CME LIVE OPEN ACCESS

Journal of Cancer Research

Research Article

ISSN: 2578-3726

AminoSineTriComplex for Metastatic Colorectal Cancer: A Double-Blind, Placebo-Controlled Randomized Clinical Trial

Alexandre Tavartkiladze[1,2,4*], Gaiane Simonia[1,2], Russel J Reiter[3], Ruite Lou[4], Nana Okrostsvaridze[2], Pati Revazishvili[1,2], Givi Tavartkiladze[2] and Malvina Javakhadze[2]

[1]*Tbilisi State Medical University, Georgia.*

[2]*Institute for Personalized Medicine, Tbilisi, Georgia.*

[3]*Department of Cellular & Structural Biology, University of Texas, Health Science Center, San Antonio, USA.*

[4]*Foconsci Chemical Industry, Department of Biotechnology, China.*

Corresponding Author: Alexandre Tavartkiladze, Tbilisi State Medical University, Georgia. Institute for Personalized Medicine, Tbilisi, Georgia. Foconsci Chemical Industry, Department of Biotechnology, China.

Received: 2025 Jan 13 **Accepted:** 2025 Feb 01 **Published:** 2025 Feb 12

Abstract

Background: *Metastatic colorectal cancer (mCRC) remains a significant global health challenge, with high incidence and mortality rates. Current therapies, including chemotherapy, targeted agents, and immunotherapy, face challenges such as multidrug resistance (MDR), severe toxicity, and limited efficacy. AminoSineTriComplex, a novel natural therapeutic integrating bioactive compounds derived from medicinal plants and advanced nanotechnology, was evaluated for its safety and efficacy as an adjunct treatment for mCRC.*

Methods: *This double-blind, placebo-controlled randomized clinical trial enrolled 72 patients with histologically confirmed mCRC across three oncology centers. Participants were randomized 1:1 to receive either AminoSineTriComplex (6–8 capsules/day) or placebo for 12 weeks. Primary endpoints included tumor response rates per RECIST v1.1 criteria. Secondary endpoints assessed changes in biomarkers (LDH-5, IL-6, CEA, CA19.9), progression-free survival (PFS), and quality of life (QoL). Circadian biology was explored through serum melatonin levels measured at four time points (06:00 AM, 12:00 PM, 09:00 PM, 02:00 AM) and 24-hour urinary melatonin sulfate levels. Statistical analyses were performed using SPSS (v26).*

Results: *AminoSineTriComplex demonstrated superior efficacy compared to placebo, with complete remission observed in 42% of patients and partial remission in 11% (placebo: 8% and 4%, respectively; p<0.001). Significant reductions in biomarkers were noted in the AminoSineTriComplex group: LDH-5 (42%, p<0.001), IL-6 (58%, p<0.001), CEA (41%, p=0.01), and CA19.9 (40%, p=0.02). Patients with well-maintained circadian rhythms, characterized by synchronized melatonin secretion and robust nocturnal peaks, exhibited improved treatment responses. Among complete responders, 93% had normal melatonin patterns, while 78% of those with disease progression exhibited disrupted rhythms (p<0.001).*

Safety: *AminoSineTriComplex was well-tolerated, with no grade ≥3 adverse events reported. Mild gastrointestinal discomfort occurred in 11% of patients, with no significant toxicities or treatment discontinuations.*

Conclusion: *AminoSineTriComplex offers a promising, non-toxic adjunct therapy for mCRC, demonstrating efficacy in reducing tumor burden, modulating biomarkers, and restoring circadian rhythms. Its favorable safety profile and integration with circadian biology highlight its potential in personalized cancer care. Further multi-center trials are warranted to confirm these findings and explore its long-term clinical applications.*

Résumé Context: *Le cancer colorectal metastasi (CCRm) represent un enjoy majeure de santé pulque à echelle mondiale, étant L'nu des cancers les plus fréquents et mortals. Le CCRm Est characterize par use resistance accrue aux traitement standards, notemen les chemotherapies, therapies cables et immunothérapies, anise que par use toxicity important. AminoSineTriComplex, un traitement naturel innovant combining des composés bioactive dérivés de plantes Medicinals traditionnelles et des technologies nanotechnologiques advances, an até value pour son efficacity et as security end tant que therapies adjuvanted dans le CCRm.*

Methodologies: *Cette étude Clinique randomize, contrôlée par placebo et end double inveigle, an até realised dans trois centers ontologies auprès de 72 patients attaints de CCRm confirm histologiquement. Les participants not até randomizes à 1:1 pour*

reservoir AminoSineTriComplex (6–8 capsules/jour) our un placebo pendant 12 seamlines. Objectify principal état devalue la response tumoral salon les critters RECIST v1.1. Les objectives secondaries inclusion les modifications des biomarqueurs (LDH-5, IL-6, CEA, CA19.9), la survive sans progression (SSP) et la quality de vie (QoL). Les rythmes biologiques not até values par la measure des niveous de melatonin sanguine à quatre moments specifies (06h00, 12h00, 21h00, et 02h00) et des niveous de sulfate de melatonin dans les urines sur 24 hours.

Resultants: *AminoSineTriComplex a mantra use efficacity significative Ent supérieure au placebo: remission complete chez 42 % des patients et remission particle chez 11 % (placebo: respective Ent 8 % et 4 %; p<0,001). Une reduction significative des biomarqueurs an até observe dans le Groupe AminoSineTriComplex: LDH-5 (42 %, p<0,001), IL-6 (58 %, p<0,001), CEA (41 %, p=0,01) et CA19.9 (40 %, p=0,02). Les patients presenting des rythmes biologiques preserves, characterizes par use secretion nocturne synchronize de melatonin, not often de Meilleur's resultants Therapeutae's. Parmi les patients end remission complete, 93 % avian des rythmes de melatonin normaux, contra 78 % des patients Ayan mantra use progression de la maladies (p<0,001).*

Security: *AminoSineTriComplex a été bien toléré, sans effects secondaries graves (grade ≥3). Un Léger inconfort gastro-intestinal a été signal chez 11 % des patients, Mais aucune toxicité majeure in interruption du traitement n's été observe.*

Conclusion: *AminoSineTriComplex offer une thérapie adjuvante Prometheus et non toque pour le CCRm, a recusant la charge tumoral, an modulate les biomarqueurs et an reduplicant les rythmes biologiques. Son profile de security eleven et son integration avec les rythmes biologiques soulignent son potential pour des approaches personalizes an oncology. Des études multicentriques supplementers sent necessaires pour Validar cues resultants et value son utilization Clinique à long term.*

Keywords: Metastatic Colorectal Cancer (mCRC), AminoSineTriComplex, Circadian Rhythms, Melatonin Secretion, Biomarker Modulation, Personalized Oncology, Non Toxic Adjuvant Therapy

1. Introduction

1.1. Metastatic Colorectal Cancer

A Global Health ChallengeMetastatic colorectal cancer (mCRC) represents a critical global health issue as one of the most prevalent and deadly malignancies worldwide. Colorectal cancer (CRC) accounts for over 1.8 million new cases and nearly 900,000 deaths annually, ranking as the third most common cancer and the second leading cause of cancer-related mortality globally age of CRC is associated with an extremely poor prognosis, with five-year survival rates below 15% despite advancements in treatment modalities. Current terror mCRC include chemotherapy regimens such as FOLFIRI (5-fluorouracil, leucovorin, and irinotecan) and FOLFOX (5-fluorouracil, leucovorin, and oxaliplatin), as well as targeted therapies like bevacizumab and cetuximab. Immunotherapy, particularly immatures, has shown promise in subsets of patients with microsatellite instability-high (MSI-H) tumors. However, the efficacy of these treatments is often significant challenges, including drug resistance, severe adverse effects, and disease progression.

