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REVIEW

A CRITICAL ANALYSIS OF
THE ROLE OF FASTING
IN CLINICAL ONCOLOGY
AND AGEING:
DISPELLING A MYTH

ORIGINAL ARTICLE

EXPLORING
AUTONOMIC NERVOUS
SYSTEM RESPONSES
DURING COGNITIVE
STRESS TEST
FOR AUTOMATIC
PAIN ASSESSMENT
IN CANCER PATIENTS

ORIGINAL ARTICLE

SHOULD ITERATIVE
CYTOREDUCTIVE
SURGERY
AND HYPERTHERMIC
INTRAPERITONEAL
CHEMOTHERAPY
BE CONSIDERED
THE BEST TREATMENT
OF RECURRENT
PSEUDOMYXOMA
PERITONEI (PMP)?

MEETING REPORT

SBUR 2025 ANNUAL
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REVIEW

A CRITICAL ANALYSIS OF THE ROLE OF FASTING IN CLINICAL ONCOLOGY AND AGEING: DISPELLING A MYTH

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ABSTRACT: Over the past few decades, the role of nutrition as a vital element of health has gained significant recognition. Both the quality and quantity of dietary intake play a pivotal role in preventing age-associated diseases, such as cardiovascular conditions, neurodegenerative disorders, metabolic syndromes, and cancer. Adequate nutrition can improve the condition of cancer patients, who are often malnourished, by enhancing their response to anti-tumor therapies and reducing drug side effects, thereby improving their quality of life. Calorie restriction and periodic fasting have recently been the focus of extensive preclinical research due to their potential to extend lifespan and enhance the efficacy of anti-tumor therapies in mouse models. These dietary interventions have garnered significant attention on social media and in the media, gaining public support despite the lack of clinical validation. In this review, we examine clinical studies on dietary restriction within the contexts of oncology and longevity, with the aim of clarifying the concrete advantages these interventions may offer. As anticipated in our previous analyses, the results of clinical studies have been disappointing, as these interventions fail to provide significant benefits. This highlights the challenges associated with translating successful outcomes from animal models to human applications. However, the promotion of these dietary interventions in the mass media and on social media continues to spread alleged benefits that are not supported by clinical data.

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Abbreviations: **CR:** Calorie Restriction- consistent reduction in daily caloric intake without malnutrition. **FMD:** Fasting Mimicking Diet- a plant-based, low-calorie and low-protein 5-day lasting dietary intervention. **PF:** Periodic Fasting- significant calorie restriction over consecutive days, such as for 2 to 7 days at a time. **STF:** Short Term Fasting- a period of voluntary abstinence from food.

Key words: *fasting mimicking diet; cancer; aging; metabolism; cancer therapy.*

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INTRODUCTION

The nutritional status of cancer patients is compromised even at the initial stages of the disease, at the time of diagnosis. Indeed 20-70% of cancer patients experience malnutrition, a condition frequently linked to poorer prognosis, diminished treatment response, and reduced quality of life (1). Malnutrition may result from impaired food absorption due to tumor type, particularly when the gastrointestinal tract is affected; the development of cachexia, a multifactorial condition characterized by organ and tissue wasting as result of pro-inflammatory and metabolic alterations; and sarcopenia, or skeletal muscle wasting, arising from patients' physical inactivity, inflammatory processes, metabolic alterations, and hormonal imbalances, orchestrated by the tumor. It is important to emphasize that a malnourished individual is not necessarily underweight, as the accumulation of adipose tissue can mask the degradation of internal organs and the decline in lean body mass (2, 3).

In recent years, clinical research has focused on this pathological condition, aiming to explore and elucidate the mechanisms involved in this process to develop novel dietary or pharmacological interventions capable of preventing malnutrition and enhancing the nutritional status of cancer patients (4). Despite the concerning clinical picture regarding the nutritional status of cancer patients, several preclinical studies, primarily conducted on mouse models, have reported promising and encouraging results regarding the benefits of calorie restriction (CR), such as periodic fasting (PF), in enhancing the efficacy of anticancer therapies, in some instances even achieving complete tumor eradication and permanent cancer remission in mice, as well as extending the lifespan of healthy mice (5-16).

Considering that cancer patients often experience malnutrition, the imposition of CR may exacerbate their clinical condition. In light of the remarkable antitumor effects observed in preclinical studies involving PF, several clinical trials have been undertaken to assess the efficacy of such dietary restrictions in cancer patients (7, 17-29). A primary challenge in implementing these dietary interventions in humans is the difficulty in ensuring compliance, given the highly restrictive nature of the regimen. Moreover, these dietary interventions significantly impact the metabolism and physiology of animal models, and if such changes were to occur in humans, they could be dramatic and poten-

tially life-threatening. Consequently, as discussed in our previous work, it is improbable that the beneficial effects of these dietary interventions can be effectively translated to humans due to the inability to replicate the same physiological and metabolic changes induced by severe caloric restriction in mice (30).

In this review, we will focus on and analyze the outcomes of clinical studies conducted on cancer patients and healthy individuals wherein the PF diet has been tested both as an independent intervention and in conjunction with various therapies. In this analysis, we will critically evaluate the results and address any uncertainties regarding the true effectiveness of such stringent dietary regimes in humans and the transferability of the benefits observed in mice to human subjects.

PERIODIC FASTING IN CANCER PATIENTS FAILS TO MEET EXPECTATIONS.

The clinical oncological trials analyzed in this review are detailed in **Table 1**, which specifies the sample size, outcomes, and confounders for each study. The research primarily involved patients with breast cancer (HR⁺ and TNBC), with additional studies conducted on other cancers, including skin cancer, colorectal cancer, lumbar duct cancer, and hematologic cancers such as myeloma and CLL. Many of these studies are single-arm, Phase I pilot studies, which are small-scale trials assessing the feasibility and safety of Fasting Mimicking Diet (FMD) in cancer patients undergoing anticancer therapies, and they do not include a control group. These preliminary studies produced conflicting and inconclusive outcomes due to the limited number of participants (**Table 1**).

An early study involving ten patients with various malignancies indicated that a 2-3-day fast, conducted before and after therapy, mitigated chemotherapy side effects such as fatigue and weakness, and completely prevented vomiting and diarrhea. Although fasting induced dizziness, hunger, and headaches (26), it was generally well tolerated by the patients. However, it is crucial to acknowledge that the small sample size lacked sufficient statistical power. Additionally, the study relied on patient self-reporting of toxicity symptoms, raising the possibility that the effects might be overestimated and attributable to a placebo effect (26).

Table 1. Clinical trials on fasting/FMD and cancer.

TRIAL NAME-AUTHOR	INTERVENTIONS	CANCER TYPE	TREATMENT	SAMPLE SIZE	PRIMARY OUTCOMES	COFOUNDERS	REF
Safdie F.M. et al. -2009	Fasting (water only) 48-140h pre CHT 5-56 post-CHT	Breast, Esophagus, Lung Uterus, Ovary, Prostate	Docetaxel + Cyclophosphamide; Docetaxel + carboplatin + 5FU; Docetaxel; Carboplatin + Paclitaxel; Gemcitabine (day1)+ GMZ Docetaxel (Day8);	N:10	Patient-reported outcomes on CHT toxicity indicate a notable decrease in fatigue, weakness, and gastrointestinal side effects among six patients.	- Patient self-reporting of toxicity symptoms	(26)
NCT00936364	Fasting (water only) Arm A: 24 h pre CHT Arm B: 48 h pre CHT Arm C: 48h pre CHT-24h postCHT	Urothelial, NSCLC, Ovarian,Uterine, Breast	Gemcitabine + Cisplatin Carboplatin + Paclitaxel Docetaxel + Carboplatin + Trastuzumab	N:17 Arm A: 4 Arm B: 6 Arm C: 7	- Feasibility: Safety concerns are limited to toxicities of sG2, including fatigue, headache, and dizziness. - Effect on blood cells: There is a non-significant reduction in leukocyte DNA damage and fewer instances of G3 or G4 neutropenia in Arm B and C. - Effect on tumor growth factors: A decrease in IGF-1 levels is observed.	- No control group with normal diet - IGF1 reduction during refeeding may be from chemotherapy - Different chemotherapy regimen between 24 and 72 hours starvation and blood collection timing could affect hematology toxicity differences	(20)
NCT01954836	Modified fasting: daily caloric intake <350 kcal Arm A: modified fasting during the first half of CHT cycles (1 and 2 of four or 1-3 of six cycles) Fasting started 36 h before and ended 24 h after chemotherapy (60 h-fasting period)	Breast Ovary	Chemotherapy	N:34 Arm A: N=18 Arm B: N=16	- Modifies fasting is safe and feasible - Improved QoL - Fatigue reduction - Maintenance of body weight	- Patient self-reporting of toxicity symptoms	(17)
NCT01304251	48 hours Short term fasting-STF (water only) with TAC Arm A: Fasting 24 hours before and 24 hours after administration of chemotherapy) Arm B: diet based on guidelines for healthy nutrition	HER2-negative stage II/ III BC	Docetaxel + Doxorubicin + Cyclophosphamide	N:13 Arm A: N=7 Arm B: N=6	- STF is safe and feasible - Higher erythrocytes/ platelets day 7- DNA damage recovery signal in PBMCs- Non-hematologic toxicity similar.	-Dexamethasone treatment reverses the metabolic effects of fasting	(19)
DRKS00011610	Modified short-term fasting Arm A: 4-day fasting period during 2 to 3 cycles of chemotherapy followed by NC (normal diet period, normocaloric) during the next 2 to 3 cycles of chemotherapy Arm B: 10-day dietary intervention period including 6-day FSD (fasting supportive diet) + 4-day fasting followed by NC (normal diet period, normocaloric) during the next 2 to 3 cycles of chemotherapy Arm C: NC (normal diet period, normocaloric) during the 2 to 3 cycles of chemotherapy followed by F = 4-day fasting period during 2 to 3 cycles of chemotherapy Arm D: NC (normal diet period, normocaloric) during the 2 to 3 cycles of chemotherapy followed by FAD+F = 10-day dietary intervention period including 6-day FSD (fasting supportive diet) + 4-day fasting	Breast, Ovarian, Endometrial and Cervical.	Paclitaxel + Carboplatin Epirubicin + Cyclophosphamid Docetaxel + Cyclophosphamid	N: 51 Arm A: 11 Arm B: 16 Arm C: 4 Arm D: 20	- Modified STF is safe and feasible - Reduced incidences of stomatitis, headaches, and weakness, along with a lower total toxicity score - Fewer chemotherapy delays following mSTF - Significant decreases in insulin and IGF-1 levels	- Non randomized clinical trial	(31)
NCT02126449	4-day FMD cycles with neoadjuvant chemo vs. control diet Arm A: FMD, a plant-based low amino-acid substitution diet, comprising soups, broths, liquids, and tea. - Day 1: ~1200 kcal (CHO/protein/fat energy ratio of ~3.5/1-2) - Days 2-4: ~200 kcal (CHO >80 % of energy) +TAC Arm B: regular diet +TAC + Dexamethasone	HER2-negative stage II/ III BC	Cyclophosphamide, Doxorubicin, Docetaxel OR Paclitaxel +/- Dexamethasone	N: 129 Arm A: N=65 Arm B: N=64	No notable difference in toxicity: inability to decrease CHT AEs - Primary pCR endpoint not improved - Low adherence - Several metabolic changes (IGlucose/insulin/IGF-1) and higher CRP in FMD arm- Design confounded by differential dexamethasone	Low adherence to FMD- Dexamethasone treatment in control group could impact lymphocyte DNA damage and pathological response.	(18)

