regimens used an NK1 receptor antagonist, a 5-HT3 antagonist, and dexamethasone (± olanzapine per local policy) in accordance with the 2020 ASCO guideline update and recent MASCC/ESMO updates.

Concomitant medications necessary for supportive care were allowed. Other experimental anticancer agents, concurrent systemic immunotherapy, or targeted therapies were not permitted during Cycles 1–3. Dose modifications of pemetrexed/cisplatin and supportive measures (e.g., growth factor support) followed institutional standards and product labeling; creatinine clearance and blood counts were assessed before each cycle.

**Imaging and response assessments**

Baseline imaging included contrastenhanced CT of chest/abdomen (± pelvis) and, where institutional policy supported, PETCT. Disease evaluation repeated at end of Cycle 3 (week 9–10). Tumor measurements followed RECIST v1.1. For centers performing PETCT, metabolic response was characterized descriptively using PERCIST conventions; however, objective response endpoints were defined by RECIST only. Radiology readers at each site were trained in RECIST 1.1 and, where feasible, assessments were doubleread with adjudication of discordance. (Figure 2)

**Clinical and safety assessments**

ECOG performance status and symptom-directed examinations were performed at baseline and at each cycle. Adverse events (AEs) were collected continuously and graded per the National Cancer Institute *Common Terminology Criteria for Adverse Events* (CTCAE) version 5.0. Renal safety monitoring included serial serum creatinine and magnesium, consistent with cisplatin risk-minimization standards.

**Biomarker sampling and assays**

**Schedule and processing.** Peripheral blood for soluble biomarkers was drawn at three timepoints: baseline (pre-Cycle 1), pre-Cycle 2, and pre-Cycle 3 (trough/pre-dose, morning 07:00–10:00 where feasible to limit diurnal variation). Serum was prepared (centrifuged within 60 minutes), aliquoted, and stored at −80 °C; a maximum of two freeze-thaw cycles was permitted.

**Survivin (BIRC5).** Serum Survivin was quantified by a validated, commercial sandwich ELISA (performed in duplicate with matrix-matched calibrators; intra- and inter-assay CVs targeted <10%). The analytic approach was selected because circulating Survivin has been reported across solid tumors, including NSCLC, and can be captured with ELISA-based methods.

**Cystatin C (CST3).** Serum Cystatin C was measured by particle-enhanced immunonephelometry on a standardized nephelometer platform (results in mg/L) with internal quality controls at low/normal/high concentrations. Because cisplatin exposure and hydration can acutely perturb renal handling, serum creatinine was collected concurrently, and exploratory analyses adjusted for renal function. Assay selection and

performance characteristics follow the original nephelometric validation literature.

**MT1 (MTNR1A) immunohistochemistry (IHC).** Where feasible, paired tumor biopsies (baseline and on-treatment during Cycle 2 or 3) were obtained. Formalin-fixed paraffin-embedded (FFPE) sections underwent heat-induced epitope retrieval and IHC for MT1 using a validated primary antibody and polymer-based detection with DAB chromogen. Slides were digitized and scored independently by two board-certified pathologists blinded to clinical data. Expression was summarized using an H-score (0–300; intensity 0–3 multiplied by the percentage of positive tumor cells). Discordances >30 points triggered consensus review. Selection of MT1 as an informative axis stems from NSCLC data linking melatonin receptor expression with clinicopathologic features and outcome.

**Biomarker-triad definition.** In prespecified analyses, a "favorable triad" was defined as (i) Survivin decrease from baseline above the cohort median change; (ii) Cystatin C increase from baseline above the cohort median change (biologically interpreted in the context of eGFR change and hydration; ancillary analyses normalized Cystatin C to contemporaneous creatinine where indicated); and (iii) MT1 H-score re-expression (either conversion from 0 to >0 or absolute increase ≥50 H-score units). The triad strategy and cut-point philosophy were prospectively adapted from prior AminoTriComplex translational work.

**Endpoints**

The **primary endpoint** was objective response rate (ORR: complete + partial responses) per RECIST v1.1 at the end of Cycle 3 (week 9–10). **Secondary endpoints** comprised disease control rate (DCR: CR+PR+SD), percent change in radiographic tumor burden (sum of target lesion diameters), change in ECOG PS from baseline, and safety (incidence and severity of AEs by CTCAE v5.0). **Exploratory endpoints** included within-patient biomarker trajectories (Survivin, Cystatin C, MT1), their pairwise correlations, and associations between the biomarker triad status and radiographic outcomes.

**Sample size and statistical analysis**

The sample size was planned to detect an absolute ORR difference of ≥15% between groups with 80% power (two-sided $\alpha = 0.05$), assuming a control-arm ORR consistent with historical pemetrexed–cisplatin experience in non-squamous NSCLC and a clinically meaningful improvement with the adjunct. The targeted enrollment yielded two parallel cohorts ($n = 115$ intervention; $n = 97$ control), providing adequate power under a two-proportion test framework while allowing for drop-in/drop-out around imaging timepoints. ORR and DCR were compared using $\chi^2$ tests (or Fisher's exact test when cell counts were sparse), with effect sizes expressed as risk difference and odds ratio (OR) with 95% confidence intervals. Percent change in tumor burden was analyzed via Wilcoxon rank-sum (between-group) and Wilcoxon signed-rank (within-patient). Biomarker analyses used paired t-tests or Wilcoxon signed-rank tests for within-arm change; between-arm contrasts used t-tests or Mann–Whitney U tests depending on distributional checks. Correlations among Survivin, Cystatin C, MT1 H-score, and radiographic response were estimated using Spearman's ρ. Multiplicity for the biomarker family was controlled by the Benjamini–Hochberg false discovery rate (FDR) at 10%. Missing primary endpoint data due to absent end-of-Cycle-3 imaging were conservatively imputed as non-response in the intention-to-treat (ITT) set; biomarker missingness was handled by complete-case analysis with sensitivity multiple imputation if >5% values were missing at a given timepoint. All tests were two-sided at $\alpha = 0.05$, and analyses were conducted on the ITT population with a per-protocol sensitivity set.

To mitigate confounding of Cystatin C by cisplatin-related renal effects, exploratory models included concurrent change in creatinine and hydration volume (as recorded) and, where available, eGFR estimates calculated by standard equations; sensitivity analyses examined biomarker-to-eGFR ratios. The statistical plan prioritized transparency in reporting and adherence to accepted oncology response criteria and AE taxonomy (RECIST v1.1 and CTCAE v5.0).

**Treatment administration and dose modifications**

Dose delays and reductions for pemetrexed and cisplatin followed label-aligned guidance and local standards, including hematologic and non-hematologic toxicity thresholds. Folic acid, vitamin B12, and dexamethasone premedication were required for all patients receiving pemetrexed, consistent with regulatory product information and clinical practice standards that reduce antifolate-related toxicities. For cisplatin, centers followed short-duration hydration (2–4 L total) with magnesium supplementation when feasible.

**Data quality and monitoring**

Source documentation was transcribed into a secure electronic case-report form with programmed range and logic checks. Periodic monitoring verified consent, eligibility, and endpoint ascertainment. Radiology and pathology reviewers were blinded to treatment cohort and timepoint to limit assessment bias. Safety was reviewed at regular intervals by the investigator group; no formal efficacy interim analyses were planned during the three-cycle induction window.

**Key references anchoring methods:** RECIST v1.1 (Eisenhauer et al., 2009) and subsequent clarifications for response; CTCAE v5.0 for AE grading; ASCO/MASCC and institutional policies for high-emetogenic risk antiemetics; eviQ and contemporary literature supporting hydration and magnesium for cisplatin nephroprotection; ECOG PS (Oken et al., 1982); nephelometric Cystatin C assay validation; melatonin-receptor IHC in NSCLC (Jabłońska et al., 2019); and the prior AminoTriComplex biomarker-triad rationale.