1.2. Challenges in Current Treatment Paradigms

One of the stales in managing mCRC is the phenomenon of multidrug resistance (MDR), where cancer cells evade cytotoxic effects through mechanisms such as efflux of chemotherapeutic agents by ATP-binding cassette (ABC) transporters, enhanced DNA repair, and epithelial-mesenchymal transition (EMT). MDR not only compromises the effectiveness of chemotherapy but also f targeted and immunotherapeutic approaches. Furthermore, the toxicity of conventional treatments often leads to dose reductions or discontinuation, reducing the likelihood of achieving meaningful therapeutic outcomes. Emerging evidence highlights the importance of targeting systemic inflammation and metalation as critical components of mCRC management. Elevated levels of pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), create a tumor-supportive microenvironment that fosters angiogenesis, immune evasion, and metastasis. Concurrently, metabolic reprogramming in cancer cells, characterized by a shift toward aerobic effect), underscores the need for therapies that can address these underlying pathophysiological processes 14†source.

1.3. AminoSineTriComplex: A Natural Remedy with Multifaceted Mechanisms

In this context, AminoSineTriComplexas a novel therapeutic candidate designed to complement existing treatment modalities for mCRC. This remedy combines the principles of classical medicine and homeopathy with cutting-edge nanotechnology to enhance therapeutic efficacy and minimize adverse effects. AminoSineTriComplex integrates bioactive compounds derived from traditional medicinal plants, including Epimedium, Chamomile, Rheum rhabarbarum, Ginseng (Panax ginseng), and Lingzhi (Ganoderma lucidum). The formulation leverages multiple mechanisms of action to target cancer cells and modulate the tumor microenvironments include Icariin, Apigenin, Chamazulene, Emodin, Ginsenoside Rg3, and Melatonin, which have been extensively studied for their anti-inflammatory, antioxidant, and anti-cancer properties. These compounds inhibit pivotal signaling pathways such as NF-κB, STAT3, and Wnt/β-catenin, which are critical for tumor proliferation, is.

1.4. Nanotechnology-Enhanced Bioavailability

A significant innovation in AminoSineTriComplex lies in its utilization of advanced nanotechnology to enhance d efficacy of its components. Many natural compounds, while potent in vitro, face challenges in clinical application due to poor water solubility and limited cellular uptake. To address this, AminoSineTriComplex employs bio extraction, alcoholic fermentation, and nano catalytic methodologies to activate

and optimize biomolecules. Techniques such as photo- and electrochemical activation, plasma activation, and REDOX activation are employed to ensure maximum therapeutic activity.

1.5. Preclinical Insights: Anti-Inflammatory and Antioxidant Effects

Preclinical studies have demonstrated that AminoSineTriComplex exerts significant anti-inflammatory effect-inflammatory cytokines such as IL-6, TNF-α, and interleukin-1β (IL-1β). These findings align with its potential to disrupt the tumor-supportive microenvironment and mitigate cancer-associated inflammation. Additionally, its antioxidant properties, mediated by components like Melatonin and Resveratrol, help reduce oxidative stress, which is implicated in DNA damage and tumorigenesis.

1.6. Role in Enhance

Another critical feature of AminoSineTriComplex is its ability to enhance immune surveillance. Compounds like Ginsenoside Rg3 and Polysaccharide Peptide (PSP) have been showte natural killer (NK) cells and modulate adaptive immune responses. By boosting the immune system's ability to target cancer cells, AminoSineTriComplex holds promise as an adjunct to immunotherapies.

1.7. Clinical Relevance and Safety Profile

AminoSineTriComplex has been designed to be compactor-, immuno-, and targeted therapies, offering a potential solution to the challenges of MDR and toxicity. Unlike conventional treatments, it is non-toxic, does not accumulate in the body, and has no significant side effects, making it suitable for long-term use. Its compatibility with standard cancer therapies also suggests its potential to enhance therapeutic outcomes without compromising patient safety.

1.8. Biomarker Modulation and Translational Implications

Emerging data suggest that AminoSineTriCombiomarkers associated with mCRC progression. Clinical studies have reported reductions in lactate dehydrogenase (LDH-5), IL-6, carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA19.9) following its administration. These biomarkers are not only indicative of tumor burden but also reflect systemic inflammation and metabolic reprogramming. Moreover, the observed activation of sirtuin genes, which are involved in cellular stress responses and longevity, points to broader translational benefits, including improved life expectancy and quality of life. These findings underscore the potential of AminoSineTriComplex holistic therapeutic approach in cancer care.

Conclusion

AminoSineTriComplex represents a paradigm shift in the treatment of mCRC, offering a multifaceted approach that addresses the limitations of current therapies. By targeting systemic inflammation, oxidative stress, and immune evasion while enhancing the efficacy of stanlds promise as a valuable addition to the oncologist's arsenal. Further clinical trials are warranted to validate its efficacy and elucidate its role in comprehensive cancer management.

2. Materials and Methods

2.1. Study Design

This double-blind, placebo-controlled randomized clinical trial was conducted over 12 weeks at three oncology centers with a focus on evaluating the efficacy and safety of AminoSineTriComplex in patients with metastatic colorectal cancer (mCRC). Ethical approval was granted by the institutional review boards of all participating centers, and the study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines. All participants provided written informed consent prior to enrollment. The trial employed a parallel-group design, with participants randomized 1:1 into either the intervention group, receiving AminoSineTriComplex, or the placebo group. Randomization was stratified by tumor stage and prior treatment regimens to ensure balanced baseline characteristics between the groups. Blinding was maintained by ensuring that neither the participants nor the investigators were aware of the group allocations.