(Continued on next page)

TRIAL NAME-AUTHOR	INTERVENTIONS	CANCER TYPE	TREATMENT	SAMPLE SIZE	PRIMARY OUTCOMES	COFOUNDERS	REF
NCT03595540	FMD: low-calorie and low-protein plant-based diet. - Day 1: ~1099 kcal (11% protein, 46% fat and 43% CHO) - Day 2-5: 717 kcal (9% protein, 44% fat and 47% CHO) Between CHT cycles: a personalized recovery diet (20-30 kcal/kg) with a protein intake of 1.2-1.5 g/kg and physical activity	Breast, colorectal, prostate, glioma, melanoma, ovarian, NSCLC, pancreatic, anal, bladder, multiple myeloma acute lymphoblastic leukemia, chronic myeloid leukemia.	doxorubicin, paclitaxel, carboplatin, cisplatin, capecitabine, temozolomide, cyclophosphamide, vinorelbine, eribulin, gemtactabine, taxol, mitomycin C, etoposide, oncocarbide, XELOX, FOLFOLX, letrozole, exemestane and anastrozole, tamoxifen, fulvestrant and GnRH analogues, ruxolitinib, nintedanib and nilotinib, abemaciclib, ribociclib and palbociclib, carfilzomib, lenalidomide, trastuzumab, T-DM1, pertuzumab and bevacizumab, pembrolizumab, ipilimumab and durvalumab, dexamethasone	N: 90 Arm A: 90	- Feasibility: 90% of enrolled patients completed at least one FMD cycle, and 72% completed the study, with the number of FMD cycles ranging from 2 to 21. - Safety: 52% experienced mild and transient AEs (G1-2), with no G3-5 AEs reported. - Maintenance of stable body weight and handgrip strength. - Increase in bioidipendence phase angle and fat-free mass. - Decrease in fat mass, confirmed by CT when available. - Effect on circulating growth factors, adipokines, and cytokines/chemokines.	- Absence of a control arm and experimental arm to assess effects of physical activity and protein supplementation	(7, 32)
NCT03340935	FMD: a plant-based, calorie-restricted, low-CHO, and low-protein diet - Day 1: up to 600 kcal - Day 2-5: up to 300 kcal 5 days FMD + concomitant treatment followed by a refeeding period of 16-23 days	Breast, colorectal, Lung, Prostate Pancreas, Melanoma, Germinal, Ovary, Thyroid, Chronic lymphocytic leukemia, Non-Hodgkin lymphoma, Uterus, Sarcoma, Multiple myeloma, Stomach, Kidney, Mesothelioma	Chemotherapy, Endocrine (± targeted therapies), Immunotherapy, Targeted therapy, Radiotherapy, Radionuclide treatment	N: 101 Arm A: 101	- Safety: G3-4 FMD-related AEs 12.9%. - Feasibility: Global compliance 91.8% per cycle. - Effects on systemic metabolism: blood glucose/ growth factors reduced - Effects on antitumor immunity: There was an enhancement of IFN-γ activating immune signatures, Th1/cytotoxic responses, and tumor-infiltrating CD8+ T cells with PD-1 upregulation, as well as macrophages CD68+ and NK cells. Additionally, there was a downregulation of the immunosuppressive circulating cells, including exhausted T cells and Tregs. - Five patients achieved complete and durable clinical responses. - Effect on body composition: FMD significantly reduces the Skeletal Muscle Index (SMI) and Visceral (VAT) and Subcutaneous (SAT) Adipose Tissues.	- Tumor heterogeneity: No control arm-High CRP could induce aspecific T and NK activation - Control arm heterogeneity with mixed CP and CG - Imbalance in anthracycline and taxane exposure between groups - Higher proportion of de novo cases in FMD group vs relapsed controls	(Ligorio et al., 2024, 2022; Sposetti et al., 2025b, 2025; Vernieri et al., 2022)
NCT04248998	FMD:Cyclic, 5-day, calorie-restricted (600 Kcal on day 1; 300 Kcal on days 2-5), low-carbohydrate, low protein diet every three weeks Arm A: FMD + doxorubicin+cyclophosphamide and paclitaxel; Arm B: FMD + doxorubicin+cyclophosphamide and paclitaxel + metformin	stage-II and III triple negative breast cancer (TNBC)	doxorubicin 60 mg/mq plus cyclophosphamide 600 mg/mq, followed by twelve consecutive cycles of weekly paclitaxel 80 mg/mq plus minus metformin 850 mg twice a day	N: 30 Arm A: 13; Arm B: 17	- Safety: 70% of patients experienced Grade 3-4 adverse events (AEs); severe AEs attributable to the FMD occurred in 3% of patients, while serious adverse events (SAEs) were observed in 6.7% of all patients. - Feasibility: a full compliance rate of 63.3%. - Effects on antitumor response: excellent pathologic complete response (pCR) rates (primary endpoint) and favorable long-term clinical outcomes (secondary endpoints). - Effects on cancer metabolism: FMD is associated with the downmodulation of the glycolytic pathway and pyruvate metabolism, correlating with the pCR of highly glycolytic cancer cells.	-Lack of internal control- External control design differs from experimental group-Lack of statistically significant data-High pCR variability in experimental and external control groups- High tumor heterogeneity and chemotherapy regimen variability between groups- Higher proportion of node-negative (N0) patients in experimental vs external control-Higher BRCA mutation percentage in experimental vs external control	(21)
NCT04387084	Short term fasting (STF) for 47-48 hours prior to immunotherapy and for 24 hours after immunotherapy	Skin carcinoma	Atezolizumab, Avelumab, Cemiplimab, Durvalumab, Nivolumab, Pembrolizumab	N:10	- Feasibility: 70% of patients were able to fast for at least two-thirds of the recommended fasting intervals. - Safety: No unacceptable toxicities related to fasting or treatment were observed; - Effects on antitumor response: The efficacy of PD-1 was consistent with historical outcomes for cutaneous malignancies.		(Lin et al., 2025)

Another prospective study, conducted on 20 patients with various malignancies divided into three experimental groups and subjected to a 24-hour and 48-hour fast, either before platinum-based chemotherapy or 48 hours before and 24 hours after chemotherapy, demonstrated that fasting reduced drug toxicity, including fatigue, headache, and dizziness. Nevertheless, this study also faced significant limitations (20): it lacked a control group of patients on a normal diet, the sample size was small, and the diversity of tumors further constrained statistical validity. Adherence to the 72-hour fast was low, complicating the ability to draw definitive conclusions and affirm the protective effect of fasting due to the study's limited power. Furthermore, analysis of IGF1 levels, which are hypothesized to play a key role in protecting against nonspecific chemotherapy toxicity, revealed that although fasting reduces IGF1 levels, these levels remain reduced even during the refeeding phase. Moreover, no significant differences in IGF1 reduction were observed between a 24-hour and a 72-hour fast. Consequently, the absence of an internal control precludes determining whether the reduced IGF1 levels are attributable to chemotherapy in addition to fasting and whether this reduction in IGF1 could be associated with a reduction in side effects. Additionally, fasting was found to reduce chemotherapy-induced DNA damage in lymphocytes, particularly in the 72-hour fasting group compared to the 24-hour fasting group. However, it is important to note that the chemotherapy regimens differed between the experimental groups, as the 24-hour fasting group received a higher dose of gemcitabine/cisplatin compared to the 48- and 72-hour fasting cohorts. Furthermore, leukocytes collected during different chemotherapy cycles may not be comparable, as patients were enrolled even when they had already undergone chemotherapy cycles (20).

A randomized clinical trial involving 34 patients diagnosed with breast and ovarian cancer demonstrated that a stringent calorie restriction of 350 kcal per day, akin to fasting, mitigated the toxicity associated with chemotherapy and enhanced the patients' quality of life. It is noteworthy that this study relied on patient self-reported toxicity, which may introduce a degree of subjectivity and potential placebo effect, as the psychological state of the patients could influence their perception and reporting of these effects (17).