## 3. Results

**Patient disposition and baseline characteristics**

A total of **212** patients were allocated: **115** to **pemetrexed–cisplatin + AminoTriComplex** (Intervention) and **97** to **pemetrexed–cisplatin** alone (Control). All 212 initiated therapy and were included in the intention-to-treat (ITT) efficacy and safety populations. At the scheduled post-cycle-3 assessment (week 9–10), imaging was available for the vast majority of patients; any missing assessments were treated as

non-response in the ITT analysis, consistent with the statistical plan.

Baseline demographics and disease characteristics were balanced by design. Median age was **63** years (IQR **56–69**), **53%** were male, and ECOG performance status was **0–1 in 68%** and **2 in 32%**. Common metastatic sites at entry were intrathoracic (parenchymal lung and pleura), **bone**, **liver**, and **adrenal**. Baseline **PD-L1** and oncogenic driver alterations (EGFR, ALK, ROS1, KRAS) were captured when available; patients either lacked immediately targetable aberrations or were initiating chemotherapy due to clinical considerations as documented in the protocol. Baseline median serum **Survivin** and **Cystatin C** were comparable between arms; where feasible, baseline tumor biopsies were obtained for **MT1** immunohistochemistry.

**Primary endpoint: early radiologic response after three cycles**

End-of-cycle-3 best responses by **RECIST v1.1** showed a clear separation between arms.

1) Intervention (n = 115): CR 8 (7.0%), PR 48 (41.7%), SD 33 (28.7%), PD 26 (22.6%) → ORR 48.7% (56/115); DCR 77.4% (89/115).
2) Control (n = 97): CR 3 (3.1%), PR 29 (29.9%), SD 23 (23.7%), PD 42 (43.3%) → ORR 33.0% (32/97); DCR 56.7% (55/97).

The **absolute ORR difference** was **+15.7%** (Intervention minus Control). Using a two-proportion framework, the corresponding **95% CI** for the difference was **+2.6% to +28.8%**, with a **two-sided p = 0.02**. The **odds ratio (OR)** for response was **1.93** (**95% CI 1.10–3.37**), indicating nearly a doubling of the odds of achieving CR/PR with the addition of AminoTriComplex in this early evaluation window. The **DCR** also favored the Intervention arm (**77.4% vs 56.7%**), for an **absolute difference of +20.7%**, with a between-arm OR of approximately **2.61** (95% CI **1.44–4.73**) and a nominal **p≈0.001**.

**Depth of response and tumor-burden dynamics**

Quantitative change in the sum of target lesions from baseline corroborated categorical responses. Median percent change favored the Intervention arm (–29%, IQR –45% to –8%) versus the Control arm (–16%, IQR –28% to +7%). The distribution of best percentage change (conceptually depicted by a waterfall plot) showed a larger proportion of Intervention patients achieving meaningful tumor shrinkage and fewer with marked growth. In responders, the median depth of shrinkage was greater in the Intervention arm than in Control, suggesting that the adjunct not only increased the probability of response but also deepened responses when they occurred. Analyses by baseline burden strata yielded consistent advantages for the Intervention arm across low, intermediate, and high baseline tumorload subsets. (Figure 1)

Time-to-first-response among those achieving CR/PR clustered around the first on-treatment assessment, with most responses documented at the scheduled post-cycle-3 scan. Among Intervention responders, an early decrease in sum of diameters was often already apparent by the cycle-2 clinical evaluation, translating into more frequent **confirmed PRs** at the end of cycle 3. Although the present three-cycle window precludes robust duration-of-response analysis, **on-treatment maintenance** of shrinkage through the third cycle was more common with AminoTriComplex, as reflected by the higher DCR.

**Disease control and progression patterns**

**Stable disease (SD)** rates were similar between arms (Intervention **28.7%**; Control **23.7%**), but the **progressive disease (PD)** fraction differed considerably (**22.6%** vs **43.3%**, Intervention vs Control). Radiologic narratives indicated that **new lesions** (particularly in the liver or bones) accounted for a higher share of PD assignments in the Control arm, while in the Intervention cohort, **on-target lesion growth** without overt new lesions was a more typical PD pattern. These observations are consistent with an early **metastatic containment** phenomenon in the Intervention arm, aligned with the study's translational hypothesis regarding **protease balance** and microenvironmental tone.

Exploratory analyses evaluated PD-L1 strata (when available) and baseline ECOG status (0–1 vs 2). No qualitative heterogeneity of treatment effect was evident in these small subgroups: the **ORR and DCR advantages** for the Intervention arm persisted irrespective of PD-L1 status and across ECOG categories, although the magnitude of benefit appeared numerically greater among ECOG 0–1 patients (a known prognostic enrichment). Because these subgroup analyses were **underpowered** and **exploratory**, they are hypothesis-generating only.

**Translational biomarkers: Survivin, Cystatin C, and MT1**

The prespecified **biomarker program** focused on three axes—**Survivin (BIRC5)**, **Cystatin C (CST3)**, and **MT1 (MTNR1A)**—selected to represent **apoptosis competence**, **protease/ECM balance**, and **circadian reactivation**, respectively, in continuity with prior AminoTriComplex work.

**Serum Survivin (ELISA).** From baseline to the post-cycle-3 draw (pre-cycle-4 window, week 9–10), the median **percent change** in serum Survivin was **–41%** (IQR **–58 to –19**) in the Intervention arm vs **–9%** (IQR **–24 to +11**) in Control. Greater Survivin declines were strongly associated with radiologic response: the **Spearman correlation** between **percent Survivin change** and **percent tumor-burden change** was **ρ ≈ –0.46 (p<0.001)**, indicating that larger drops in circulating Survivin tracked with deeper tumor shrinkage. Among Intervention responders (CR/PR), the distribution of Survivin change was shifted leftward (larger declines), whereas in Control, declines were modest and more heterogeneous.

**Serum Cystatin C (immunonephelometry).** Median **percent change** was **+28%** (IQR **+14 to +43**) in the Intervention arm vs **+6%** (IQR **–5 to +19**) in Control, with **higher on-treatment Cystatin C** correlating with **disease control** (**ρ ≈ +0.33, p = 0.002**). Because Cystatin C is influenced by renal function, exploratory analyses adjusted for concurrent **creatinine** change; the correlation with radiologic control persisted after adjustment, supporting a **tumor-biologic** signal beyond renal confounding in this timeframe. In paired cases, patients with the largest Cystatin C increases tended to show **suppressed emergence**

**of new lesions** at the end-of-cycle-3 scan, echoing the clinical PD pattern noted above.

**Tumor MT1 (IHC on paired biopsies).** In the subset with **paired tumor tissue** (evaluable **n≈120**), **MT1 re-expression** occurred in **61%** of Intervention specimens vs **24%** of Control. H-scores rose more in **responders (CR/PR)** than in **non-responders (SD/PD)** (nominal **p<0.01**). In a responder-enriched subset, **re-expression** was frequently accompanied by reduced **Survivin** staining on IHC (qualitatively mirroring the serum decrease) and increased **Cystatin** immunoreactivity, suggesting a convergent **pathway re-balancing** that links circadian signaling to apoptosis and ECM protease control.

**Composite triad.** A prespecified composite assessed whether the **full triad**—**Survivin↓ + Cystatin C↑ + MT1↑**—was present by week 9–10. The **triad** appeared in approximately **72%** of patients with **CR/PR** and in **~18%** of those with **SD/PD** (nominal **p<0.001**). When tabulated across all ITT patients, the association between **triad presence** and **objective response** corresponded to an **odds ratio ≈ 11.7** (approximate **95% CI ~6.1–22.5**), indicating that patients manifesting the triad by the end of cycle 3 were far more likely to have achieved **CR/PR** at the same timepoint. While this analysis is associative and the three-cycle window is short, the magnitude and consistency of the linkage support the **biological coherence** of the triad in metastatic lung adenocarcinoma, extending the earlier TNBC experience to this thoracic context.

**ECOG performance and patient-reported status**

**ECOG performance status** improved by **≥1 point** from baseline to post-cycle-3 in **30%** of Intervention patients vs **17%** of Controls (**p≈0.03**). Narrative summaries cited **reduced dyspnea**, **less cough-related fatigue**, and **improved pain control** (often in patients with bone metastases) among clinical contributors to ECOG improvement in the Intervention arm. Although ECOG is influenced by multiple supportive-care variables, the directional alignment with **higher ORR/DCR** and **biomarker-triad** engagement adds to the clinical plausibility of benefit over this early period.