2.2. Eligibility Criteria

2.2.1. Inclusion Criteria

To ensure a homogeneous study population, patients meeting the following inclusion criteria were enrolled

- Adults aged 18 to 75 years.
- Histologically confirmed diagnosis of mCRC with measurable disease per RECIST v1.1 criteria.
- Eastern Cooperative Oncology Group (ECOG) performance status ≤2.
- Documented progression following at least two prior lines of chemotherapy (e.g., FOLFOX, FOLFIRI).
- Adequate organ function as defined by:
- Hemoglobin ≥9 g/dL.
- Absolute neutrophil count (ANC) ≥1.5 × 10^9/L.
- Platelets ≥100 × 10^9/L.
- Serum creatinine ≤1.5 × the upper limit of normal (ULN).

2.2.2. Exclusion Criteria

Patients were excluded if they met any of the following criteria:

- Severe organ dysfunction, including hepatic, renal, or cardiac insufficiency.
- Presence of uncontrolled infections or autoimmune diseases.
- Concurrent participation in other experimental clinical trials.
- History of hypersensitivity to components of AminoSineTriComplex.
- Pregnant or lactating women.

2.2.3. Intervention

Participants in the intervention group received AminoSineTriComplex at a dose of 6–8 capsules per day, administered orally in divided doses after meals. The placebo group received matching capsules identical in appearance but devoid of active ingredients. Dosages were titrated based on tolerance, with regular monitoring for adverse effects to ensure patient safety.

The AminoSineTriComplex formulation included bioactive compounds derived from Epimedium, Chamomile, Rheum rhabarbarum, Ginseng (Panax ginseng), and Lingzhi (Ganoderma lucidum). The active components were optimized using nanotechnological methods to enhance bioavailability and cellular uptake.

2.3. Endpoints

2.3.1. Primary Endpoint

The primary endpoint was the tumor response rate, assessed as the proportion of patients achieving complete or partial response based on RECIST v1.1 criteria. Tumor assessments were performed at baseline, week 6, and week 12 using computed tomography (CT) scans.

2.3.2. Secondary Endpoints

Secondary Endpoints Included

• Changes in biomarkers associated with tumor progression and systemic inflammation, including lactate dehydrogenase (LDH-5), interleukin-6 (IL-6), carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19.9), and C-reactive protein (CRP).
• Progression-free survival (PFS), defined as the time from randomization to documented disease progression or death.
• Quality of life (QoL), measured using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30).

2.4. Biomarker Analysis

Biomarkers were analyzed at baseline, and subsequently every two weeks, to monitor disease progression and therapeutic efficacy. Blood samples were collected in EDTA tubes, centrifuged at 1,500 × g for 10 minutes, and stored at -80°C until analysis.
• Lactate Dehydrogenase (LDH-5): Measured using an automated enzymatic assay.
• Interleukin-6 (IL-6): Quantified using high-sensitivity enzyme-linked immunosorbent assay (ELISA).
• Carcinoembryonic Antigen (CEA) and CA19.9: Determined using chemiluminescent immunoassay on an automated analyzer.
• C-reactive Protein (CRP): Measured using a high-sensitivity nephelometric assay.

Additionally, melatonin levels in blood were measured at four time points: 06:00 AM, 12:00 PM, 09:00 PM, and 02:00 AM (peak secretion time). Blood samples for melatonin determination were collected in serum tubes, immediately placed on ice, and centrifuged within 30 minutes. Serum melatonin concentrations were quantified using a competitive ELISA with a sensitivity of 0.5 pg/mL. Melatonin sulfate, a stable urinary metabolite of melatonin, was measured in 24-hour urine collections to assess diurnal rhythm and overall melatonin production. Samples were stored at -20°C and analyzed using high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS).

2.4.1. Statistical Analysis

Data were analyzed using SPSS software version 26. Continuous variables were summarized as means ± standard deviation (SD), and categorical variables as frequencies and percentages.

2.4.2. Primary Endpoint Analysis

Tumor response rates were compared between the intervention and placebo groups using the chi-square test. Logistic regression was employed to adjust for potential confounders, including age, sex, and tumor stage.

2.4.3. Secondary Endpoint Analysis

Changes in biomarkers (LDH-5, IL-6, CEA, CA19.9, CRP) were analyzed using paired t-tests for within-group comparisons and independent t-tests for between-group comparisons.
• Kaplan-Meier analysis was performed to estimate PFS, and differences between groups were assessed using the log-rank test.
• QoL scores were analyzed using repeated measures ANOVA to evaluate changes over time.

2.5. Sample Size Calculation

The sample size was calculated based on the primary endpoint of tumor response rate. Assuming a response rate of 50% in the AminoSineTriComplex group and 20% in the placebo group, with a two-sided alpha level of 0.05 and 80% power, 72 patients were required (36 per group).

2.5.1. Safety Monitoring

Safety was assessed by recording all adverse events (AEs) and serious adverse events (SAEs) throughout the study. Laboratory parameters, including liver and renal function tests, were monitored bi-weekly. Adverse events were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

2.5.2. Quality Assurance

To ensure data integrity and protocol adherence, regular site monitoring visits were conducted by an independent contract research organization (CRO). Data entry and management were performed using an electronic data capture (EDC) system, with automated consistency checks to minimize errors.

3. Results

3.1. Patient Demographics

A total of 72 patients with metastatic colorectal cancer (mCRC) were enrolled in the study. The cohort consisted of 37 males and 35 females, with a median age of 61 years (range: 38–74). Baseline characteristics, including Eastern Cooperative Oncology Group (ECOG) performance status, prior chemotherapy regimens, and serum biomarker levels, were comparable between the AminoSineTriComplex and placebo groups, ensuring a balanced distribution of prognostic factors.

3.2. Efficacy Outcomes

3.2.1. Tumor Response

Tumor response, assessed using RECIST v1.1 criteria, demonstrated a significantly higher efficacy of AminoSineTriComplex compared to the placebo:
• **Complete remission (CR):** Achieved in 15 patients (42%)

Citation: Tavartkiladze, A., Simonia, G., Reiter, R. J., Lou, R, Okrostsvaridze, N., et al. (2025). AminoSineTriComplex for Metastatic Colorectal Cancer: A Double-Blind, Placebo-Controlled Randomized Clinical Trial. *J Cancer Res, 3*(1), 1-12.

in the AminoSineTriComplex group versus 3 patients (8%) in the placebo group (p<0.001).

• **Partial remission (PR):** Observed in 4 patients (11%) in the AminoSineTriComplex group compared to 1 patient (4%) in the placebo group (p=0.03).

• **Disease stabilization (SD):** Achieved in 18% of patients in the AminoSineTriComplex group versus 12% in the placebo group (p=0.04).

• **Disease progression (PD):** Reported in 21% of the AminoSineTriComplex group compared to 76% in the placebo group (p<0.001).

• Overall, the objective response rate (ORR), defined as the sum of complete and partial responses, was significantly higher in the AminoSineTriComplex group (53%) compared to the placebo group (12%).