A randomized study involving 13 patients with HER2-negative breast cancer demonstrated that a

48-hour water-only fast (24 hours before and after chemotherapy) mitigated the hematological toxicity associated with the docetaxel/doxorubicin/cyclophosphamide regimen, in comparison to a control group on a standard diet (19). The fasting cohort exhibited an increase in erythrocytes and platelets, although no significant differences were noted in lymphocytes and neutrophils. Additionally, a reduction in DNA damage was observed, as measured by FACS detection of γ -H2AX phosphorylation in CD45⁺CD3⁻ myeloid cells 30 minutes post-therapy, and in CD45⁺CD3⁺ lymphocytes and CD45⁺CD14⁺CD15⁻ monocytes 7 days post-therapy. However, it is noteworthy that no significant differences in the onset of side effects were detected between the two groups, nor were there differences at the metabolic level. For instance, glucose and insulin levels were comparable between the groups, as were IGF-BP3 and TSH levels, while a slight but significant reduction in IGF-I and an increase in the pro-inflammatory marker C-reactive protein (CRP) were observed in the fasting group. The absence of metabolic differences between the groups may be attributed to dexamethasone treatment, which could have mitigated the effects of fasting. Consequently, this study is subject to several limitations, including the small sample size and the use of dexamethasone, which, by reversing the metabolic effects of fasting, precludes a comprehensive explanation and justification of the protective effect of fasting on chemotherapy-induced DNA damage. In this context, it is crucial to acknowledge that the data on DNA damage in leukocytes may be imprecise or contingent upon the speed of blood sample processing. Given the rapid repair of DNA damage, the absence of a swift and efficient protocol for the isolation and fixation of peripheral blood mononuclear cells (PBMCs) may have influenced the quantification of DNA damage, as highlighted by the authors (19).

A pilot study involving 30 patients with gynecological tumors demonstrated that four cycles of 96-hour fasting (48 hours before and after chemotherapy) significantly reduced grade I/II side effects, such as stomatitis [-0.16 ± 0.06 ; 95% CI $-0.28 - (-0.03)$; $P = 0.013$], headaches [-1.80 ± 0.55 ; 95% CI $-2.89 - (-0.71)$; $P = 0.002$], and weakness [-1.99 ± 0.87 ; 95% CI $-3.72 - (-0.26)$; $P = 0.024$] (31). Additionally, fasting improved tolerance to therapy by decreasing the frequency of chemotherapy postponements. However, this study did not observe a reduction in gastrointestinal toxicities, such as nausea, vomiting, and diarrhea, as reported by Safdie et al. and

Dorff et al (20, 26). Contrary to the findings of De Groot and Dorff (19, 20), fasting cycles in this study did not affect erythrocyte, thrombocyte, and neutrophil counts, thereby not supporting the hypothesis that fasting protects against leukocyte depletion and DNA damage. Furthermore, unlike previous studies by De Groot and Dorff, this study found that fasting cycles significantly reduced insulin and free T3 levels, while confirming the reduction in IGF-1 and increase in free T4 observed in prior research. A limitation of this study is the small sample size, which restricts its statistical power, along with low compliance with fasting protocols. Additionally, the study was not randomized, as patients were selectively assigned to study groups, potentially influencing their psychological state (31).

In a randomized clinical trial involving 131 patients with HER2-negative stage II/III breast cancer, FMD, administered for 96 hours (24 hours prior to and 48 hours following neoadjuvant chemotherapy), did not demonstrate efficacy in enhancing pathological complete response (pCR) (10.8% in the FMD group versus 12.7% in the control group; OR 0.830, 95% CI 0.282–2.442, $P = 0.735$) or in mitigating grade III/IV chemotherapy side effects compared to the control group (27.7% FMD vs 23.8% control, $P = 0.580$) (18). Consequently, the study was terminated prematurely. Additionally, the rate of chemotherapy discontinuation was comparable between the control and FMD groups, with no significant differences observed in quality of life or overall distress. Notably, patient adherence to FMD was suboptimal, with only 50% of participants completing two cycles and 33.8% completing four cycles. Data analysis indicated that radiological complete and partial responses, assessed via MRI and ultrasound prior to surgery, were approximately three times higher in the FMD group compared to the control group, with improvements correlating with FMD compliance. However, it is important to emphasize the discrepancy between pathological and radiological responses, despite similar trends. Therefore, the radiological response data, while promising, should be interpreted with caution as it does not correspond with the pathological response. FMD was found to significantly reduce insulin, glucose, and IGF levels after three or more cycles. Furthermore, the FMD group exhibited a significant increase in the pro-inflammatory marker CRP, consistent with findings from other studies. A protective effect of FMD against chemotherapy-induced DNA damage was also observed in CD45⁺CD3⁺ T lymphocytes, as evidenced by a

reduction in phosphorylated H2AX levels 30 minutes post-chemotherapy in the FMD group. A major limitation of this study is the experimental design, as dexamethasone was administered throughout the treatment course in the control group but omitted in the FMD group. Previous studies have shown that dexamethasone alters metabolic markers such as insulin, glucose, and IGF-1, potentially amplifying in this study the observed differences between the control and FMD groups. However, these metabolic differences do not appear to correlate with a reduction in chemotherapy side effects or improvements in quality of life, as previously hypothesized. Additionally, dexamethasone may induce DNA damage in lymphocytes, potentially exacerbating chemotherapy toxicity and explaining the differences in H2AX phosphorylation between the control and FMD groups. Finally, it is possible that dexamethasone administration may influence the differences in radiological responses between the control and FMD groups by modulating the immune response (18). In this context, it is noteworthy that a recent Phase III clinical trial, initiated in 2023, involving HR⁺ HER2⁻ breast cancer patients has been suspended. The data indicate that FMD is unlikely to provide a significant or meaningful benefit to patients (<https://www.clinicaltrials.gov/study/NCT05503108>).

The FMD was demonstrated to be safe and effective in improving various metabolic markers in a single-arm, phase I/II clinical trial (NCT03595540) involving 90 patients with solid and liquid tumors (7, 32). The regimen, which included cycles of FMD combined with 20–30 minutes of daily physical activity and a protein supplement of 1.2–1.5 g/kg daily during the three-four week refeeding period, resulted in reduced circulating levels of growth factors, adipokines, and cyto/chemokines, as well as serum c-peptide, IGF1, IGF1BP3, and leptin, while adiponectin and IGF1BP1 levels increased. These alterations persisted several weeks post-FMD cycle, during the refeeding phase. Additionally, patients undergoing FMD maintained their weight and handgrip strength, reduced fat mass, and increased lean mass. Although the study presents promising findings, as the reduction in growth factors such as insulin and IGF-1 may influence tumor growth and enhance the efficacy of anti-tumor therapies, significant limitations exist due to the sample size, absence of a control arm, and lack of an experimental arm to assess the effects of physical activity and protein supplementation on metabolic markers and body composition changes. Nonetheless, adherence to FMD

was observed to decrease with the progression of cycles (7, 32).

In a single-arm clinical study (NCT03340935) involving 101 participants, primarily with solid tumors but also including healthy subjects, cycles of FMD demonstrated potential in enhancing antitumor responses (29). This was achieved by reducing the percentage of immunosuppressive myeloid and regulatory T cells both systemically and intratumorally, while concurrently promoting the activation of CD8 T cells and natural killer (NK) cells. Consistent with prior research, the FMD cycles resulted in a systemic reduction in glucose and other growth factor levels, replicating metabolic changes similar to those observed in preclinical studies, albeit less pronounced, which may facilitate a more effective antitumor response. Notably, compliance with FMD in this study was significantly higher than in previous studies, achieving a rate of 91.8% when evaluating individual FMD cycles. Specifically, patients completed 404 out of the 440 cycles that were scheduled. Importantly, these fasting cycles did not adversely affect the patients' nutritional status, as all participants regained their weight during the 3-week refeeding period. Despite the promising nature of the data, the study is limited by its small sample size, tumor heterogeneity, and absence of a control arm, necessitating caution in data interpretation. FMD-induced immunomodulation was observed in both cancer patients undergoing therapy and healthy individuals. While the activation of cytotoxic T lymphocytes and cytolytic NK cells in peripheral blood may suggest an enhanced antitumor response in cancer patients, the reason for similar activation in healthy individuals remains unclear and may be attributed to a stress response, as fasting cycles have been associated with increased levels of the proinflammatory factor CRP, a stress indicator, in several clinical studies. Additionally, the study indicates that T lymphocyte activation in cancer patients follows a cyclical pattern, increasing during the fasting phase and returning to baseline during the refeeding phase. This suggests that the immune response activation is not tumor-specific, as it would otherwise exhibit a constant pattern throughout the therapeutic course, irrespective of the fasting/refeeding cycles. Regarding the increased intratumoral immune response, it is noteworthy that the immune signature associated with enhanced Th1/cytotoxic responses and enrichment of IFN γ signaling was identified through comparative RNA sequencing analysis of tumor biopsy samples taken before and after FMD and chemo-

therapy cycles. However, the study lacks a control, such as RNA sequencing analysis of biopsies from patients on a normal diet before and after chemotherapy. Consequently, it is not possible to ascertain whether the favorable antitumor immune signature observed in FMD-treated samples is attributable to FMD, chemotherapy, or potentially linked to the wound healing effect of the biopsy on pro-inflammatory and immune cell activation. Therefore, these data should be interpreted with caution (29). Moreover, the remarkable responses observed in this study among a limited number of patients lack statistical significance, as they may represent outliers commonly found in clinical trials (23).