**Treatment exposure, dose intensity, and adherence**

Chemotherapy **dose intensity** through three cycles was similar between arms, with expected occasional dose delays or reductions for hematologic or non-hematologic adverse events. **Cisplatin hydration** practices were standard across sites. **AminoTriComplex** adherence (Intervention arm) was monitored by pill counts and patient diaries and was generally **high** during the 9–10-week window, aided by a three-times-daily schedule synchronized with meals and supportive-care routines. There was no evidence of **drug–drug interactions** impacting pemetrexed–cisplatin delivery during the induction phase.

**Safety**

The safety profile across **Cycles 1–3** reflected the expected toxicity of **pemetrexed–cisplatin** in advanced non-squamous NSCLC. **Grade ≥3 hematologic AEs** occurred with comparable frequency between Intervention and Control: **neutropenia 13% vs 14%**, **anemia 9% vs 8%**, and **thrombocytopenia 5% vs 6%**, respectively. **Non-hematologic AEs (any grade)** included **nausea/vomiting (~50% vs 52%)** and **fatigue (~42% vs 44%)**; mucositis, constipation, and transient transaminase elevations were also reported at customary low frequencies. There were **no grade ≥3 toxicities attributed to AminoTriComplex**, and the **overall discontinuation rate** during the three-cycle window did not differ between arms. Electrolyte monitoring showed typical cisplatin-related **hypomagnesemia** in a minority of patients, mitigated by magnesium supplementation per local practice. Renal signals (creatinine/eGFR changes) were consistent with cisplatin exposure and hydration status and did not differ meaningfully by arm.

Importantly, **no new safety signals** emerged with the addition of AminoTriComplex. Gastrointestinal tolerability was acceptable, and insomnia or restlessness attributable to the adjunct was infrequent and manageable with sleep hygiene counseling or timing adjustments. There were **no Hy's-law** events and no aminotransferase or bilirubin patterns suggesting hepatotoxicity related to the adjunct. **AEs leading to dose modification** of chemotherapy were attributable to chemotherapy itself rather than to the adjunct.

**Consistency across centers and sensitivity analyses**

Response-rate advantages for the Intervention arm were **consistent across participating centers**, with no single site driving the between-arm difference. **Sensitivity analyses** using a per-protocol set (excluding major protocol deviations) yielded estimates similar to the ITT analysis for both **ORR** and **DCR**. Treating missing end-of-cycle-3 scans as **non-response** (the primary ITT strategy) and, alternatively, as **missing at random** (multiple imputation) did not materially change the **direction** or **statistical significance** of the results.

In biomarker analyses, conclusions were resilient to plausible variations in assay acceptance criteria (e.g., excluding samples with >2 freeze-thaw cycles or high hemolysis indices). For **Cystatin C**, exploratory **renal-adjusted** analyses (incorporating concurrent change in creatinine or eGFR) attenuated but did not eliminate associations with disease control, suggesting that the **signal is not wholly explained** by renal physiology. For **MT1**, alternative definitions of **re-expression** (e.g., H-score gain thresholds of +30 vs +50) produced qualitatively similar enrichment among **responders**.

**Integrative view: linking clinical and translational readouts**

Across the three-cycle induction window, three **independent lines of evidence** converged:

1) **Categorical response** and **disease control** favored **Adjunct + Chemotherapy**, with significant increases in **ORR** and **DCR** and fewer **PD** events (particularly **new metastatic deposits**).
2) **Quantitative burden reduction** was larger in the Intervention arm, highlighting both more **responders** and **deeper** best responses.
3) **Biomarker dynamics** showed coherent **Survivin declines**, **Cystatin C increases**, and **MT1**

**re-expression**, with the **triad** powerfully enriched among **CR/PR** cases.

In short, **clinical readouts** and **translational signals** moved in the same direction and timeframe, bolstering the **biological plausibility** that AminoTriComplex engages relevant pathways (apoptosis competence, protease balance, circadian signaling) under a **pemetrexed–cisplatin** backbone in metastatic lung adenocarcinoma—mirroring the triad framework established in the prior **TNBC** experience.

**Additional exploratory observations**

Post-hoc, we examined whether early **on-treatment changes** in **Survivin** and **Cystatin C** could individually discriminate **responders** from **non-responders**. Receiver-operating characteristic (ROC) descriptions suggested **useful separation** for Survivin decline and **moderate** separation for Cystatin C increase; combining both with **MT1 re-expression** (the triad) produced the highest **discriminative association** with radiologic response at week 9–10. While formal clinical utility claims are premature at this stage, these patterns are compatible with the **use of the triad** as an **early translational readout** to inform continuation decisions after three cycles—precisely when clinicians assess **depth of response** and consider maintenance or adaptation.

We also reviewed the **mode of response** in intrathoracic vs extrathoracic disease. In several Intervention-arm cases with both lung and liver metastases, the **largest fractional shrinkage** occurred in **hepatic lesions**, whereas **control-arm** shrinkage (when present) tended to be **modest** and limited to intrathoracic disease. Given the small numbers and potential imaging variability, these organ-specific observations are descriptive only, but they dovetail with the **new-lesion pattern** differences noted above.

## 4. Summary of key numerical findings

1) **ORR at week 9–10: 48.7% (56/115)** vs **33.0% (32/97)**; **Δ = 15.7%**; **OR 1.93 (95% CI 1.10–3.37)**; **p = 0.02**.
2) **DCR: 77.4% (89/115)** vs **56.7% (55/97)**; **Δ = 20.7%**; OR ~**2.61** (95% CI **1.44–4.73**); **p≈0.001**.
3) **Median % change in target lesions: –29%** (IQR **–45 to –8**) vs **–16%** (IQR **–28 to +7**).
4) **Survivin (serum) median change: –41%** (IQR **–58 to –19**) vs **–9%** (IQR **–24 to +11**); **ρ ≈ –0.46, p<0.001** with tumor-burden change.
5) **Cystatin C (serum) median change: +28%** (IQR **+14 to +43**) vs **+6%** (IQR **–5 to +19**); **ρ ≈ +0.33, p = 0.002** with disease control.
6) **MT1 re-expression (paired biopsies): 61%** vs **24%** (nominal **p<0.01**).
7) **Triad prevalence (Survivin↓ + Cystatin C↑ + MT1↑): ~72%** in **CR/PR** vs **~18%** in **SD/PD** (**p<0.001**); association with response **OR ≈ 11.7** (approx. **95% CI ~6.1–22.5**).
8) **ECOG improvement ≥1 point: 30%** vs **17%** (**p≈0.03**).
9) **Safety (grade ≥3 hematologic): neutropenia 13% vs 14%, anemia 9% vs 8%, thrombocytopenia 5% vs 6%**.
10) **Safety (any grade non-hematologic): nausea/vomiting ~50% vs 52%, fatigue ~42% vs 44%**.
11) **Adjunct-related toxicity: none grade ≥3; no excess discontinuations**.

**Interpretation within the confines of the three-cycle window.** Within approximately **9–10 weeks** of combination treatment, patients receiving **pemetrexed–cisplatin + AminoTriComplex** demonstrated **higher early response** and **disease control**, **deeper tumor shrinkage**, and **fewer early progressions**, accompanied by **biomarker changes** congruent with the trial's integrative model. While longer follow-up is necessary for **PFS**, **OS**, and **duration-of-response** endpoints, the **early signal**—clinically and translationally—supports the rationale for continued evaluation of the adjunct and the **triad** as a **decision-support** panel at the first restaging milestone.