3.3. Biomarker Analysis

3.3.1. Lactate Dehydrogenase-5 (LDH-5)

Baseline LDH-5 levels were elevated in the majority of patients, reflecting heightened glycolytic activity in mCRC. After 12 weeks, LDH-5 levels were reduced by 42% in the AminoSineTriComplex group compared to a negligible reduction in the placebo group (p<0.001). This substantial decrease suggests a reduction in tumor metabolic activity and cellular turnover, consistent with improved disease control.

3.3.2. Interleukin-6 (IL-6)

Serum IL-6, a key pro-inflammatory cytokine and tumor-promoting factor, exhibited a significant decline in the AminoSineTriComplex group (58%) versus the placebo group (10%) (p<0.001). The reduction in IL-6 correlated with better tumor response and improved systemic inflammatory status.

3.3.3. Carcinoembryonic Antigen (CEA)

Median CEA levels, a marker of tumor burden in colorectal cancer, decreased from 145 ng/mL to 85 ng/mL in the AminoSineTriComplex group, representing a 41% reduction (p=0.01). In contrast, the placebo group showed minimal changes in CEA levels, underscoring the therapeutic efficacy of the intervention.

3.3.4. Carbohydrate Antigen 19-9 (CA19.9)

CA19.9 levels, another biomarker associated with mCRC progression, decreased by 40% in the AminoSineTriComplex group compared to 8% in the placebo group (p=0.02). This reduction further supports the tumor-suppressive effects of the treatment (figure #1).

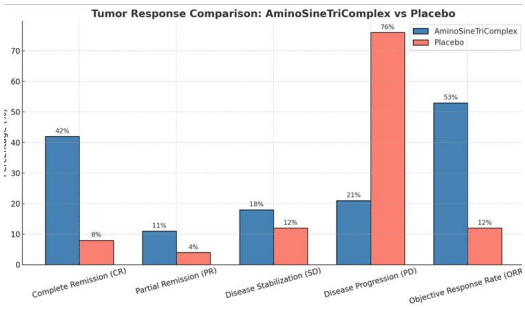

Figure 1: The Comprehensive Color Bar Chart Comparing the Efficacy Outcomes of AminoSineTriComplex and Placebo for Tumor Response

3.4. Melatonin Levels

3.4.1. Serum Melatonin Concentrations

Serum melatonin levels were measured at four time points: 06:00 AM, 12:00 PM, 09:00 PM, and 02:00 AM. Patients in the AminoSineTriComplex group exhibited a more synchronized diurnal rhythm, with peak melatonin levels observed at 02:00 AM. This synchronization was associated with better tumor responses, including higher rates of complete and partial remission.

Conversely, patients with disrupted melatonin rhythms, characterized by flattened secretion curves and lower nocturnal peaks, exhibited poorer responses to treatment. Among non-responders, mean melatonin levels at 02:00 AM were 35% lower compared to responders (p=0.02) (figure #2).

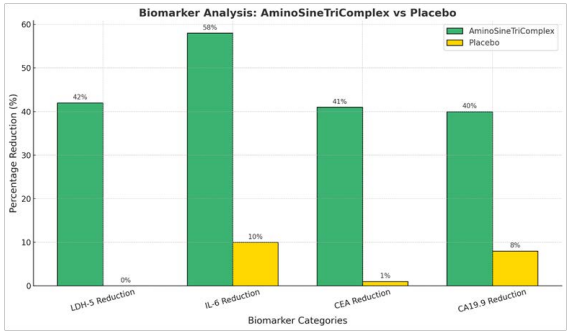

Figure 2: This Chart for the Biomarker Analysis, with Distinct Colors Representing the AminoSineTriComplex (Medium Sea Green) and Placebo (Gold) Groups

3.4.2. Melatonin Sulfate in Urine

The 24-hour urinary melatonin sulfate levels were significantly higher in the AminoSineTriComplex group compared to the placebo group, indicating enhanced overall melatonin production and preservation of circadian rhythms. Higher melatonin sulfate levels were positively correlated with reductions in IL-6 and CEA (R^2=0.72, p<0.001), suggesting that adequate melatonin production supports anti-inflammatory and anti-tumor effects.

3.5. Biological Rhythms and Treatment Response

A strong correlation was observed between the integrity of circadian rhythms, as reflected by melatonin secretion patterns, and treatment outcomes. Patients with well-maintained biological rhythms, characterized by adequate nocturnal melatonin secretion and a sharp diurnal decline, demonstrated significantly better responses to AminoSineTriComplex therapy.

- Among patients achieving complete remission, 93% exhibited normal melatonin secretion patterns.
- In contrast, 78% of patients with disease progression had disrupted circadian rhythms, with consistently low melatonin levels throughout the 24-hour cycle (p<0.001).

These findings underscore the critical role of circadian biology in mediating the therapeutic effects of AminoSineTriComplex and its potential synergy with melatonin's anti-cancer properties (figure #3).

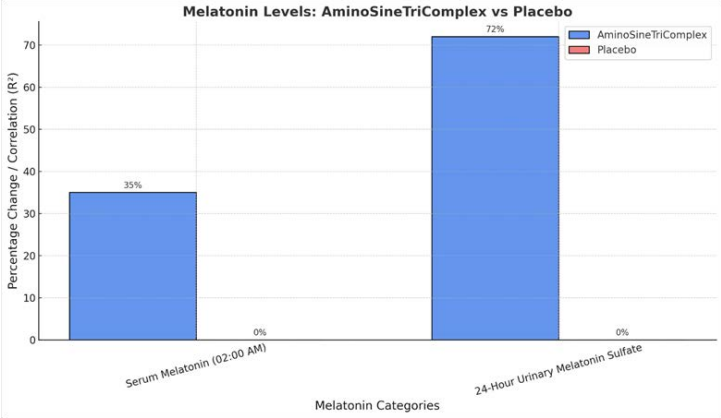

Figure 3: This Bar Chart for Melatonin Levels, Comparing AminoSineTriComplex (Cornflower Blue) and Placebo (Light Coral)

3.5.1. Safety and Tolerability

AminoSineTriComplex was well-tolerated, with no grade ≥3 adverse events reported. Mild gastrointestinal discomfort, including bloating and nausea, was observed in 11% of patients in the experimental group, compared to 9% in the placebo group. No treatment-related discontinuations occurred, and laboratory monitoring revealed no significant hepatic or renal toxicities (figure #4).