A subanalysis of the NCT03340935 clinical trial indicates that FMD cycles may enhance overall survival (OS) but not progression-free survival (PFS) in patients with advanced triple-negative breast cancer undergoing first-line carboplatin-gemcitabine therapy (22). The OS for 14 patients who underwent FMD and were treated with carboplatin-gemcitabine (CG) was 30.3 months (95% CI 18-NR). In contrast, the OS for 76 patients treated with either carboplatin plus gemcitabine or carboplatin plus paclitaxel (CP) was 17.2 months (95% CI 15.3-25.1), with a log-rank P value of .041(22). This subanalysis yielded promising data; however, certain biases must be acknowledged. In the control group, only 25% of patients received carboplatin-gemcitabine, while 75% received carboplatin plus paclitaxel. This distribution could affect OS outcomes, as the authors found patients receiving first-line carboplatin-gemcitabine showed superior PFS and OS compared to those receiving carboplatin-paclitaxel. When comparing OS of patients in the FMD group with those of the 19 control patients treated with carboplatin-gemcitabine, differences are not statistically significant, though trending toward better OS in the FMD group (median OS: 30.3 months, 95% CI: 18.0-NR, vs. 15.3 months, 95% CI 13.7-31.6, log-rank P value = .052). Even more importantly, it is worth emphasizing that, in the overall control group, 80% had received anthracyclines and 75% taxanes, while in the control subgroup treated with carboplatin-gemcitabine, all patients had previously received treatment with taxanes and 79% anthracyclines, compared with 57% in the FMD group. The choice between carboplatin-paclitaxel and carboplatin-gemcitabine likely depends on clinical considerations, such as prior taxane use, rather than randomization. Therefore, the selection and combination of these treatments in the control group is not random and this clinically significant disparity increases con-

founder risk. Patients previously treated with anthracyclines and taxanes may present with more chemorefractory disease or cumulative toxicities affecting OS in first-line metastatic therapy, while those without prior therapies may be more chemosensitive. To interpret the outcomes of first line therapy, considering previous therapies is essential. In the FMD group, 36% of metastases are de novo, versus 5.3% in the control group. Within the CG control subgroup, most metastases are recurrent. Patients with de novo metastases typically experience better outcomes compared to those with recurrent metastases, especially when the disease-free interval after adjuvant therapy is short. The FMD group comprises many de novo cases, while the CG control subgroup consists of relapses, which are presumably harder to treat. This meta-analysis does not permit definitive conclusions regarding FMD efficacy in enhancing outcomes in advanced TNBC. This is due to confounding factors, including control arm heterogeneity with mixed CP and CG, imbalance in prior exposure to anthracyclines and taxanes between groups, and higher proportion of de novo cases in the FMD group versus relapsed controls, which may have influenced the analysis and presumed FMD benefits (22). A recent clinical study (NCT04248998) published in *Cell Metabolism* reports that cycles of FMD administered every three weeks in conjunction with chemotherapy (anthracycline-cyclophosphamide-taxane chemotherapy, with or without metformin) prior to surgery significantly enhance the rate of pCR and prolong event-free survival (EFS) in patients with early-stage triple-negative breast cancer (TNBC) (21). The study involved 30 patients, divided into two experimental cohorts (group A: 13 patients; group B: 17 patients), both receiving FMD and anthracycline-cyclophosphamide-taxane chemotherapy, with the distinction that metformin treatment was excluded in group A but included in group B. The pCR rates were 53.9% in group A and 58.8% in group B, with an overall mean of 56.6% across both groups. Due to the absence of an internal control, pCR rates were compared with those from previous studies with similar experimental designs. This comparison indicated that the overall pCR rate in the FMD group exceeded that of other control studies, particularly among overweight women who achieved weight loss by limiting food consumption and energy intake through FMD. Additionally, the 3-year EFS in the FMD group surpassed that of the external control group (86.7% vs. 65.8%, respectively). Omics analyses of tumor biopsies suggested that FMD may potentiate the effects

of chemotherapy by inhibiting glycolytic metabolism, particularly in TNBC characterized by high glycolytic activity, and to a lesser extent in TNBC with oxidative phosphorylation (OXPHOS) metabolism. The influence of FMD on glycolytic metabolism results in a significant reduction in lactate dehydrogenase (LDH), detectable in the blood primarily after the first FMD cycle, which may correlate with an enhanced anti-tumor immune response. The authors observed too an increase in immune signature, as detected by RNA sequencing analysis of tumor biopsies, indicating activation and enrichment of cytotoxic T lymphocytes and cytolytic NK cells exclusively in patients with glycolytic TNBC who responded to therapy and achieved complete pathological response, whereas this signature was absent in non-responsive TNBC tumors (21). However, the study is limited by its small sample size and, notably, the lack of an internal control, which precludes obtaining statistically significant data to substantiate the hypothesis that FMD augments the efficacy of chemotherapy in TNBC patients. Firstly, the standard deviations of the pCR for both individual and combined experimental groups are notably high, therefore achieving statistically significant results would likely require a considerable increase in the sample even with an internal control. Second, given that pCR rates in clinical trials used as external controls vary significantly, ranging from 14% to 48.5% (33-40), the inclusion of an internal control is crucial for ensuring the validity and reliability of experimental results. Third, although pCR in studies analogous to the one under discussion vary between 30% and 39% (33, 36, 38), it is worth highlighting that the percentage of patients with node-negative (cN0) disease enrolled in this study (NCT04248998) is significantly higher than in other studies used as external controls and this could positively influence pCR. Indeed, a Dutch retrospective study presented at the ESMO Congress 2024, showed that neoadjuvant anthracycline-taxane-based chemotherapy administered to 1,144 patients with stage 1 cT1c TNBC from 2012 to 2022, as recorded in the Dutch Cancer Registry, achieved a pCR in 57.6% of patients. Notably, no significant differences were observed between patients receiving platinum-based therapy and those treated solely with anthracyclines and taxanes (57.1% versus 57.6% for patients not treated with platinum; $p = 0.9$) (41). The findings of this Dutch study contest and refute the conclusions regarding the advantages of FMD in augmenting the effectiveness of chemotherapy in patients with TNBC. Fourth, the percentage of patients with BRCA pathway mutations

enrolled in NCT04248998 is notably higher, with only 50% of patients being wild-type (WT), 25% carrying mutations in the BRCA pathway, and the remaining 25% untested, compared to 10% in the external control studies. This aspect is pertinent, as tumor cells with BRCA pathway mutations exhibit a high glycolytic metabolism (42), and glycolytic tumor cells have been found to be more sensitive to FMD combined with chemotherapy. Furthermore, it is noteworthy that tumors with mutated BRCA respond more favorably to anthracycline/taxane therapy than BRCA wt tumors, suggesting that a higher prevalence of patients with mutated BRCA may have positively influenced the pCR and EFS observed in the study (43, 44). Anyway, it is possible that FMD may enhance therapeutic responses, particularly in individuals with overweight or obesity, by modulating metabolic and endocrine functions. By decreasing adiposity in these patients, FMD could potentially lower estrogen production, hyperinsulinemia, and insulin resistance, thereby preventing the activation of the phosphatidylinositol-3-kinase/AKT/mammalian target of rapamycin (mTOR) pathway and mitigating the chronic proinflammatory state, both of which are adverse prognostic factors associated with elevated body mass index (BMI). Although this hypothesis is plausible, a study (NCT01627067) involving overweight and obese patients with metastatic, hormone receptor-positive, HER2-negative breast cancer indicated that treatment with metformin (an antidiabetic drug), everolimus (an mTOR inhibitor), and exemestane (an aromatase inhibitor), targeting these specific pathways altered in overweight individuals, has moderate clinical benefits. Consequently, the absence of an internal control represents a significant limitation that may have also affected the various omics analyses conducted in the study, given that the external controls employed differed in terms of chemotherapy type, timing and cycles of drug administration, as well as the timing of biopsy collection and the biological samples used for analyses. These discrepancies could serve as confounding factors potentially influencing the final results and conclusions. For instance, a notable distinction between this study and the preceding one pertains to the influence of FMD on immunomodulation (21, 29). The previous study asserted that FMD enhances the presence of cytotoxic T lymphocytes and cytolytic NK cells within the tumor, based on the immune signature derived from RNA sequencing of biopsies obtained solely from the experimental FMD group (29). In contrast, the current study identified the immune signature

exclusively in tumors that exhibited a pCR to therapy, and not in those that did not achieve such a response (21). Consequently, this data raises questions regarding whether the immune signature and intratumoral enrichment of cytotoxic lymphocytes are primarily effects related to the response to chemotherapy, independent of fasting. Similarly, the peripheral blood immunomodulation observed in the previous study may be more attributable to fasting-induced stress rather than a tumor-specific response (29). Therefore, while the reported results and their interpretations offer significant insights, they are not necessarily substantiated by statistical evidence, given the absence of internal controls, a limited sample size that constrains statistical power, and various inaccuracies in the experimental design. These findings necessitate prospective, randomized studies with homogeneous control arms and predefined stratifications to determine whether FMD confers a genuine survival benefit in TNBC.

The fragility of clinical data regarding the benefits of FMD in oncology practice is further supported by a pilot study (NCT04387084) presented at ASCO 2025 (45). This study demonstrated that a 72-hour FMD (200 calories/day), administered 48 hours before and 24 hours after immunotherapy every three weeks to 10 oncology patients (70% with melanoma, 20% with cutaneous squamous cell carcinoma, and 10% with basal cell carcinoma), did not enhance the efficacy of anti-PD-1-based therapy (45). This finding aligns with some preclinical studies (46) but contradicts others that have reported its efficacy (47). The results were consistent with historical outcomes for cutaneous malignancies, showing a 30% overall response rate (10% complete responses and 20% partial responses), 40% stable disease, and 20% progressive disease. Full compliance with the FMD regimen (9 out of 9 cycles) was achieved by 50% of patients, while 70% achieved partial compliance (i.e., 6 out of 9 cycles). The study's limitations are evident due to the small sample size of skin cancer patients, who, despite being among the most responsive to immunotherapy, did not exhibit any improvement in their therapeutic response (45). Additionally, the impact of FMD on body composition in cancer patients warrants consideration. An analysis of 36 patients enrolled in a phase IB clinical study (NCT03340935) indicated that FMD cycles reduce visceral (VAT) and subcutaneous (SAT) adiposity, as well as the Skeletal Muscle Index (SMI), potentially leading to sarcopenia and an increased risk of mortality in cancer patients (48).

IS FASTING AN EFFECTIVE APPROACH TO COMBAT AGING? THE PERSPECTIVE IS CHANGING

Research on aging faces inherent limitations in tracking long-term effects of interventions on health, quality of life, and longevity. Existing studies have focused on interventions over shorter periods (3 months to 2 years), examining their impact on cardiometabolic, inflammatory, or epigenetic markers linked to aging. No research has conclusively demonstrated any intervention's effectiveness in halting aging. The positive outcomes in short-term studies on aging-related biomarkers suggest potential to lower disease risks associated with aging. However, long-term physiological effects remain uncertain. In this review, **Table 2** outlines notable preclinical studies and limited clinical studies on healthy individuals over brief periods, with details on sample sizes, primary outcomes, and adverse effects. Dietary strategies, including CR and FMD, have been tested on overweight or obese populations, who showed the most significant benefits.