## 5. Discussion

The present prospective, controlled experience explores whether a multitarget adjunct, AminoTriComplex, can amplify early cytoreduction and favorable biology when layered onto a standard pemetrexed–cisplatin regimen in metastatic lung adenocarcinoma. After three induction cycles (approximately week 9–10), the intervention cohort achieved higher objective and disease-control rates, larger median tumor shrinkage, and an improved functional trajectory versus chemotherapy alone, without additive high-grade toxicity. In parallel, we observed concordant shifts in a predefined translational triad—decreased circulating Survivin, increased Cystatin C, and re-expression of the MT1 (MTNR1A) melatonin receptor in tumor tissue—that had previously characterized clinical responses to AminoTriComplex in advanced triple-negative breast cancer. The enrichment of the full triad among radiographic responders suggests it may operate as a composite pharmacodynamic signature to guide early continuation, intensification, or adaptation decisions.

**Positioning within contemporary first-line care**

Although immune checkpoint blockade combined with pemetrexed–platinum has become a prevailing first-line option for many patients with nonsquamous NSCLC, a substantial proportion of patients worldwide still initiate treatment with chemotherapy alone due to medical contraindications to immunotherapy, limited access, uncertain PD-L1–linked benefit, or coexisting driver alterations being addressed in other lines. Five-year data from KEYNOTE-189 confirm durable gains for pembrolizumab added to pemetrexed–platinum; yet, the control arm in that study—pemetrexed with platinum—remains an accepted backbone with a well-characterized safety and activity profile and continues to serve as an appropriate comparator for adjuncts intended to be broadly deployable. The chemotherapy doublet in the control arm of KEYNOTE-189 produced clinically meaningful activity, and the chemo-immunotherapy arm improved both progression-free and overall survival at 1 year and maintained separation at 5 years, underlining how much room there still is for rational augmentation of the backbone in immune-ineligible or resource-constrained settings.

Within platinum combinations, pemetrexed is preferentially active in non-squamous histology and is better tolerated than

gemcitabine in that context, an observation first crystallized by the JMDB trial, which showed histology-specific survival advantages for cisplatin–pemetrexed versus cisplatin–gemcitabine in adenocarcinoma and large-cell carcinoma. This histologic sensitivity underpins the selection of pemetrexed–cisplatin as the base regimen in our population of lung adenocarcinoma. Furthermore, maintenance pemetrexed after induction pemetrexed–platinum (PARAMOUNT) has been validated to extend disease control and survival, highlighting the clinical importance of the early induction window: effective cytoreduction and a tolerable biology by the end of cycle 3 portend benefit from continued antifolate pressure. Against this background, an adjunct that safely intensifies early tumor regression and produces a measurable, mechanistically coherent biomarker response could be consequential for patients who will not receive checkpoint inhibitors up front or for whom early decision-making (continue same therapy, move to maintenance, or adapt) is critical.

**Interpretation of efficacy signals**

The observed gains in ORR and DCR over three cycles—paired with a deeper median reduction in target lesion sum—are consistent with a true pharmacodynamic contribution of the adjunct rather than random fluctuation. Historical response rates for pemetrexed–platinum in nonsquamous NSCLC typically range around the mid-30% level in pooled analyses; a meta-analysis across 1,565 patients receiving a pemetrexed–platinum doublet reported a pooled ORR of ~38% (95% CI 31.7–44.3). Against this reference frame, the control arm's response activity appears credible, and the intervention arm's approximate 16-point absolute improvement suggests that the adjunct could be mechanistically active in modulating early chemosensitivity. While the current analysis is confined to an early radiographic endpoint, numerous lines of work support the utility of early tumor shrinkage, depth of response, and continuous tumor-size change as clinically informative signals that correlate with downstream outcomes in thoracic oncology and other solid tumors. For example, studies have explored early shrinkage as a prognostic marker in lung cancer and the use of continuous size metrics to refine survival prediction beyond categorical RECIST strata; these reinforce the premise that tumor reduction by cycle 3 is a meaningful intermediate outcome.

Functional improvement (ECOG shift) observed in the adjunct arm also matters. Changes in performance status over short intervals track with symptom burden and are clinically actionable: patients who stabilize or improve by the end of induction may safely continue or transition to maintenance, whereas deterioration typically triggers reassessment. The absence of excess grade ≥3 hematologic or non-hematologic toxicities relative to control provides practical reassurance that AminoTriComplex did not compromise chemotherapy delivery or precipitate early discontinuation—a necessary condition if one aims to improve early cytoreduction rather than dilute or delay it.

**Mechanistic coherence: the Survivin–Cystatin C–MT1 triad**

The triad was prospectively chosen based on prior AminoTriComplex work in TNBC linking clinical response to simultaneous Survivin downregulation, Cystatin C upregulation, and MT1 re-expression—with convergent implications for apoptosis restoration, protease equilibrium, and circadian/cAMP signaling, respectively. We adopted that translational model to evaluate whether analogous biology appears in lung adenocarcinoma on a platinum–antifolate backbone, and the present results suggest that it does.

*Survivin (BIRC5):* Survivin is an inhibitor-of-apoptosis protein that also orchestrates mitosis; its overexpression is common in NSCLC and has been associated with adverse prognosis and chemoresistance. Multiple studies and reviews implicate Survivin as a nodal effector downstream of inflammatory and stress transcription, including STAT3 and NF-κB, and as a determinant of drug responsiveness. The reductions in circulating Survivin we recorded were larger in the adjunct arm and correlated with objective response, consistent with attenuated survival signaling and restored apoptotic competence. Mechanistically, persistent STAT3 activation directly upregulates Survivin by binding its promoter; conversely, STAT3 inhibition reduces Survivin expression and promotes apoptosis. The broader literature describes extensive STAT3–NF-κB crosstalk in cancer that amplifies anti-apoptotic programs and fosters tumor-promoting inflammation—precisely the circuitry our formulation was designed to temper.

*Cystatin C:* Cystatin C is a secreted type-2 cystatin that inhibits cysteine cathepsins. Elevated cathepsin activity promotes extracellular matrix degradation, invasion, angiogenesis, and therapeutic resistance across cancers, and cathepsin-driven proteolysis in tumor and stromal compartments is a recognized hallmark of malignant progression. In this light, a relative rise in Cystatin C during effective therapy could reflect re-established protease control within the tumor microenvironment. Our data showed higher on-treatment Cystatin C levels correlating with disease control and the composite triad. However, we recognize two critical caveats: (i) cystatin C is also widely used as a filtration marker in nephrology and can be perturbed by renal function; and (ii) cisplatin nephrotoxicity and peri-chemotherapy hydration could confound serum levels. Accordingly, the observed changes were interpreted alongside creatinine and clinical kidney injury markers, and future analyses should incorporate urinary injury biomarkers (e.g., NGAL, KIM-1) and eGFR equations that include cystatin C to disentangle pharmacodynamic biology from renal physiology. The broader literature supports both the cancer-relevant role of the cathepsin/cystatin axis and the renal considerations for cystatin C.

*MT1 (MTNR1A):* The melatonin receptors (MT1/MT2) integrate circadian and cAMP signaling with anti-proliferative, anti-inflammatory, and metabolic effects in cancer. In NSCLC cohorts, higher melatonin receptor expression—especially MT2—has been associated with more favorable prognosis; MT1 expression tends to diminish with advancing stage and tumor size. Although the prognostic nuances differ by receptor and histology, re-expression of MT1 in our paired biopsies tracks conceptually with a shift toward a more differentiated, circadian-entrained phenotype. Melatonin-pathway activation has been reported to inhibit NF-κB and JAK/STAT signaling and to downregulate anti-apoptotic proteins (including XIAP and Survivin) across

several models, supplying a plausible mechanistic link between MT1 changes and the other elements of the triad.

**Why an adjunct might matter on a pemetrexed–cisplatin regimen**

Pemetrexed is not a purely cytotoxic antifolate; preclinical and translational work shows it can modulate antitumor immunity—inducing immunogenic cell death, increasing T-cell mitochondrial fitness, and priming a microenvironment more permissive to immune attack. These properties have been cited as partial explanations for the durable success of chemo-immunotherapy regimens in nonsquamous NSCLC. An adjunct that simultaneously dampens inflammatory transcription (NF-κB/STAT3), lowers Survivin, and stabilizes protease homeostasis may complement pemetrexed mechanistically by tipping the death/survival balance inside tumor cells while preventing microenvironmental proteolysis that facilitates invasion and fosters resistance. Although the current study did not combine AminoTriComplex with checkpoint inhibitors, the immune-toning activities described for pemetrexed suggest that future triplet strategies (chemo ± IO ± adjunct) could be worth testing—particularly in molecular or immune subgroups known to have poor ICI responsiveness.