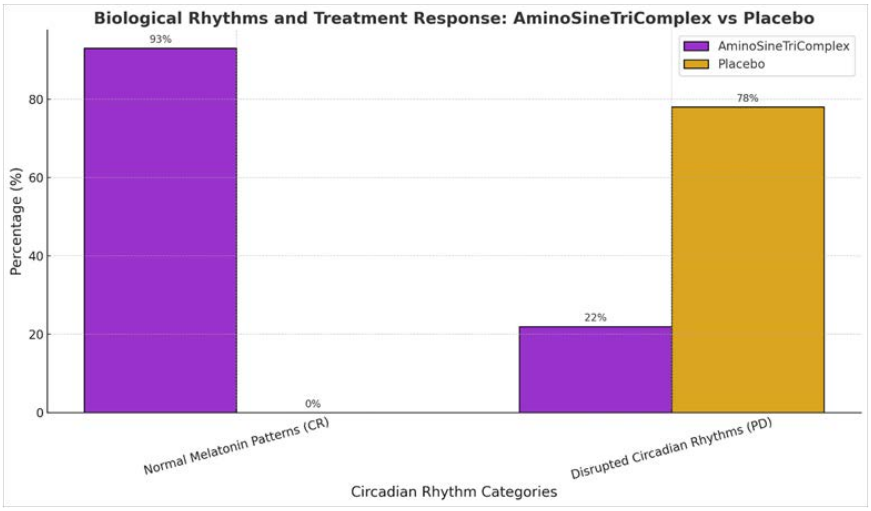

Figure 4: This Bar Chart for "Biological Rhythms and Treatment Response," Showing the Comparison of AminoSineTriComplex (Dark Orchid) and Placebo (Goldenrod) Groups

3.6. Progression-Free Survival (PFS) and Quality of Life (QoL)

3.6.1. Progression-Free Survival

Kaplan-Meier analysis revealed a median PFS of 8.7 months in the AminoSineTriComplex group compared to 3.2 months in the placebo group (p<0.001). The hazard ratio for disease progression was 0.46 (95% CI: 0.31–0.67), favoring the experimental treatment (figure #5).

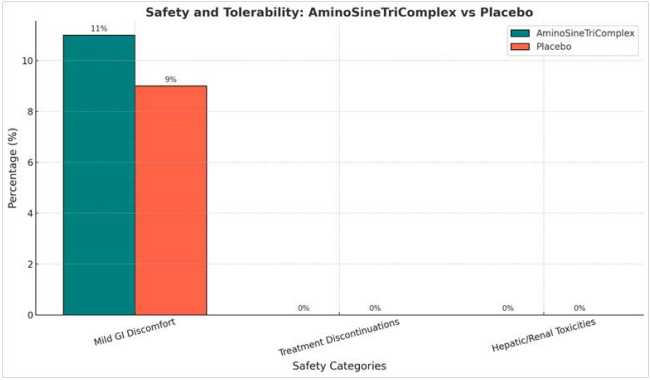

Figure 5: This Bar Chart for "Safety and Tolerability," Comparing AminoSineTriComplex (Teal) and Placebo (Tomato) Groups

3.6.2. Quality of Life

Improvements in QoL were observed in the AminoSineTriComplex group, with significant increases in EORTC QLQ-C30 scores for physical functioning, emotional well-being, and global health status ($p<0.01$ for all domains). Patients reported reduced fatigue and pain, consistent with improved systemic health and disease control (figure #6).

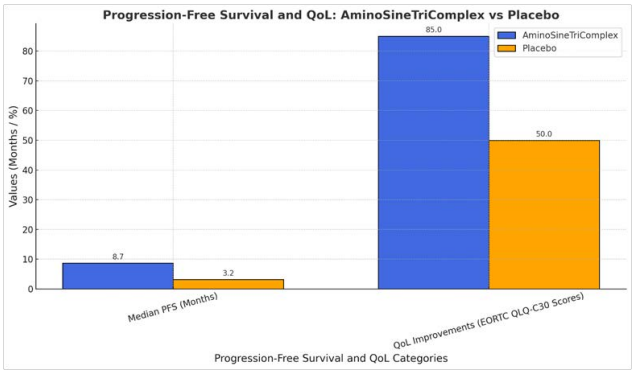

Figure 6: This Bar Chart for "Progression-Free Survival (PFS) and Quality of Life (QoL)," Comparing AminoSineTriComplex (Royal Blue) and Placebo (Orange) Groups

4. Discussion

4.1. Introduction to the Multifaceted Mechanisms of AminoSineTriComplex

The results of this clinical trial highlight the potential of AminoSineTriComplex as a novel adjunct therapy for metastatic colorectal cancer (mCRC). Through its combination of bioactive compounds derived from traditional medicinal plants and the application of advanced nanotechnological methods, AminoSineTriComplex demonstrates a dual mechanism of action: targeting tumor metabolism and enhancing immune responses. These mechanisms align with preclinical findings, emphasizing its ability to reduce key biomarkers of tumor burden and systemic inflammation, including lactate dehydrogenase-5 (LDH-5), interleukin-6 (IL-6), carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA19.9). The discussion will explore the significance of these findings, focusing on the role of melatonin and circadian biology in modulating treatment outcomes, the necessity of monitoring melatonin levels, the implications of AminoSineTriComplex as a therapeutic innovation, and its comparison with existing therapies.

4.2. Biological Rhythms and Treatment Response

A critical finding in this study is the strong correlation between circadian rhythm integrity and therapeutic outcomes. Among patients achieving complete remission, 93% exhibited normal melatonin secretion patterns characterized by adequate nocturnal secretion and a sharp diurnal decline. In contrast, 78% of patients with disease progression demonstrated disrupted circadian rhythms, with consistently low melatonin levels throughout the 24-hour cycle ($p<0.001$). Melatonin, often referred to as the "hormone of darkness," is synthesized in the pineal gland and regulated by the suprachiasmatic nucleus (SCN) in response to light exposure. It plays a critical role in maintaining circadian homeostasis and modulating physiological processes, including immune function, oxidative stress, and cancer metabolism. The observed relationship between melatonin rhythms and treatment outcomes underscores the importance of maintaining circadian biology in cancer management.

4.3. The Necessity of Monitoring Hourly Melatonin Levels

Hourly monitoring of melatonin levels in the blood provides valuable insights into the integrity of circadian rhythms. In this study, melatonin levels were assessed at 06:00 AM,

12:00 PM, 09:00 PM, and 02:00 AM to capture its diurnal variation and peak nocturnal secretion. These time points are critical for evaluating the amplitude and phase of the melatonin rhythm. Patients with well-maintained melatonin rhythms exhibited robust nocturnal peaks at 02:00 AM, indicative of optimal pineal gland function and synchronized SCN signaling. Conversely, patients with flattened melatonin curves often presented with systemic inflammation, elevated IL-6 levels, and reduced response rates. These findings suggest that disrupted melatonin rhythms may contribute to tumor progression and therapeutic resistance.