Numerous preclinical investigations have demonstrated that CR represents the most potent non-pharmacological intervention capable of extending the maximum lifespan in mice (49, 50). The advantageous effects on aging are directly proportional to the extent of CR, with more stringent dietary regimens yielding greater anti-aging outcomes. A study published in *Nature* in 2024 revealed that a 40% calorie restriction can extend the maximum lifespan in mice by 30%, whereas a less restrictive regimen, such as fasting for 1-2 days per week, enhances median lifespan but does not affect maximum lifespan. The impact of CR on aging is contingent upon the mouse strain, thereby being intricately linked to the genetic and epigenetic characteristics of each strain. A notable finding from this study is that the health benefits of CR are not necessarily correlated with lifespan extension. For instance, metabolic shifts induced by calorie restriction, such as improved fasting blood glucose levels, reduced adiposity, increased energy expenditure, and preservation of metabolic flexibility (delta respiratory quotient), did not predict lifespan extension. Consequently, the health benefits of CR may not translate into a significant extension of lifespan. In fact, life extension was observed in mice that maintained better body weight retention, exhibited a high proportion of lymphocytes, and had immune cells in a physiological resting state, such as CD4⁺ and CD8⁺ naive T cells and immature

NK cells. Conversely, immune cells displaying activation or mature phenotypes, such as CD4⁺ and CD8⁺ effector T cells and CD11⁺ memory B cells, were generally associated with a reduced lifespan. Furthermore, certain adverse effects of dietary restriction may be detrimental to other aspects of physiological health, such as lifelong loss of lean mass, lower body temperature, increased food-seeking behavior (indicative of hunger), and alterations in the immune repertoire that could potentially increase susceptibility to infection (51). These findings in mice raise concerns regarding the potential risks of extreme dietary restriction for humans. Therefore, as discussed and highlighted in our previous review, given the differences in metabolic rates between humans and mice, it is improbable that the beneficial effects of dietary restriction interventions can be replicated and applied to humans (30) (**Figure 1A**).

Aging is associated with epigenetic drift, which is characterized by alterations in DNA methylation at various sites. The methylation status of several age-related methylated (ARM) genes in the blood has been demonstrated to correlate with aging, thereby serving as biomarkers for assessing biological age, as exemplified by the PhenoAge and GrimAge tests (52). Research conducted on animal models has indicated that CR of 40% and 30% can reduce biological age in mice and monkeys (53). However, the effects of CR on biological age in humans are not as pronounced as those observed in animal models. In the CALERIE study, where normal-weight subjects underwent an average 12% calorie restriction for two years, no significant differences in biological age, as measured by PhenoAge and GrimAge, were observed between the experimental and control groups (54), although improvements in certain cardiometabolic markers associated with aging were scored. Conversely, three cycles of the FMD, conducted once a month for 6 months, were shown to reduce biological age by an average of 2.5 years in predominantly overweight or obese patients enrolled in the NCT02158897 and NCT02158897 studies. Simultaneously, FMD improved several age-related cardiometabolic markers, such as insulin resistance, reduced hepatic fat, and an increased lymphoid-to-myeloid ratio, in overweight or obese participants (55). Nevertheless, this final observation is intuitive, considering the established understanding that weight gain and obesity contribute to elevated blood pressure, cholesterol, and inflammation—three factors that increase the risk of numerous life-threatening diseases, including type 2 diabetes, cardiovascular dis-

Table 2. Preclinical study and clinical trials on fasting/FMD and caloric restriction on longevity.

INTERVENTIONS	MODEL SYSTEM	SAMPLE SIZE	PRIMARY OUTCOMES	ADVERSE EFFECTS	CLINICAL TRIAL	REF
Caloric Restriction	Mouse (preclinical)	-	Increased expression of stress response gene and metabolic shift in muscle tissue	-	-	(49)
Caloric Restriction	Mouse (preclinical)	-	10 to 20 percent increases in mean and maximum survival times compared to the control mice.	-	-	(50)
Caloric Restriction and Intermittent Fasting	Mouse (preclinical)	960 genetically diverse female mice	caloric restriction extends maximum lifespan; intermittent fasting improves slightly the mean lifespan. Retention of body weight, high lymphocyte proportion, low red blood cell distribution width and high adiposity in late life are associated with increased lifespan. Reduced adiposity and lower fasting glucose, were not associated with increased lifespan	-Loss of lean mass, hypothermia, and changes in the immune repertoire with increased susceptibility to infections	-	(51)
Caloric Restriction (30-40%)	Rhesus monkeys and Mouse (preclinical)	N: 57 rhesus monkeys; 43 mice	CR prolongs lifespan in mice and monkeys, markedly delays methylation drift and results in a significantly younger "methylation age"	-	-	(53)
Caloric Restriction (12%) Arm A: Normal Diet ad libitum Arm B: 12% CR	Human Normoweight	N: 218 Arm A: 75; Arm B: 143	CR intervention did not affect the PhenoAge and GrimAge DNAm clocks.	-Lack of gold standard measure of biological aging	CALERIE- NCT00427193	(54)
Fasting Mimicking Diet (FMD). 5-day FMD cycles: FMD, a plant-based low amino-acid substitution diet, comprising soups, broths, liquids, and tea. - Day 1: ~1200 kcal (CHO/protein/fat energy ratio of ~3.5/1-2) - Days 2-4: ~200 kcal (>80% of energy)	Human (>60%-Overweight or Obese)	NCT02158897 N: 100 Normal Diet: N=48 FMD: N=52; NCT04150159 N: 84 Mediterranean Diet: N=40 FMD: N=44	FMD cycles reduce the median biological age by 2.5 years. FMD improves insulin resistance, reduces hepatic fat, and increases lymphoid-to-myeloid ratio in overweight or obese participants	-Low sample size -25% FMD dropout; -participants in the trial, characterized by favorable social, economic, behavioral, and health attributes, do not constitute a homogeneous or representative sample of the general population.	Multi-cycle Prolon Diet- NCT02158897; Evaluation of a Fasting Mimicking Diet- NCT04150159	(55)
Intermittent Fasting and Caloric Restriction. Arm A: CR 75% energy intake daily; Arm B: IF 24-hour fasting with 150% energy intake on alternate days for 3 weeks; Arm C: IF 24-hour fasting without net energy restriction, with 200% energy intake on alternate days	Human (lean and healthy individuals)	N: 36 Arm A: 12; Arm B: 12; Arm C: 12	Caloric restriction resulted in a reduction in body mass (-1.91±0.99 kg), primarily attributable to fat loss (-1.75±0.79 kg). Limiting energy intake through fasting (0:150) also led to a decrease in body mass (-1.60±1.06 kg), albeit with a less pronounced reduction in body fat (-0.74±1.32 kg). In contrast, fasting without energy restriction (0:200) did not significantly affect either body mass (-0.52±1.09 kg) or fat mass (-0.12±0.68 kg).	- The proportion of males and females was not equal between groups. - compliance was self-reported -Small sample size	Impacts of Intermittent Fasting on Energy Balance and Associated Health Outcomes NCT02498002	(56)
Intermittent Fasting (2-3 nonconsecutive day of complete fasting per week for 6 moth) Arm A: Intermittent Fasting Arm B: Normal Diet	Human (overweight)	N: 50 Arm A: 28 Arm B: 22	- Intermittent fasting results in an 8% reduction in body weight. - Intermittent fasting does not alter the serum levels of C-reactive protein, cytokines, or chemokines -IF improve oral glucose tolerance test (OGTT)-derived insulin sensitivity indexes significantly, but this improvement is clinically irrelevant.	- Small number of participants	-	(57)
The Impact of Nutrition on Human Health from Birth to 28-30 Years of Age Arm A: individuals with a normal BMI; Arm B: individuals who experienced the onset of obesity during adolescence; Arm C: individuals who exhibited obesity from early childhood	Human (normoweight and overweight)	N: 205 Arm A: 89; Arm B: 43; Arm C: 73	- Obesity increases aging markers in adults aged 28 to 31 years, causing epigenetic alterations, telomere attrition, chronic inflammation, impaired nutrient sensing, mitochondrial stress, and impaired intercellular communication.	- Selection bias of participants arises from a non-random subset of the original cohort due to budgetary constraints. -Utilizing BMI as the primary exposure does not accurately reflect body fat distribution or quantity.	-	(58)