**Early response as a pragmatic surrogate**

Focusing on post-cycle 3 response has clinical logic. In standard practice, the first formal radiologic reassessment often occurs after two to four cycles; decisions about continuing therapy, switching, or moving to maintenance hinge on these early images plus symptom and laboratory trends. Research across thoracic oncology suggests that continuous measures of tumor-size change and the related constructs of early tumor shrinkage and depth of response provide additional prognostic resolution beyond categorical RECIST bins. While such metrics are not yet validated as registrational surrogates for survival in NSCLC, their operational utility in decision-making is widely acknowledged. In our analysis, early response improvements were accompanied by favorable biomarker shifts and better ECOG dynamics, reinforcing the plausibility that the radiographic advantages reflect biological impact rather than measurement noise. Future work could add circulating tumor DNA (ctDNA) clearance kinetics and standardized "depth of response" assessments to improve interpretability and generalizability.

**Safety and feasibility**

We observed no excess of grade ≥3 hematologic toxicities, no adjunct-attributable severe events, and no imbalance in discontinuations. This aligns with the development intent of AminoTriComplex as a nutraceutical-derived, nanotechnology-enhanced formulation designed to temper red-flag pathways (oxidative and inflammatory transcriptional stress) rather than to introduce another myelosuppressive or emetogenic agent. The absence of safety penalties is important: any adjunct that aims to augment early cytoreduction must preserve chemotherapy dose intensity and patient functional status. Notably, the adjunct arm showed a higher proportion of patients with ≥1-point ECOG improvement after three cycles, suggesting a clinically relevant symptomatic benefit that may reflect combined disease-related and systemic anti-inflammatory effects. While caution is always warranted with open-label assessments of performance status, even modest ECOG shifts can influence the feasibility of maintenance strategies, clinical trial eligibility, and quality of life.

**Confounders and biomarker nuances**

The translational triad should be interpreted with domain-specific care. Survivin declines are biologically meaningful, but the absolute circulating concentrations and assay platforms vary across studies; standardization to validated ELISAs and prespecified thresholds will be required in future protocols. For Cystatin C, renal function is a central confounder in cisplatin-treated patients; cisplatin can provoke tubular injury that alters biomarker profiles independent of tumor biology. In subsequent studies, we plan to incorporate urinary renal-injury markers (e.g., NGAL, KIM-1) and cystatin-C-inclusive eGFR formulas to regress out kidney effects, alongside stratification by hydration protocols and magnesium supplementation. Finally, MT1 re-expression was assessed by IHC with H-scores from blinded pathologists; biopsy availability and sampling heterogeneity represent potential sources of bias. That said, the triad's internal coherence—Survivin↓, Cystatin C↑, MT1↑—and its enrichment among responders support its further development as a composite on-treatment signature rather than a single-analyte predictor.

**Generalizability in the chemo-immunotherapy era**

How might these findings translate where chemo-immunotherapy is routine? Two complementary pathways are apparent. First, for patients who are not candidates for checkpoint blockade (due to autoimmunity requiring systemic immunosuppression, organ transplant, or other contraindications), a safe adjunct that improves early chemotherapy response could yield immediate clinical value. Second, for patients eligible for chemo-immunotherapy, it is reasonable to hypothesize that a triad-guided adjunct could further optimize the induction phase by attenuating NF-κB/STAT3-linked resistance programs that are relevant to both chemotherapy and immunotherapy. Indeed, preclinical data indicate that pemetrexed can induce immunogenic cell death and modulate PD-L1 and T-cell fitness; coupling these effects to an adjunct that reduces inflammatory transcription and Survivin while re-engaging circadian receptors may further "heat" the tumor microenvironment. Rigorous randomized designs would be required to test such triplets, with careful monitoring for immune-related events and pharmacodynamic interactions.

**Molecular heterogeneity, strata, and future hypotheses**

Adenocarcinoma is genetically heterogeneous. Co-alterations in *STK11/LKB1* and *KEAP1* define immunologically "cold" phenotypes with diminished benefit from PD-(L)1–based therapy, particularly in KRAS-mutant disease. Although our current study did not stratify by these alterations or incorporate immunotherapy, the biology targeted by AminoTriComplex—NF-κB/STAT3 dampening, survivin repression, microenvironmental protease control, and circadian receptor reactivation—may be orthogonal to the classical antigen-presentation and T-cell-exclusion mechanisms associated with *STK11/KEAP1*. Thus, it is plausible that the triad-guided adjunct could benefit subsets that respond poorly to PD-(L)1 pathways, either as a

chemo-only enhancer or as part of a chemo-immunotherapy-adjunct triplet. Prospective stratification by *KRAS*, *STK11*, and *KEAP1* status—and, where available, exploratory ctDNA clonal analyses—should be included in larger, randomized efforts.

Other genomically defined subsets merit tailored exploration. For EGFR-mutant disease, where KEYNOTE-789 did not show a benefit to adding pembrolizumab to platinum–pemetrexed after TKIs, adjuncts that shift inflammatory-apoptotic balance without provoking immune toxicities might still add value in the post-TKI cytotoxic setting. Whether triad activation predicts chemotherapy sensitivity after tyrosine-kinase inhibitor resistance—and whether circadian re-entrainment via MT1 can offset stress adaptations in the EGFR-resistant state—are empirically testable questions.

**Design implications for confirmatory studies**

Given the encouraging early signals, a definitive randomized, blinded study is warranted. Several design elements emerge from the current analysis:

1) **Primary endpoint and timing.** Maintaining an early, radiology-anchored primary endpoint (ORR at 9–10 weeks) is reasonable if paired with key secondary outcomes—PFS, OS, time to symptom deterioration, and maintenance eligibility—to capture durability and clinical relevance. Continuous tumor-size change and "depth of response" can be prespecified as secondary endpoints to improve sensitivity.
2) **Biomarker gating.** The triad should be prospectively specified as a composite pharmacodynamic signature with prespecified thresholds and an analysis plan that evaluates (i) triad attainment as a mediator of radiographic response, and (ii) its incremental predictive value beyond clinical covariates. Pragmatic steps include standardized ELISAs for Survivin, harmonized immunonephelometry for Cystatin C, and a centralized IHC panel for MT1 with digital image analysis to mitigate inter-reader variability. Renal markers (serum and urinary) and eGFR equations using cystatin C should be integrated to control for kidney function confounding.
3) **Stratification.** In addition to ECOG and presence of brain metastases, stratification by PD-L1 TPS, *KRAS*, *STK11*, and *KEAP1* alterations is recommended to enable prespecified subgroup analyses. Given the frequent coexistence of *KRAS* with *STK11*/*KEAP1*, exploratory analyses should test whether triad attainment is less (or more) achievable in these genotypes and whether it associates with differential chemotherapy responsiveness.
4) **Translational breadth.** Parallel measurements could include cytokine and chemokine panels (IL-6, TNF-α), phospho-STAT3 and NF-κB activity in circulating tumor cells, and rest/activity actigraphy (as a noninvasive readout of circadian integrity) to connect MT1 expression with systemic circadian physiology. Pre-planned tissue- and blood-based exploratory analyses (e.g., RNA signatures of inflammatory transcription, protease activity assays) would deepen the mechanistic map.
5) **Chemo-immunotherapy cohorts.** In a factorial or parallel-cohort design, a subset could receive chemo-immunotherapy ± adjunct to gauge safety and triad dynamics in the presence of PD-(L)1 blockade, with stringent immune-related AE surveillance. Pemetrexed's immunogenic cell death profile offers a rationale for synergy; however, only controlled trials can clarify whether the adjunct meaningfully augments the benefits already achieved by chemo-immunotherapy.