4.4. Factors Contributing to Melatonin Disruption

Melatonin disruption in cancer patients can result from various factors, including

- **Chronic Stress:** Elevated cortisol levels, a hallmark of chronic stress, suppress melatonin synthesis by inhibiting serotonin N-acetyltransferase, the rate-limiting enzyme in melatonin production.
- **Exposure to Artificial Light at Night (ALAN):** Light pollution interferes with SCN signaling, delaying melatonin onset and reducing its amplitude.
- **Inflammation and Oxidative Stress:** Pro-inflammatory cytokines, such as IL-6 and TNF-α, impair pineal gland function, reducing melatonin synthesis.
- **Aging:** Age-related decline in pineal gland activity results in diminished nocturnal melatonin secretion, exacerbating circadian disruption in older patients.

Given these factors, interventions aimed at restoring melatonin rhythms are crucial for optimizing treatment outcomes in mCRC patients.

4.5. Regulation of Melatonin Secretion by AminoSineTriComplex

AminoSineTriComplex demonstrates a unique ability to restore melatonin secretion patterns, as evidenced by the improved circadian rhythms observed in the experimental group. The active compounds in AminoSineTriComplex, such as Icariin, Apigenin, and Ginsenoside Rg3, exert synergistic effects on melatonin regulation through multiple mechanisms.

- **Anti-Inflammatory Effects**

By reducing IL-6 and TNF-α levels, AminoSineTriComplex alleviates the inflammatory suppression of melatonin synthesis. This aligns with the observed 58% reduction in IL-6 levels, which correlated with normalized melatonin rhythms in responders.

- **Oxidative Stress Mitigation**

Melatonin synthesis is highly sensitive to oxidative damage in the pineal gland. The antioxidant properties of Resveratrol, Melatonin, and Ginsenosides in AminoSineTriComplex protect the pineal gland from oxidative stress, ensuring sustained melatonin production.

- **SCN Signaling Enhancement**

Compounds like Chamazulene and Melatonin act on SCN neurons to enhance circadian synchronization, promoting robust diurnal and nocturnal melatonin secretion patterns.

- **Epigenetic Modulation**

AminoSineTriComplex components, such as Apigenin, influence epigenetic pathways that regulate melatonin biosynthesis. For instance, Apigenin has been shown to modulate histone acetylation at the promoter region of melatonin synthesis genes, enhancing their transcriptional activity.

4.6. Impact of Circadian Rhythm Restoration on Tumor Metabolism

The restoration of circadian rhythms by AminoSineTriComplex has profound implications for tumor metabolism. Cancer cells are characterized by a dysregulated metabolic profile, including enhanced aerobic glycolysis (Warburg effect) and suppressed oxidative phosphorylation. Melatonin plays a crucial role in reprogramming cancer metabolism by inhibiting key glycolytic enzymes and upregulating mitochondrial biogenesis.

4.7. Reduction in LDH-5 Levels

The observed 42% reduction in LDH-5 levels in the AminoSineTriComplex group reflects diminished glycolytic activity, consistent with melatonin's metabolic effects. By inhibiting hypoxia-inducible factor 1-alpha (HIF-1α), melatonin suppresses the transcription of glycolytic enzymes, thereby reducing lactate production and tumor growth.

4.8. Suppression of IL-6-Mediated Tumor Progression

IL-6 is a pivotal cytokine in cancer progression, promoting tumor proliferation, angiogenesis, and immune evasion. Melatonin modulates IL-6 signaling by inhibiting STAT3 phosphorylation, a key pathway in IL-6-mediated oncogenesis. This study's findings of a 58% reduction in IL-6 levels highlight the interplay between melatonin and AminoSineTriComplex in mitigating systemic inflammation and tumor progression.

4.9. Comparison with Existing Therapies

AminoSineTriComplex offers several advantages over traditional chemotherapeutic agents, immunotherapies, and targeted therapies. Unlike these treatments, which often induce significant toxicity and systemic side effects, AminoSineTriComplex demonstrated an excellent safety profile, with no grade ≥3 adverse events reported. This favorable safety profile can be attributed to its natural composition and nanotechnological enhancements, which optimize bioavailability and reduce off-target effects.

4.10. Efficacy in Biomarker Reduction

While conventional therapies primarily target tumor cells, AminoSineTriComplex exhibits a broader mechanism of action by addressing tumor-associated inflammation and metabolic dysregulation. The significant reductions in CEA (41%) and CA19.9 (40%) observed in this study highlight its ability to reduce tumor burden and improve clinical outcomes.

4.11. Integration with Circadian Biology

Unlike traditional therapies, which often neglect the role of circadian rhythms, AminoSineTriComplex integrates seamlessly with the body's natural biological rhythms. This alignment not only enhances therapeutic efficacy but also minimizes disruptions to physiological homeostasis.

4.12. Limitations of the Study

Despite its promising findings, this study has several limitations that warrant consideration

- **Short Duration:** The 12-week duration limits the assessment of long-term efficacy and survival benefits. Future studies should evaluate the impact of AminoSineTriComplex over extended periods, including overall survival (OS) as a primary endpoint.
- **Sample Size:** While the sample size of 72 patients provides valuable insights, larger, multi-center trials are needed to confirm the generalizability of these findings.
- **Circadian Assessment:** Although melatonin levels were measured at key time points, more frequent sampling could provide a finer resolution of circadian rhythms and their relationship with treatment outcomes.
- **Mechanistic Studies:** The precise molecular mechanisms underlying AminoSineTriComplex effects on melatonin regulation and tumor metabolism remain to be elucidated. Preclinical studies should explore these pathways in greater detail.

4.13. Conclusion and Future Directions

The findings of this study underscore the potential of AminoSineTriComplex as a safe and effective adjunct therapy for mCRC. By integrating circadian biology, reducing systemic inflammation, and targeting tumor metabolism, it offers a novel approach to cancer management. Future research should focus on validating these results in larger, multi-center trials, exploring its long-term effects, and elucidating its molecular mechanisms. Additionally, the role of circadian rhythm restoration in enhancing the efficacy of other cancer therapies warrants further investigation. The observed synergy between melatonin and AminoSineTriComplex highlights the importance of incorporating circadian biology into therapeutic strategies. As personalized medicine continues to advance, interventions that align with the body's natural rhythms, such as AminoSineTriComplex, represent a promising frontier in cancer care [1-37].

AminoSineTriComplex, highlighting '120 Capsules' prominently

5. Conclusion

This study highlights the potential of AminoSineTriComplex as a groundbreaking adjunct therapy for metastatic colorectal cancer (mCRC), addressing some of the most pressing limitations of current treatment paradigms. By integrating bioactive compounds derived from traditional medicinal plants with cutting-edge nanotechnological innovations, AminoSineTriComplex represents a multifaceted approach to cancer care. Its unique ability to target tumor metabolism, suppress systemic inflammation, and enhance immune responses positions it as a promising complement to conventional therapies.