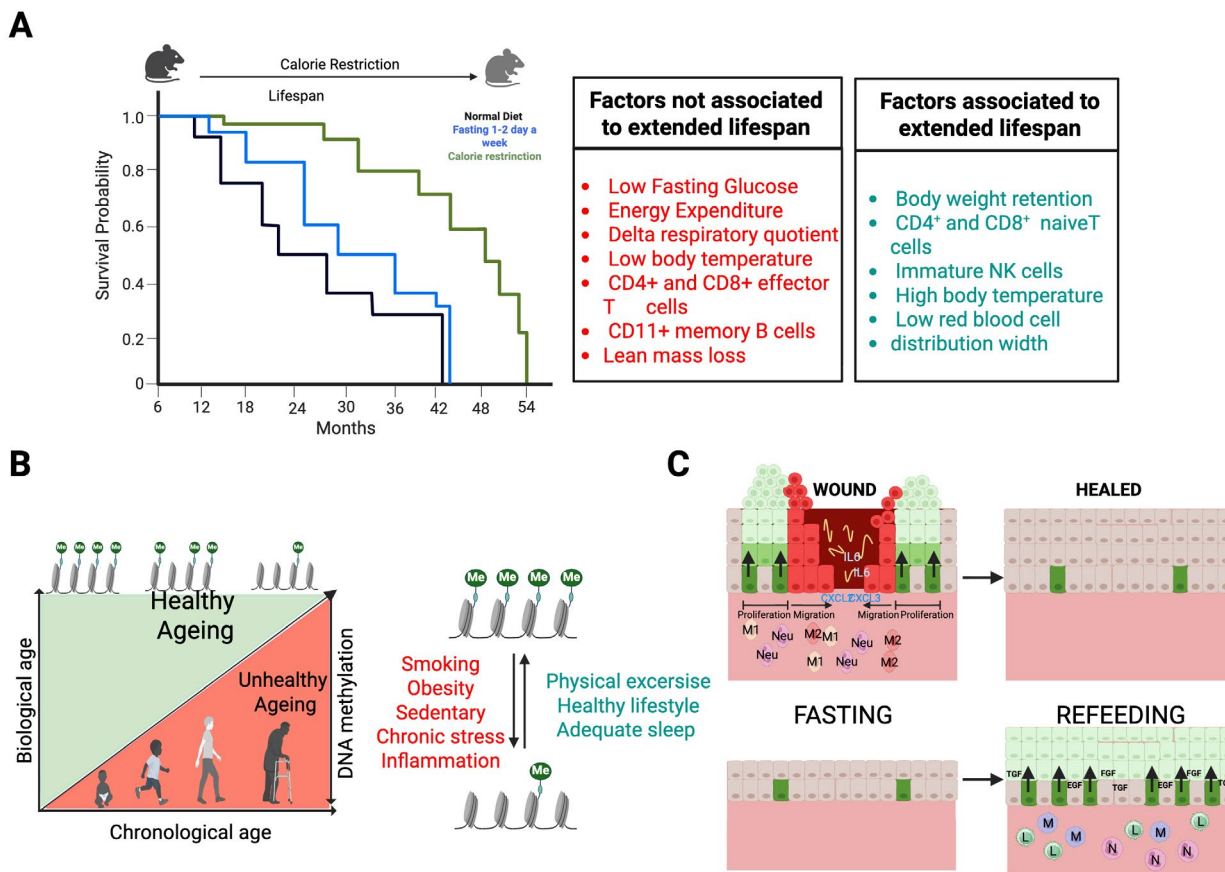


Figure 1. The Impact of Caloric Restriction and Fasting on Lifespan, Biological Age, and Stem Cells.

A) Caloric restriction, as opposed to fasting for 1–2 days per week, has been shown to extend the maximal lifespan in mice. B) Biological age, an indicator of cellular aging determined by DNA methylation, which diminishes with age, is affected by various environmental factors and lifestyle choices. C) In mice, severe caloric restriction, such as periodic fasting, leads to significant organ shrinkage due to limited nutrient availability. Upon refeeding, the provision of nutrients and the release of growth factors restore the original tissue dimensions, facilitating the enrichment of stem cells and their differentiation into the diverse cell types that comprise the tissue. The impact of nutrient deprivation on tissues is analogous to a tissue wound and its subsequent repair process. L: Lymphocytes; M: Macrophages; Neu: Neutrophils; EGF: epidermal growth factor; FGF: fibroblast growth factor; CXCL2-3: Chemokine (C-X-C motif) ligand; IL6: Interleuchin 6; TNF: Tumor Necrosis Factor.

eases such as myocardial infarction and stroke, and various cancers. Consequently, the advantageous effects of FMD may not be linked to the modulation of specific molecular pathways or cellular mechanisms, as it does not provide significant benefits in normal-weight individuals. Instead, these benefits could primarily result (56) from weight loss and, more importantly, the reduction of adipose tissue, thereby mitigating the principal risk factors associated with ageing diseases.

Extensive preclinical research has consistently shown that CR stands as the only dietary intervention capable of extending lifespan, surpassing FMD in its efficacy to enhance cardiometabolic markers. Given

that the benefits of fasting in humans are largely attributed to reduced caloric intake and not to the regulation of fasting dependent mechanism (autophagy, ketone bodies) (56), CR is often favored over fasting diets due to its primary role in promoting fat mass reduction, whereas fasting regimen leads to substantial lean mass loss (56). Additionally, fasting interventions in humans has been found to exert no influence on the expression of critical cardiometabolic markers, such as glucose tolerance and insulin sensitivity, nor on stress and proinflammatory markers, including CRP and a range of cytokines and chemokines, in obese individuals (57). Therefore the reasons behind the pronounced differ-

ences in biological age observed in the two studies remain elusive.

The Santiago Longitudinal Study, which monitored 1,000 individuals from birth until they reached ages 28 to 31, offers a solution to this issue(58). The study's primary advantage is its extensive duration and its examination of a cohort from birth, which facilitates the comparison between biological and chronological age. This approach differs from prior clinical studies, which often assessed intervention effects over shorter durations and lacked a clearly defined control group. The Santiago study revealed that biological age, assessed through two epigenetic clock tests (Horvath and GrimAge), increased from 2.23 years to 4.68 years in individuals who maintained a high BMI from early childhood or adolescence, as opposed to those with a normal BMI. The study involved 205 participants, who were categorized into three distinct groups: 89 individuals with a normal BMI, 43 individuals who developed obesity during adolescence, and 73 with obesity from early childhood. As a result, this study challenges the conventional understanding of CR's impact on aging by demonstrating, for the first time, that overweight and obesity initiate metabolic and physiological processes that accelerate DNA methylation linked to aging (59). Contrary to prevailing hypotheses, CR does not reduce biological age but rather mitigates the acceleration of aging associated with overweight and obesity (60). In murine models, restricting nutrients may extend lifespan by preventing overweight and obesity in rats and mice that are fed *ad libitum*. This restriction helps avoid the accumulation of fat and weight, which can lead to a pro-inflammatory state and the development of dermatitis and ulcerative conditions that contribute to age-related diseases. In the study examining the impact of various dietary restrictions on the longevity of diversity outbred female mice (51), it was observed that the control group females attained a peak weight of 45 grams at 20 months, significantly exceeding their typical weight range of approximately 25-30 grams. Conversely, the dietary interventions under investigation successfully reduced weight, maintaining it within the normal range of 25-35 grams (51). Thus, the enhancement of lifespan and healthspan is not contingent upon the mechanisms regulated by calorie restriction or fasting, but rather on the altered metabolism and inflammatory processes linked to excess fat mass and weight in *ad libitum* fed mice which may result in a decreased life expectancy. If the mice in the control group of calorie restric-

tion studies, which have been published so far, are found to be overweight or obese, it would necessitate a complete reevaluation of the benefits of calorie restriction on longevity. To improve health and reduce disease risk, maintaining a healthy weight through attention to dietary quality and quantity, as well as lifestyle choices that favor physical activity and exercise, is sufficient. It is noteworthy that, although the biological clock is currently regarded as the optimal tool for measuring biological aging in gerontology, it is not yet considered a reliable and accurate system due to conflicting opinions regarding its clinical validity. The variability in biological aging is influenced by numerous factors, including obesity, genetic variants, diet quality, tobacco use, and environmental pollutants, which may affect epigenetic remodeling. However, current epigenetic analyses are still unable to fully decipher and encompass all aspects of epigenetic changes, and the data are not easily interpreted in a clear and unequivocal manner (61) (**Figure 1B**).

Proponents of CR and PF propose a hypothesis that these dietary interventions may counteract aging by enhancing tissue regeneration. This process is thought to occur through the stimulation of self-renewal and the enrichment of stem cells, alongside the potential reprogramming of differentiated cells back into stem cells (62-65). Severe CR appears to promote the enrichment of stem cells, as the deprivation of nutrients, growth factors, and cytokines results in a notable reduction in organ size within animal models. This phenomenon arises from the necessity to curtail metabolic activity and energy expenditure by entering a conservation state, thereby activating autophagy to derive energy from the degradation of organelles and macromolecules. Additionally, the body's inability to adequately synthesize all necessary metabolites and molecules to maintain the structural integrity of organs such as the spleen, liver, immune system, and intestine contributes to this effect. In studies involving mice, even short-term fasting significantly impacts their metabolism and physiology, leading to a notable decrease in leukocyte levels and a substantial slowdown in the differentiation and regeneration of the intestinal epithelium. Extended fasting further exacerbates these effects, causing significant changes in the microvilli and nutrients absorption (30, 66). During the refeeding period, this tissue and organ "damage" is repaired as nutrient intake supports the production of factors crucial for the activation and proliferation of stem cells. Thus, the increase

in hematopoietic and intestinal stem cells observed post-fasting is a physiological response to the tissue “damage” induced by fasting, without involving any genetic reprogramming or conversion of differentiated cells into stem cells (63, 67-69). Consequently, the enrichment of stem cells during severe dietary restriction may be regarded as a physiological response to the “damage” inflicted on organs by insufficient nutrient availability. This adaptation to limited nutrient availability also serves as a biological alert mechanism, ready to be activated when nutritional conditions improve. The proliferation of stem cells and their differentiation into various cell types during refeeding facilitate the restoration of organ structures to their full size. Therefore impact of severe dietary restriction on stem cells mirrors a scenario akin to tissue damage induced by a wound. Indeed, lesions of the epidermis or intestinal epithelium, as well as hemorrhages, release factors that promote the activation of hematopoietic stem cells (HSCs) and, more broadly, an increase in the stem cells required to repair the damage and restore the tissue. However, during the healing phase, the increase in stem cells necessary for tissue regeneration is not attributed to the epigenetic reprogramming of differentiated cells into stem cells. Instead, it results from the stimulation of self-renewal in existing stem cells (70, 71) (**Figure 1C**).