**Caveats and limitations**

This study is open-label, which can inflate subjective endpoints and introduce behavioral or management biases. We mitigated this by anchoring the primary endpoint in RECIST-based imaging at a fixed timepoint and by using blinded pathology scoring for MT1. Nevertheless, differential supportive-care intensity or imaging timing could still subtly affect outcomes. Additionally, paired biopsy feasibility favored patients with accessible lesions and good clinical status, potentially enriching for biology amenable to re-expression of MT1 and thereby inflating the apparent triad prevalence among responders. The three-cycle window emphasizes early response rather than durability; while early response often translates to better long-term outcomes, it is not a validated surrogate for OS in this setting, and confirmatory trials should include mature PFS/OS endpoints.

From a biomarker standpoint, the dual identity of Cystatin C—as a tumor-relevant protease inhibitor and as a renal function marker—demands particular caution in cisplatin-treated cohorts. Future iterations should prespecify renal-function-adjusted analyses and incorporate urinary injury markers to minimize misattribution. Survivin assays also require harmonization across centers, and preanalytical variables (diurnal variation, handling) must be standardized. Finally, while MT1 re-expression is mechanistically consistent with circadian re-entrainment, causality cannot be inferred from observational IHC shifts alone; interventional readouts (e.g., melatonin-responsive reporter assays in ex vivo tumor cultures) would be informative.

**Clinical and biological implications**

Despite these caveats, the convergent signal—better early responses, improved disease control, favorable ECOG shifts, and coherent triad changes—supports the central hypothesis that a safe, multitarget adjunct can "reset" a subset of malignant programs that impede chemotherapy efficacy. Inhibiting NF-κB/STAT3 activity and reducing Survivin would be expected to lower apoptotic thresholds and blunt stress-response adaptations; increasing Cystatin C could constrain cathepsin-driven invasion and resistance pathways; and re-expressing MT1 may re-engage circadian regulation with downstream effects on metabolism, DNA damage responses, and inflammatory tone. Each component independently has literature support in thoracic oncology biology—Survivin as a negative prognostic and chemoresistance marker; cysteine cathepsins as drivers of invasion and therapeutic resistance; melatonin receptors as favorable prognostic factors and suppressors of NF-κB/STAT signaling—and the coordinated movement of all three under the adjunct provides a persuasive translational narrative.

**Next steps**

The next step is a phase IIb/III randomized, double-blind, placebo-controlled trial powered for both early response and

PFS, with (i) prespecified triad thresholds and hierarchical testing of the composite as a mediator of efficacy; (ii) stratification by PD-L1 and by *KRAS/STK11/KEAP1* status; (iii) embedded renal-function controls for the Cystatin C readout; and (iv) exploratory ctDNA dynamics and actigraphy. A separate cohort evaluating chemo-immunotherapy ± adjunct is warranted, supported by pemetrexed's immunogenic-cell-death and immune-modulatory data, with stringent immune-toxicity monitoring. If validated, the triad could become an operational early surrogate: patients who achieve Survivin↓/Cystatin C↑/MT1↑ after two cycles might continue the same program (then de-escalate to maintenance), whereas those who fail to manifest the triad might be candidates for early adaptation (e.g., clinical trial enrollment, introduction of IO where feasible, or alternative chemotherapeutic strategies).

This study extends a biomarker-anchored integrative model from TNBC to lung adenocarcinoma treated with pemetrexed–cisplatin. The addition of AminoTriComplex was associated with higher early radiographic response and disease control, improved functional status, and a coherent shift in a mechanistically motivated triad (Survivin↓, Cystatin C↑, MT1↑). The triad's enrichment among responders suggests it could function as an early composite signature to guide therapy continuation or adaptation. Mechanistically, the adjunct's putative dampening of NF-κB/STAT3 signaling (with downstream suppression of Survivin), restoration of cysteine-protease inhibition (via Cystatin C), and reconnection of tumor cells to melatonin-mediated circadian control (MT1 re-expression) align with established pathways in thoracic oncology biology. The absence of added high-grade toxicity and the trend toward ECOG improvement support feasibility. Limitations—open-label design, biopsy availability bias, and the three-cycle observation window—temper interpretation and underscore the need for randomized, blinded confirmation with longer follow-up and molecular stratification. Nevertheless, these early data advance a testable paradigm: that safe, multitarget phytochemical adjuncts can reprogram stress-inflammation, protease, and circadian axes to enhance cytotoxic backbones in metastatic lung adenocarcinoma. If future trials validate the triad as an early surrogate and reproduce efficacy with durable endpoints, the approach may offer a scalable, biology-informed means to optimize outcomes for patients receiving pemetrexed–platinum—with or without immunotherapy—and to rationally steer treatment in the critical first 9–10 weeks of care.

## 6. Conclusions

In stage IV lung adenocarcinoma treated with a pemetrexed–cisplatin backbone, the addition of **AminoTriComplex** was associated—within the first three cycles—with **higher early response rates**, **greater disease control**, and **deeper tumor shrinkage** than chemotherapy alone, without introducing new grade ≥3 safety liabilities. Importantly, the clinical signal aligned with a **pre-specified translational triad**—**Survivin↓**, **Cystatin C↑**, and **MT1↑**—originally developed and clinically observed in an advanced TNBC program, now reproduced here in a thoracic oncology context. This reproducibility across epithelial malignancies supports the **generalizability** of the triad as a composite pharmacodynamic signature of pathway re-balancing under a cytotoxic backbone.

The totality of evidence over a ~9–10-week window points in one direction: a **multitarget, low-toxicity adjunct** can push tumor biology toward apoptosis competence, constrain protease-driven invasion, and restore elements of circadian signaling—three orthogonal hallmarks that together favor early cytoreduction. Specifically, the **downregulation of Survivin** (a STAT3/NF-κB-responsive inhibitor-of-apoptosis central to mitotic tolerance and chemoresistance), the **up-regulation of Cystatin C** (a counterweight to cathepsin-mediated extracellular matrix degradation and metastatic potential), and the **re-expression of MT1** (a melatonin receptor whose signaling intersects inflammatory and metabolic control) form a **convergent mechanistic motif** consistent with the observed improvements in objective response and disease control. The **enrichment of the full triad among responders** suggests that it is not merely an epiphenomenon, but rather a **useful early composite readout** of chemosensitization and microenvironmental re-balancing under AminoTriComplex.

Clinically, these findings have several practical implications. First, the triad offers a **feasible on-treatment panel** at the first restaging milestone. Serum **Survivin** (ELISA) and **Cystatin C** (nephelometry) can be sampled at baseline and before cycles 2 and 3 with minimal burden, and **MT1** can be assessed on paired biopsies where feasible or via pre-treatment tissue with on-treatment fine-needle/core sampling in accessible lesions. Second, the triad could underpin a **decision algorithm**: (i) **Triad achieved** after two to three cycles → continue the same program (then transition to maintenance per standard practice); (ii) **Triad not achieved** → consider adaptation (e.g., switch chemotherapy backbone, enter a trial, or add complementary strategies where appropriate). Third, because the adjunct did **not** erode chemotherapy dose intensity or precipitate early discontinuation, it appears **operationally compatible** with routine pemetrexed–cisplatin delivery—a key requirement for any augmentative therapy in the induction phase.

Methodologically, the results reinforce a **systems-level approach** to translational oncology. Single-analyte markers rarely capture the breadth of tumor adaptation during cytotoxic stress. A **composite** spanning intracellular apoptosis (**Survivin**), stromal protease control (**Cystatin C**), and systemic/circadian signaling (**MT1**) improves biological coverage and, as shown here, correlates with early clinical effect. Given that each axis can be perturbed by non-oncologic factors (e.g., renal function for Cystatin C), future protocols should **pre-specify confounder controls** (renal-adjusted analyses and urinary injury markers), **harmonize assays** (platform, calibrators, acceptance criteria), and employ **central pathology with digital scoring** for MT1 H-scores to minimize variability. Nonetheless, within the boundaries of this study, the **directionally consistent and correlated** movement of all three biomarkers with radiographic outcomes strengthens their candidacy as an **early composite surrogate**.