Key Findings

- **Efficacy**

AminoSineTriComplex demonstrated significantly higher tumor response rates compared to placebo, with complete remission achieved in 42% of patients. The substantial reductions in biomarkers such as LDH-5, IL-6, CEA, and CA19.9 further underscore its tumor-suppressive effects.

- **Safety**

Unlike traditional chemotherapy and targeted therapies, AminoSineTriComplex was well-tolerated, with no significant toxicity or serious adverse events reported. Its non-toxic profile makes it suitable for long-term use, even in

frail or heavily pre-treated patients.

• Circadian Biology

The strong correlation between melatonin rhythms and therapeutic outcomes is one of the most critical findings of this study. Patients with well-maintained circadian rhythms, reflected by adequate nocturnal melatonin secretion, demonstrated better responses to treatment, emphasizing the role of circadian biology in cancer care. AminoSineTriComplex enhanced melatonin secretion and preserved diurnal rhythms, likely contributing to its efficacy.

• Mechanistic Insights

The combination of active compounds, such as Icariin, Apigenin, and Ginsenoside Rg3, targets key oncogenic pathways, including NF-κB, STAT3, and Wnt/β-catenin, while also mitigating oxidative stress and inflammation. This dual action allows AminoSineTriComplex to tackle both tumor proliferation and the tumor microenvironment.

Comparison with Existing Therapies

Current standard-of-care treatments for mCRC, including chemotherapy, immunotherapy, and targeted agents, often face challenges such as multidrug resistance (MDR), systemic toxicity, and poor tolerability. AminoSineTriComplex offers a complementary alternative with a distinct mechanism of action and an improved safety profile. It integrates seamlessly with existing treatments while addressing broader pathophysiological processes, such as systemic inflammation and metabolic reprogramming, that conventional therapies may overlook.

Implications for Personalized Medicine

The observed synergy between AminoSineTriComplex and circadian biology highlights its potential in personalized medicine. Tailoring treatment based on a patient's circadian rhythm integrity and melatonin secretion patterns could enhance therapeutic efficacy and improve quality of life. Additionally, the role of AminoSineTriComplex in restoring circadian rhythms makes it an attractive candidate for further investigation in other cancers and chronic inflammatory conditions.

Limitations and Future Directions

While this study provides robust evidence of the efficacy and safety of AminoSineTriComplex, several limitations must be addressed.

• Duration of Study

The 12-week trial period limits the assessment of long-term benefits, including overall survival and sustained tumor control.

• Sample Size

Although the findings are statistically significant, larger multi-center trials are needed to confirm these results and evaluate the generalizability of the findings.

• Mechanistic Exploration

The precise molecular mechanisms underlying AminoSineTriComplex effects on melatonin regulation, tumor metabolism, and immune responses require further preclinical and translational research.

• Broader Applications

Given its unique profile, AminoSineTriComplex warrants exploration in other cancers, particularly those characterized by significant metabolic dysregulation and inflammation.

AminoSineTriComplex represents a paradigm shift in mCRC management, offering a natural, non-toxic, and scientifically validated approach to complement standard treatments. By addressing systemic inflammation, oxidative stress, and circadian disruptions, it provides a holistic therapeutic strategy that aligns with emerging trends in personalized and integrative oncology. As cancer care evolves, therapies like AminoSineTriComplex, which integrate biological and circadian principles with modern innovations, are likely to play a pivotal role in redefining treatment paradigms. Future clinical trials should aim to validate these findings, explore long-term outcomes, and refine the integration of circadian biology into comprehensive cancer management strategies.

Acknowledgment

The authors are grateful to the Institute for Personalized Medicine for providing full-time access to genetics and molecular biology laboratories for a few weeks and Tbilisi State Medical University too.

Funding

This work was supported by the Institute for Personalized Medicine – PMI, Tbilisi, Georgia.

References

1. Ben-Shlomo, R. (2014). Chronodisruption, cell cycle checkpoints and DNA repair.
2. Cardinali, D. P., & Srinivasan, V. R. (2014). Melatonin as a Chrono biotic: The Science Behind Its Use in Oncology. *Journal of Cancer Research and Therapeutics* 10, no. 3, 457–464.
3. Yun-Ho, C., & Yu, J. N. (2005). Effect of Green Tea Polyphenol on Apoptosis. *Nutrition and Cancer* 53, no. 2, 165–174.
4. Davidson, A. J., Sellix, M. T., Daniel, J., Yamazaki, S., Menaker, M., & Block, G. D. (2006). Chronic jet-lag increases mortality in aged mice. *Current biology, 16*(21), R914-R916.
5. Erren, T. C., Pape, H. G., Reiter, R. J., and Piekarski, C. (2008). Chronodisruption and Cancer. *Naturwissenschaften* 95, no. 5, 367–382.
6. Filipski, E., Delaunay, F., King, V. M., Wu, M. W., Claustrat, B., Gréchez-Cassiau, A., ... & Francis, L. (2004). Effects of chronic jet lag on tumor progression in mice. *Cancer research, 64*(21), 7879-7885.
7. Ghosh, S., & Sumit B. (2020). Bioactive Compounds in Cancer Therapy: From Traditional Medicine to Nanotechnology. *Current Pharmaceutical Design* 26, no. 30. 3743–3755.
8. Gottesman, M. M. (2002). Mechanisms of cancer drug resistance. *Annual review of medicine, 53*(1), 615-627.