What are the limitations of the hypotheses linking CR and fasting to increased stem cell activity and tissue regeneration as prerequisites for their anti-aging effects? While these theories are compelling, it is crucial to consider the complexities of human physiology. Throughout an individual's lifespan, the body undergoes continuous transformations, resulting in alterations in both appearance and physiological function. This ongoing process necessitates constant tissue remodeling, which relies on the capacity of stem cells to proliferate and regenerate tissue by producing new cells. Consequently, organisms periodically regenerate and renew their tissues and organs. A study published in *Nature Medicine* (72) indicates that the human body turns over approximately 330 ± 20 billion cells daily (equivalent to about 4 million cells per second), with blood cells, along with intestinal and gastric cells, exhibiting the highest turnover rates, accounting for approximately 96% of cell turnover. The average turnover time for intestinal cells is about 5 days, for leukocytes it ranges from 12 to 20 days (T lymphocytes 100-200 days, monocytes 1-2 days, granulocytes a few hours, platelets 7 days), while the regeneration of the skin

epithelium (epidermis) occurs within 15 days. Thus, almost of tissues and organs are in a state of constant regeneration due to the self-renewal capacity of stem cells, which diminishes with age. Therefore, if tissues and organs are capable of continuous and rapid regeneration, what advantages might severe caloric restriction confer? In the hypothetical absence of these regenerative mechanisms in humans, it would be reasonable to consider nutrient deprivation as a means to stimulate stem cell self-renewal; however, this scenario does not apply. Under physiological conditions, stem cells represent a small population of cells that self-renew through replication, maintaining a constant number (72). If this equilibrium is disrupted by adverse events such as tissue damage or nutrient deprivation, it triggers an increase in stem cell activity through the release of factors that promote their division. Therefore CR or fasting may be conceptualized as stressors that facilitate stem cell enrichment, not through the epigenetic reprogramming of differentiated and aged cells into stem cells, as posited by some scientist, but rather by activating mechanisms that promote stem cell self-renewal and expansion. The activation of these tissue regeneration processes, induced by stringent dietary restrictions, could theoretically prevent aging if the human body were incapable of self-renewing stem cells and regenerating its organs and tissues within a relatively short timeframe. Moreover, as extensively discussed in prior reviews, fasting in mice—the primary preclinical model used to study this intervention—induces significant metabolic and physiological changes, leading to a severe structural tissue and organs alterations that are not replicable in humans due to the potential for severe adverse health effects, including potentially fatal outcomes. Consequently, it is uncertain whether dietary restriction will yield the same lifespan benefits in humans as observed in mice. Although preclinical studies have shown promise, there are numerous inconsistencies, controversial interpretations regarding the effects of restriction on aging biomarkers, and challenges in translating these dietary interventions from rodents to humans. Assertions that such interventions can effectively extend human lifespans to 120 years are exceedingly imprudent and hazardous, given the absence of robust scientific evidence supporting their efficacy in humans. These claims appear more akin to marketing slogans for the longevity industry, aimed at promoting various purportedly miraculous products for profit and personal gain.

CONCLUSIONS

In general, pilot clinical studies on FMD in clinical oncology have been conducted with a limited number of participants, and the data obtained, despite lacking statistical significance, have been interpreted with optimism (73, 74). This has led to biased conclusions, occasionally resulting from data misinterpretation, which authors have emphasized without providing robust evidence for the potential benefits of fasting. It is evident that small sample sizes and the frequent lack of internal controls can substantially affect the variability of results, potentially resulting in unreliable outcomes and discrepancies between studies. These clinical studies are justified by the remarkable preclinical data of FMD in oncology studies; however, as previously discussed in our review (30), there are significant limitations in translating the benefits of fasting from animal models to humans. Furthermore, clinical studies conducted to date confirm that the metabolic changes induced by FMD, or fasting, in humans are mild compared to the mouse model. Therefore, we recommend exercising great caution as multicenter, randomized, two-arm study using the same therapeutic regimen, with aligned follow-up after the first infusion, and with a careful balancing of key prognostic factors has not yet been conducted. Such clinical trial would allow analysis of FMD's sensitivity and efficacy, and definitively establish its efficacy in oncology, excluding confounding factors, helping resolve the inconclusive results of initial clinical studies that tested fasting's potential benefits in oncology. Moreover, it will be essential to evaluate the potential adverse effects of FMD on malnourished cancer patients. As highlighted in a recent review(2), 30-70% of cancer patients are malnourished at diagnosis, even if their weight appears normal or above normal. Malnutrition is particularly common in patients with advanced cancers. Sarcopenia is also prevalent among overweight or obese cancer patients, indicating underlying metabolic disorders and nutritional deficiencies. Malnutrition acts as a negative prognostic factor; thus, it will be crucial to assess the impact of FMD on the nutritional status of patients, especially those suffering from cachexia and sarcopenia.

In evaluating the purported advantages of dietary restriction on aging and longevity, it is imperative to acknowledge the necessity of long-term clinical studies to substantiate its efficacy and validity. Contrary to prevailing assumptions, dietary restric-

tions may extend lifespan in preclinical models primarily by preventing age-related weight gain and obesity rather than by reprogramming metabolic, genetic, and epigenetic profiles. Evidence shows that mice in control groups fed ad libitum become overweight and obese as they age. This occurs due to age-associated metabolic deceleration, limited physical activity in cages, and unrestricted food access, which contribute to excessive weight gain. For instance, the weight of an adult female mouse, typically 25-30 grams, can increase to 45 g at 20 months when fed ad libitum, representing a 50% or more increase in body mass, indicating obesity. This aspect has been overlooked by researchers, who believe that the benefits of caloric restriction, considered the most potent non-pharmacological intervention for decelerating aging and extending lifespan, are solely attributable to gene expression modulation, epigenetic reprogramming, and autophagy induction. This perspective fails to consider that the advantages of calorie restriction for extending lifespan and preventing age-related diseases may merely result from mitigating excessive weight gain in mice fed ad libitum, which were erroneously classified as having normal weight rather than being overweight and obese. Consequently, asserting that fasting or CR can enhance human longevity is a precarious and somewhat imprudent stance. Disseminating such unverified information within the scientific community could have adverse effects on the population, exacerbating eating disorders that increasingly afflict young individuals and potentially deteriorating the health of older adults, who require adequate energy and protein intake to prevent sarcopenia. Moreover, recent studies on aging indicate that both overweight and obesity may expedite the aging process. CR does not possess the capacity to "rejuvenate" the population, as it offers no benefit to individuals of normal weight. Its positive impact on aging is confined to those who are obese or overweight, not due to the activation of specific mechanisms or pathways, but rather because it facilitates weight reduction and, importantly, decreases fat mass, which appears to be a catalyst for aging through the modulation of metabolism and chronic inflammation.

In conclusion, this analysis suggests that the "secret" to improved and prolonged life lies in maintaining a healthy weight through dietary regulation and physical activity, without resorting to fad diets that, while potentially effective, are often promoted primarily for commercial and profit-driven purposes.

An important consideration involves the current condition of the scientific community and measures to avert harmful developments. Since the COVID-19 pandemic, pseudo-scientists have gained visibility through sensational claims based on weak research. These individuals, acting as science communicators and former scientists, have reached a wide audience susceptible to misinformation due to inadequate scientific literacy. This has facilitated the spread of anti-scientific ideas, leading to public confusion. The scientific community's failure to exercise oversight in information dissemination, along with insufficient efforts to counter anti-scientific narratives, has empowered those who question science, favoring persuasive stories that exploit the existing confusion and lack of scientific authority. Therefore, the scientific community must implement rigorous self-regulation to curb the spread of pseudo- and anti-scientific narratives.

METHODS

Search Strategy and Data Sources

We conducted a comprehensive literature search to identify clinical studies examining fasting and caloric restriction interventions in oncology and aging. The search was performed in PubMed/MEDLINE and Scopus databases from January 2009 through January 2025. The search strategy combined Medical Subject Headings (MeSH) terms and text words including: ("fasting" OR "fasting-mimicking diet" OR "FMD" OR "short-term fasting" OR "caloric restriction" OR "periodic fasting" OR "intermittent fasting" OR "water-only fasting") AND ("cancer" OR "neoplasm" OR "chemotherapy" OR "neoadjuvant therapy" OR "breast cancer" OR "triple-negative breast cancer" OR "aging" OR "biological age" OR "longevity") AND ("clinical trial" OR "randomized controlled trial" OR "pilot study"). No language restrictions were applied initially, though only English-language publications were ultimately included.

Study Selection

This narrative review focused on human clinical studies evaluating dietary restriction interventions. Inclusion criteria were: (1) prospective clinical trials (Phase I/II, pilot studies, or randomized controlled trials) testing fasting or fasting-mimicking diet interventions; (2) studies conducted in cancer patients receiving active treatment or in healthy individuals;

(3) studies reporting clinical, metabolic, or aging-related outcomes; and (4) full-text articles published in peer-reviewed journals. Exclusion criteria were: preclinical studies, case reports with fewer than 5 participants, review articles, editorials, and conference abstracts without full publication.

Given the narrative review design, article selection was performed by the authors based on relevance to the review objectives, with emphasis on studies that could inform understanding of the translational potential of dietary restriction from bench to bedside. The selection process was guided by the multidisciplinary expertise of the author team, incorporating perspectives from molecular biology, nutrition science, and clinical oncology.

Data Extraction and Quality Assessment

From each selected study, we extracted the following data: first author, publication year, study design, sample size, participant characteristics (cancer type and stage for oncology studies; age and health status for aging studies), intervention protocol (type, duration, and timing of dietary restriction), comparator interventions, concurrent treatments, primary and secondary outcomes, adverse events, and study limitations. For oncology trials, we specifically noted pathological response rates, toxicity grades, and survival outcomes. For aging studies, we focused on biological age assessments, metabolic markers, and body composition changes.

While formal quality assessment tools were not applied given the narrative review methodology, we critically evaluated each study for methodological rigor, including presence of control groups, randomization, blinding where applicable, statistical power, adherence rates, and potential confounding factors. Particular attention was paid to identifying design limitations that could affect interpretation of results.

Data Synthesis

We employed a narrative synthesis approach to integrate findings across studies. Studies were organized thematically into two main categories: (1) fasting interventions in cancer patients, and (2) caloric restriction effects on aging markers. Within each category, we analyzed patterns of findings, consistency of results, and methodological factors that might explain heterogeneity in outcomes.

The synthesis emphasized critical evaluation of the evidence, including assessment of the gap between preclinical promises and clinical results, identifica-

tion of common methodological limitations, and evaluation of potential biases in study design and interpretation. We specifically examined whether metabolic and physiological changes observed in animal models were replicated in human studies and whether these translated to meaningful clinical benefits.