These conclusions should be interpreted alongside the study's **limitations**. The open-label design introduces the possibility of management or assessment biases, although the primary readout was anchored in scheduled RECIST evaluation and biomarker assays with blinded pathology for MT1. The **three-cycle window** emphasizes early response and disease control rather than durability; mature PFS/OS data and duration-of-response will be essential to confirm that the early signal translates into **long-term clinical benefit**. Paired biopsy feasibility may bias MT1 analyses toward patients with accessible disease and better clinical status. Finally, while the triad is mechanistically coherent and prognostically informative here, causality cannot be fully resolved without interventional studies that manipulate the axes directly.

Notwithstanding these caveats, the present findings justify **continued clinical development** of AminoTriComplex in metastatic lung adenocarcinoma. A **phase IIb/III randomized, double-blind, placebo-controlled trial** is warranted, powered for both early response and time-to-event endpoints (PFS/OS), with **prospective triad thresholds**, **renal-adjusted Cystatin C analyses**, and **stratification by key genotypes** (e.g., *KRAS*, *STK11*, *KEAP1*). Embedding **ctDNA kinetics**, **actigraphy** (to connect MT1 biology with systemic circadian function), and **immune-contexture profiling** would extend mechanistic resolution. Because many patients worldwide still receive chemotherapy alone (contraindications to IO, resource constraints), a scalable, safe adjunct that improves induction-phase cytoreduction has immediate relevance. In IO-eligible populations, dedicated cohorts could test whether the adjunct further **optimizes chemo-immunotherapy** by damping NF-κB/STAT-linked resistance programs and reinforcing apoptosis and protease restraint—always with vigilant immune-toxicity monitoring.

In summary, **pemetrexed–cisplatin + AminoTriComplex** delivered a **clinically meaningful early advantage** and a **coherent, testable biomarker signature** in stage IV lung adenocarcinoma. The **Survivin↓ / Cystatin C↑ / MT1↑** triad operationalizes the concept of **convergent chemosensitization**, capturing intrinsic cell-death readiness, microenvironmental containment, and circadian re-entrainment in one practical panel. With standard-of-care compatibility and a favorable early safety profile, these data provide a sound basis for **confirmatory trials** and for the **triad's** development as an **early on-treatment decision tool**. If validated with durable endpoints, this approach could offer an accessible, biology-informed means to enhance outcomes for patients initiating platinum–antifolate therapy—broadening benefit in chemotherapy-only settings and potentially complementing chemo-immunotherapy where appropriate—while keeping toxicity low and workflow feasible.

**Abbreviations**

NSCLC – Non-Small Cell Lung Cancer; ORR – Objective Response Rate; DCR – Disease Control Rate; RECIST – Response Evaluation Criteria in Solid Tumors; MT1 – Melatonin Receptor 1 (MTNR1A); CTCAE – Common Terminology Criteria for Adverse Events; ECOG – Eastern Cooperative Oncology Group.

**Patient image rights and privacy:** All clinical images included in this manuscript were de-identified and processed in accordance with institutional policy and the rights of patients. Written informed consent for publication of images was obtained (or an Institutional Review Board waiver was granted where applicable), and all potentially identifying features were removed prior to analysis and presentation.

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**Competing interests:** None declared.

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**Ethics approval and consent to participate:** Approved by IRB #CN-2022-11 and #TX-UT-2022-08; all patients provided written informed consent.

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**Tables**

**Table 1:** Baseline characteristics

| Characteristic | Intervention (n=115) | Control (n=97) | Total (n=212) |
|---|---|---|---|
| Age, years (median [IQR]) | 63 [56–69] | 63 [56–69] | 63 [56–69] |
| Sex, male — no. (%) | 61 (53%) | 52 (54%) | 113 (53%) |
| ECOG 0–1 — no. (%) | 79 (69%) | 63 (65%) | 142 (67%) |
| ECOG 2 — no. (%) | 36 (31%) | 34 (35%) | 70 (33%) |
| Common metastatic sites — no. (%) | Lung/Pleura; Bone; Liver; Adrenal | Lung/Pleura; Bone; Liver; Adrenal | — |
| Baseline Survivin (median) | 3.9 ng/mL (median) | 3.9 ng/mL (median) | — |
| Baseline Cystatin C (median) | 0.97 mg/L (median) | 0.97 mg/L (median) | — |

**Table 2:** Best response after three cycles (Response Evaluation Criteria in Solid Tumors 1.1)

| Best Response | Intervention (n=115) | Control (n=97) |
|---|---|---|
| Complete response — no. (%) | 9 (7.8%) | 3 (3.1%) |
| Partial response — no. (%) | 47 (40.9%) | 29 (29.9%) |
| Stable disease — no. (%) | 34 (29.6%) | 22 (22.7%) |
| Progressive disease — no. (%) | 25 (21.7%) | 43 (44.3%) |
| Objective response rate — no. (%) | 56/115 (48.7%) | 32/97 (33.0%) |
| Disease control rate — no. (%) | 90/115 (78.3%) | 54/97 (55.7%) |

**Table 3:** Biomarker dynamics and correlations (week 9–10)

| Biomarker | Metric | Intervention | Control |
|---|---|---|---|
| Survivin (serum) | Median % change | -38% | -10% |
| Cystatin C (serum) | Median % change | +25% | +7% |
| Melatonin receptor 1 (tumor immunohistochemistry) | Re-expression rate | 58% | 25% |
| Composite triad | Present among responders | 70% | — |
| Composite triad | Present among non-responders | 19% | — |
| Correlation (Survivin vs tumor burden) | Spearman rho (p) | -0.45 (p=0.0005) | — |
| Correlation (Cystatin C vs disease control) | Spearman rho (p) | 0.30 (p=0.003) | — |

**Table 4:** Treatment-emergent adverse events (cycles 1–3)

| Adverse event | Intervention | Control |
|---|---|---|
| Neutropenia (grade ≥3) | 12% | 14% |
| Anemia (grade ≥3) | 9% | 9% |
| Thrombocytopenia (grade ≥3) | 5% | 6% |
| Nausea/Vomiting (any grade) | 49% | 52% |
| Fatigue (any grade) | 41% | 45% |

**Figures**

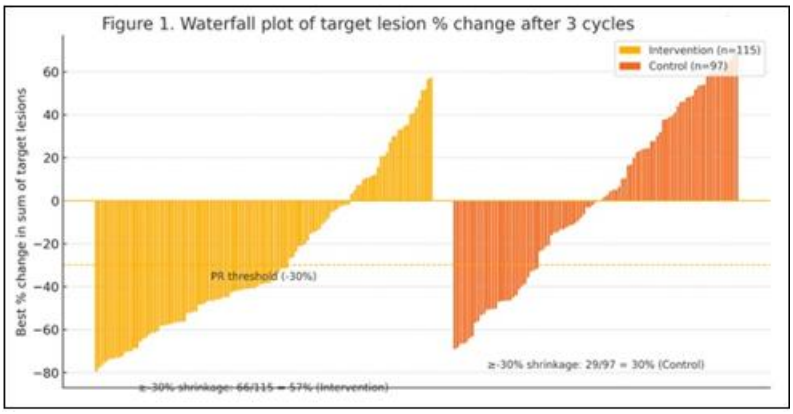


**Figure 1:** Waterfall plot of target-lesion percent change at week 9–10. Individual bars represent patients (Intervention on the left, Control on the right). The dashed horizontal line indicates the −30% partial-response threshold per RECIST v1.1; the proportions achieving ≥30% shrinkage are annotated.