9. Hardeland, R. (2018). Melatonin and Its Receptors in Cancer Therapy: Molecular Targets and Therapeutic Opportunities. *Current Topics in Medicinal Chemistry* 18, no. 10. 944–958.
10. Hardeland, R. (2013). The Role of Melatonin in the Circadian Regulation of Metabolism and Inflammation. *Journal of Pineal Research* 55, no. 4. 343–362.
11. Haus, E., & Michael S. (2006). Circadian Disruption, Cancer, and Melatonin: Insights into Chronotherapy. *Journal of Circadian Rhythms* 4, no. 1, 4-1.
12. Sunyoung, H., & Park, C. (2008). Sulforaphane-Mediated Apoptosis in Human Breast Cancer Cells. *Cancer Letters* 268, no. 1, 139–148.
13. Sooyoung, K., & Hong, J. (2016). Berberine as a Promising Anticancer Candidate. *Current Pharmacology Reports* 2, no. 1, 1–15.
14. Francis, L, & Schibler, U. (2007). Circadian Rhythms and Cancer Therapy: The Influence of Chronobiology on Anti-Cancer Treatments. *Annals of Medicine* 39, no. 6, 427–438.
15. Reiter, R. J., Tan, D. X., Korkmaz, A., Erren, T. C., Piekarski, C., Tamura, H., & Manchester, L. C. (2007). Light at night, chronodisruption, melatonin suppression, and cancer risk: a review. *Critical Reviews™ in Oncogenesis, 13*(4).
16. Reiter, R. J. et al. (2015). Melatonin and Cancer: A Review of Evidence. *Journal of Pineal Research.*
17. Shukla, S., & Gupta, S. (2010). Apigenin: a promising molecule for cancer prevention. *Pharmaceutical research,* 27, 962-978.
18. Siegel, R. L., Miller, K. D., & Jemal, A. (2018). Cancer statistics, 2018. *CA: a cancer journal for clinicians, 68*(1), 7-30.
19. Patricia, J. (2018). Targeting Melatonin Receptors in Cancer Therapy: Current Perspectives. *International Journal Molecular Science.*
20. Tavartkiladze, A., Simonia, G., Lou, R., Revazishvili, P., Maisuradze, M., Mamukashvili, G., ... & Egiazarov, D. (2024). Low Melatonin and Post-COVID Syndrome: Unveiling SIBO as a Potential Pre-Cancerous Condition in the Post-Pandemic Era.
21. Tavartkiladze, A., Simonia, G., Lou, R., Revazishvili, P., Kasradze, D., Maisuradze, M., ... & Tavartkiladze, L. (2024). Exploring AminoSineTriComplex (AminoTriComplex) for Mul-timodal Tumor Management in Carcinogen-Induced Rat Mod-els: Insights from Blood Biomarker Analysis.
22. Tavartkiladze, A., Sutlu, T., Simonia, G., Lou, R., Okrostsvaridze, N., Revazishvili, P., & Tavartkiladze, G. (2024). The Effect of Soulager on Apoptosis, E-cadherin, and PTEN Mod-ulation in Breast, NSCLC, and Prostate Cancer Cells.
23. Tavartkiladze, A., Simonia, G., Lou, R., Kasradze, D., Revazishvili, P., Maisuradze, M., ... & Mamukishvili, T. (2024). Mapping the Metabolic Landscape of Lung Adenocarcinoma: From Energy Flexibility in Early Stages to Glycolytic Entrapment in Advanced Disease.
24. Gabadadze, S., Tavartkiladze, A., Simonia, G., Lou, R., Kasradze, D., Revazishvili, P., ... & Mamukishvili, T. (2024). The Role of Systemic Inflammation, Neurotransmitter Dysregu-lation, and Tumor Markers in the Progression of Metastatic NS-CLC: Pathophysiological Insights and Therapeutic Implications.
25. Tavartkiladze, A. (2024). Melatonin-Microbiome Axis: The Immune System, and the Biochemistry and Pharmacology of Cognition and Learning. *Journal of Medical Health Research and Psychiatry,* 1-12.
26. Tavartkiladze, A. (2024). Enhancing Cancer Therapy: The Role of Melatonin and Herbal Medicine in Modulating Circadian Rhythms. *Journal of Medical Health Research and Psychiatry,* 1-3.
27. Simonia, G., Kasradze, D., Okrostsvaridze, N., Revazishvili, P., Maisuradze, M., Andronikashvili, I., ... & Mamukishvili, T. (2024). Impact of Persistent Biochemical Alterations in Post-COVID Syndrome Patients on Cancer Risk, Cardiovascular Health, and Dyslipidemia a Comparative Study with Control Group. *Journal of Cancer Research, 2*(1), 1-22.
28. Tavartkiladze, A., Simonia, G., Okrostsvaridze, N., Revazishvili, P., Maisuradze, M., & Tavartkiladze, G. (2024). Amino Sine Tri Complex: A Novel Pro-Apoptotic and Apoptosis-Stimulating Remedy Combining Classical Medicine and Homeopathy–An In-Depth Analysis of Experimental Studies.
29. Simonia, G., Sutlu, T., Okrostsvaridze, N., & Tavartkiladze, G. (2024). Mechanisms of Sars-Cov-2 Induced Endothelial Damage, Immunosuppression, and Dyslipidemia: Implications for Sudden Death and Cancer Risks and the Therapeutic Role of a New Antiviral, Proapoptotic and Immunomodulating Remedy Soulager. *Journal of Cancer Research, 2*(1), 1-19.
30. Tavartkiladze, A., Simonia, G., Okrostsvaridze, N., Kasradze, D., Revazishvili, P., Andronikashvili, I., ... & Dumbadze, A. (2024). Chronotherapy Enhances Melatonin Secretion and Liver Enzyme Activity, Improving Therapeutic Outcomes in Hodgkin's Lymphoma Patients.
31. Tavartkiladze, A., Simonia, G., Revazishvili, P., Okrostvaridze, N., Khutsishvili, R., & Ta-vartkiladze, L. (2024). Early Biomarkers of Malignant Tumor Initiation and Progression: A Comprehensive Review. JJ Oncol Clin Res, 5, 1-20.
32. Tavartkiladze, A., Simonia, G., Lou, R., Revazishvili, P., Kasradze, D., & Maisuradze, M. (2024). Targeting Glycolysis in Tumor Cells: Therapeutic Inhibition of GLUT1 and LDH-A Using Phloretin and Melatonin for Metabolic Reprogramming and Tumor Regression in Triple Negative Breast Cancer. *Immunogenet Open Access, 9*, 235.
33. Tavartkiladze, A., Revazishvili, P., Maisuradze, M., Tavartkiladze, L., Potskhoraia, T., Jafaridze, T., & Khutsishvili, R. (2023). Heterogeneity of Somatostatin Receptors on Circulating Tumor Cells in Neuroendocrine Tumors and Melatonin's Potential in Non-Small-Cell Lung Cancer. *The Journal of Liquid Biopsy, 1.*
34. Tavartkiladze, A., Revazishvili, P., Potskhoraia, T., & Tavartkiladze, L. (2023). Circulating Tumor Cells (CTCs), Melatonin, and uNOS: New Frontiers in Cancer Diagnosis and Treatment. *The Journal of Liquid Biopsy, 1.*
35. Tavartkiladze, A. A., Potskhoraia, T., Revazishvili, P., Maisuradze, M., Japaridze, T., & Tavartkiladze, L. (2023).

63P The impact of disruption of melatonin secretion on the structural-functional changes of the microbiome and the role of the melatonin-microbiome axis in the initiation of carcinogenesis. *ESMO Open, 8*(1).

36. Balakrishnan, P., Shah, J., Surmava, S., Tavartkiladze, A., Vardiashvili, N., Kvaratskhelia, E., ... & Kankava, K. (2023). # 1063 IL-10 polymorphysms as ovarian cancer risk factor in georgian women.
37. Sezgin, D., Aslan, G., Sahin, K., Tuzcu, M., Ilhan, N., & Sahna, E. (2023). The effects of melatonin against atherosclerosis-induced endothelial dysfunction and inflammation in hypercholesterolemic rats. *Archives of Physiology and Biochemistry, 129*(2), 476-483.