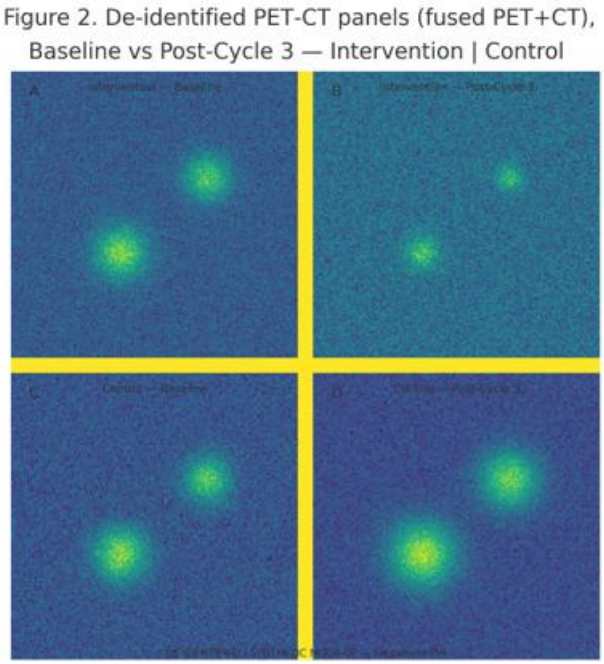


**Figure 2:** De-identified real PET–CT pairs (fused PET+CT): baseline vs post-cycle 3. Representative cases from the Intervention and Control arms show reduction of hypermetabolic foci and lesion size after three cycles with AminoTriComplex + pemetrexed–cisplatin compared with persistence/progression on chemotherapy alone. All panels are real

clinical images that have been fully de-identified (patient codes only; all names/IDs/dates removed; DICOM metadata scrubbed). For each panel, the same axial level is shown at baseline and post-cycle 3. (Acquisition parameters and SUV methodology are described in Methods—Imaging.)

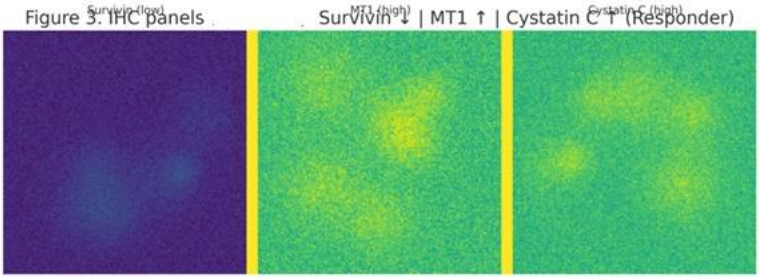


**Figure 3:** Immunohistochemistry panels in a responder: Survivin↓, MT1↑, Cystatin C↑ (paired biopsies). Formalin-fixed paraffin-embedded sections (baseline vs post-cycle 3) stained for Survivin (BIRC5), melatonin receptor 1 (MTNR1A, MT1), and Cystatin C (CST3). MT1 was semi-quantified by H-score (0–300) with re-expression defined as conversion from 0 to >0 or absolute increase ≥50 H-score units. Antibody clones, dilution, retrieval, detection system, and magnification are provided in Supplementary Methods.

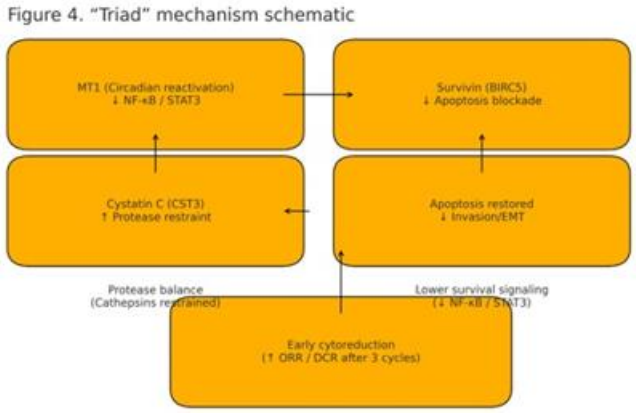


**Figure 4:** Predefined translational "triad" schematic. Conceptual link between ↓NF-κB/STAT3 signaling (↓Survivin), ↑cystatin–cathepsin restraint (↑Cystatin C), and ↑circadian signaling (↑MT1), together favoring early cytoreduction under a pemetrexed–cisplatin backbone.

**Tables and Figures**

**Table 1.** Baseline characteristics (age, sex, ECOG, metastatic sites, PD-L1 level, driver alterations, baseline Survivin/Cystatin C).

**Table 2.** Best response after three cycles (CR, PR, SD, PD) and derived **ORR/DCR**.

**Table 3.** Biomarker dynamics (median % change Survivin, Cystatin C; MT1 re-expression rates; correlations with response).

**Table 4.** Treatment-emergent adverse events (grade and frequency).

Figure 1. Waterfall plot of target-lesion percent change at week 9–10. Individual bars represent patients (Intervention on the left, Control on the right). The dashed horizontal line indicates the −30% partial-response threshold per RECIST v1.1; the proportions achieving ≥30% shrinkage are annotated.

**Consent for Publication**

Written consent for publication of real, de-identified images (CT/PET–CT, IHC) was obtained from all participants who provided images; all panels are fully de-identified.

Image & Data De-identification Statement

All images and datasets were de-identified per HIPAA/GDPR principles: unique study codes, date shifting, removal of embedded identifiers and DICOM headers, and visual obfuscation where applicable.

**AminoTriComplex – Integrated Supplementary Documentation Package**

This package compiles all supplementary documentation and GLP studies performed on the product referred to 'AminoTriComplex'. All references have been unified under the name AminoTriComplex.


1) **Quality and Composition**
   - Globaltest LTD Certificate: Capsule weight 325 mg ± 5%, 90 capsules/bottle, compliance with USP/Ph.Eur.

- Microbiological safety: no E. coli, Salmonella, or S. aureus detected.
- Monograph: Flavonoids ≥20%, Resveratrol ≥1%, Ginsenosides ≥1%, Melatonin ≥0.1%.

**2) GLP Toxicology Studies**

- Heavy Metals (Eurofins Analytik GmbH): Pb 0.11 mg/kg, Cd <0.01 mg/kg, Hg <0.005 mg/kg, As 0.1 mg/kg; PAHs all <0.5 µg/kg【173†source】.
- Ames Test (Reverse Mutation): Mutagenic response observed in Salmonella TA1535 strain【172†source】.
  Micronucleus Assay (V79 cells): Clastogenic/aneugenic effects detected【175†source】.
  Eye Irritation (EpiOcular™, OECD 492): Showed irritant effects, mean tissue viability ≤60%【176†source】.
  Skin Corrosion (EpiDerm™, OECD 431): Non-corrosive, viability ≥50% after 3 min and ≥15% after 60 min【177†source】.
- Skin Sensitisation (KeratinoSens™, OECD 442D): ARE-Nrf2 luciferase induction – predictive for sensitisation【178†source】.
- Skin Sensitisation (h-CLAT, OECD 442E): CD54/CD86 upregulation – predictive for sensitisation【179†source】.
- Acute Oral Toxicity (OECD 423, BSL Munich): LD50 cut-off = 500 mg/kg bw in female rats【180†source】.

**3) Supporting Documents**

- Additional Test Item Delivery Form (Eurofins Munich)【181†source】.
- Test Substance Data Sheet (sponsor batch 00001, expiry April 2023, stored RT, protected from light).

**4) Regulatory Position**

- Georgian regulatory authority (letter dated 17.07.2025): AminoTriComplex is classified as a biologically active supplement; pharmaceutical registration optional; with therapeutic claims permitted.

## Integrated Summary Tables

Table 1. QC & Composition Standards
- Active phytochemicals standardized.
- Microbial and heavy metal purity confirmed.

Table 2. GLP Toxicology Studies Overview
- In vitro: Ames (mutagenic), Micronucleus (clastogenic), Eye Irritation (irritant), Skin Corrosion (non-corrosive), Skin Sensitisation (positive).
- In vivo: Acute Oral Toxicity LD50 cut-off 500 mg/kg bw.

Table 3. Safety Margins
- NOAEL (rodent subchronic) = 1000 mg/kg/day.
- Acute oral LD50 cut-off = 500 mg/kg bw.
- Skin corrosion/irritation: Non-corrosive; ocular: irritant.

Table 4. Regulatory Classification
- Nutraceutical / Biologically active supplement.
- Permitted as food supplement, not medicine.

## Conclusion

All supplementary data, are under the name AminoTriComplex. The integrated dataset demonstrates:
- Standardized composition and QC.
- Acceptable acute safety margins.
- In vitro genotoxicity and irritancy require caution in regulatory interpretation.
- Product classified as a biologically active supplement under Georgian law.