A total of 19 primary studies met our inclusion criteria and were included in the final analysis: 13 clinical trials examining fasting interventions in cancer patients and 5 studies investigating caloric restriction effects on aging biomarkers, with one study addressing both domains. The narrative synthesis approach allowed us to provide a comprehensive critical assessment while acknowledging the limitations inherent in translating dietary restriction interventions from preclinical models to human clinical practice.

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ORIGINAL ARTICLE

EXPLORING AUTONOMIC NERVOUS SYSTEM RESPONSES DURING COGNITIVE STRESS TEST FOR AUTOMATIC PAIN ASSESSMENT IN CANCER PATIENTS

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ABSTRACT: Despite progress in guideline-driven cancer pain management, crucial challenges persist. Automatic Pain Assessment (APA) investigates behavioral aspects and biosignals, such as electrodermal activity (EDA) and heart rate (HR) variability (HRV), for providing objective and context-aware pain evaluation and monitoring. In this prospective, single-center study on cancer pain, we recorded EDA and HRV during a 3-block cognitive Stroop task and stratified patients by 0-10 Numeric Rating Scale (NRS) pain (<6 vs ≥6). Since chronic pain can induce cognitive and attentional alterations, the aim is to assess the performance of the autonomic nervous system during a cognitive stress test, investigating the links between nociception (the encoding of noxious stimuli through autonomic or behavioral responses) and the neurobiological consequences of pain. We extracted tonic and phasic electrodermal activity (EDA) features, including skin conductance level (SCL), skin conductance response (SCR), recovery times, and time/frequency-domain HR/HRV indices, and then we compared groups across Stroop phases. Patients with lower pain intensity showed consistently higher SCL and stronger phasic SCR components across test phases (SCL mean $p \leq 0.05$; SCR mean and SCR integral $p \leq 0.01$), whereas rise/recovery times did not differ; peak counts normalized by phase duration diverged modestly (significant in Stroop phases 1 and 3). HRV differences were limited, with a notable increase in HF power during the incongruent (phase III) block only. These exploratory findings provide initial evidence that cognitive stress-evoked autonomic responses may contribute to distinguishing pain phenotypes in cancer patients. Pipeline and results can be used for developing a multimodal APA framework aimed at personalizing cancer pain treatment.

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INTRODUCTION

Pain is one of the most common and debilitating symptoms in cancer patients. However, despite advances in pain management in oncology, many

patients still report poorly controlled pain (1). The prevalence of pain during anticancer treatment is estimated to range from 40% to 70%, reaching up to 90% in terminal stages (2). Therefore, in this vulnerable group, understanding the mechanisms and

exact pathophysiology of pain is essential for developing research approaches and personalized treatment strategies (3).

Pain assessment is commonly performed using unidimensional or multidimensional scales. These approaches, however, present several limitations, especially in individuals who are unable to properly express pain, such as newborns (4), and non-communicative patients with cognitive impairment (5) or under sedation, including those in intensive care units (6). The automatic pain assessment (APA) refers to the evaluation of pain through the application of behavioral techniques and biosignal analysis. This approach usually integrates objective physiological data, such as heart rate variability (HRV), electrodermal activity (EDA), and electroencephalography (EEG), with behavioral observations such as facial expressions and body movements, to detect and quantify pain in a standardized way (7). The aim is to provide continuous, real-time, and unbiased pain monitoring across different clinical settings (8).

Different applications of APA strategies in cancer patients have been proposed. They focus on biosignals (9, 10), facial expressions and other behaviors (11), singularly considered or through multimodal processes (12). However, several aspects still need to be clarified. These include the specific clinical setting of analysis and its correlation with the underlying oncological condition, the varying impact and reliability of measurements in acute *versus* chronic pain scenarios, the optimal model design, the reference datasets to be used, and the integration of biosignal data with clinical and instrumental information. Existing studies, for example, are often conducted in controlled laboratory settings. They address mainly acute pain and lack external validity for real-world cancer care (7). Furthermore, most APA models rely on single-modality inputs, while multimodal models, although more accurate, are not standardized for clinical deployment (12). Importantly, no consensus exists on which markers best capture objectively acute or chronic pain-related phenomena, including autonomic dysregulation (13).

Additionally, APA strategies should capture the multifaceted and dynamic features of chronic pain, also acknowledging that it reflects maladaptive neuroplastic changes involving multiple brain regions, particularly those within the limbic system (14, 15). For example, the prefrontal cortex often shows impairments in cognitive processes related to top-down regulation, contributing to altered pain modulation and emotional processing (16, 17). Chronic pain,

indeed, is associated with neurocognitive issues, including attention, inhibition, and executive control. This reduced inhibitory control may amplify the physiological cost of cognitive interference (18). Research suggested that cognitive stressors such as the Stroop task, which engages prefrontal executive control networks, may represent a valuable method for assessing pain-induced neurophysiological dysfunction (19).

The impact of chronic pain on the regulation and balance of the sympathetic-vagal axis is a key area that warrants further investigation. Chronic cancer pain involves not only peripheral nociceptive and central sensitization processes, but also dysregulation of the autonomic nervous system (ANS), particularly in the balance between sympathetic and parasympathetic activity. This autonomic dysfunction may modulate pain experience, prognosis, and response to stressors (9, 20). Consequently, investigation in this area is crucial for defining appropriate study pathways on which to base APA analyses as well as for better addressing neurocognitive aspects of pain perception (21-23).

On these premises, as part of a research project on artificial intelligence (AI) and pain in cancer patients, we investigated the phenomenology of the ANS in individuals with chronic cancer pain undergoing cognitive assessment. We assumed that the integration of a cognitive challenge with biosignal monitoring would enhance the sensitivity to detect autonomic reactivity patterns and provide a reliable approach to characterize pain-related ANS alterations. Specifically, we investigated whether EDA and HRV differ according to pain intensity across Stroop task phases. We hypothesized that patients with higher pain levels would exhibit blunted autonomic reactivity compared with those reporting lower pain, reflecting impaired sympathetic-parasympathetic modulation during cognitive interference. Ultimately, this work represents an attempt to shed light on the links between nociception (the encoding of noxious stimuli through autonomic or behavioral responses) and the neurobiological consequences of pain.

MATERIAL AND METHODS

Study design and Ethics

This investigation was carried out within the “*Refining multiple artificial Intelligence strategies for automatic pain assessment investigations (RUGGI)*” study,

designed for exploring the integration of AI in chronic pain evaluation at the AOU San Giovanni di Dio e Ruggi d'Aragona, Salerno, Italy. The study was conducted in accordance with the Declaration of Helsinki (2013 amendment) and ICH-GCP guidelines. Ethical approval for this study (Comitato Etico Territoriale Campania 2, N°2024/28590) was provided by the Ethical Committee Campania 2 (Chairperson Prof. C. Napoli) on the 3rd of April 2025. Written informed consent was obtained from all participants before enrollment.

Participants

Patients were consecutively recruited from the oncology and palliative care units of our institution. Inclusion criteria were age ≥ 18 years, diagnosis of solid cancer, presence of chronic pain (≥ 3 months), ability to communicate and provide informed consent, and completion of the full Stroop test while wearing biosignal sensors. Exclusion criteria were severe cognitive impairment, psychiatric or neurological disorders affecting autonomic function, inability to complete the cognitive task, implanted cardiac devices, arrhythmias, or medications with major autonomic impact (e.g., β -blockers at unstable dosage).

Stroop Test

In this study, the Stroop test was used as a tool to evaluate the impact of stress (here, pain) on patients' cognitive functions and emotional responses. The test, developed by John Ridley Stroop in 1935, is commonly employed in cognitive research and measures an individual's ability to suppress automatic responses while maintaining selective attention. Evidence indicates that it is an effective method to examine attentional bias in chronic pain patients (19, 24, 25). Specifically, since the test induces cognitive interference and engages executive control, it can be used to investigate pain-related attentional and inhibitory dysfunction.

The test typically involves three conditions (blocks): neutral, congruent, and incongruent conditions. Each phase lasted approximately 2 minutes, for a total Stroop duration of about 6 minutes. Specifically, the test starts with a 3-minute resting baseline with eyes closed, followed by a 2-minute open-eyes resting condition. Subsequently, in the neutral block, participants are asked to quickly read a list of words that are names of colors, but all printed in black on a white background. This portion of the test serves as a baseline control for measuring speed and accuracy in word reading. In the congruent condition,

participants read color-word names printed in the same color (for example, the word "red" printed in red). This measures the speed and accuracy when the word meaning and ink color align. In the incongruent condition, participants must read color words printed in a color that does not match the meaning of the word (for example, the word "red" printed in blue). This last block is considered the most challenging, as it requires inhibiting the automatic response to read the word and instead responding to the ink color. After the task, a 3-minute eye-closed recovery period and a final 2-minute open-eyes condition were recorded.

During the Stroop task, each block consisted of 60 stimuli (180 trials in total). Each stimulus remained on screen for 1.5 seconds, followed by a 0.5-second interstimulus interval. Behavioral performance was assessed through accuracy (%) and reaction times (ms), which were automatically recorded.

In the text, "Stroop phases" refer to the three sequential blocks of the Stroop task: neutral, congruent, and incongruent.

Biosignals

During the execution of the Stroop color-word test, we implemented the BITalino device with sensors for capturing ECG and EDA signals, as reported in (26). It is an open-source, hardware-affordable biosignal platform designed for physiological computing. Data collected with this instrument shows dependability for quantitative analysis (27). A sample rate of 1000 Hz was used to measure the signals. For EDA assessment, a fifth-order Butterworth low-pass filter with a cutoff frequency of 1 Hz was used for the processing and analysis of the signals and, after downsampling the signal by a factor of 100 to lessen the computational load of the analysis, the signals were further examined using a deconvolution approach to separate the tonic (basic level of conductance) and phasic components (short-duration changes in the presentation of a stimulus) (26). The following parameters were then considered for further analysis: tonic response (SCL), phasic response (SCR), SCR integral (*i.e.*, the mean area of the SCR peaks), number of phasic peaks normalized by the experiment duration (peaks/minute), mean rise time (measured in seconds), and recovery time (measured in seconds). Concerning HRV, a modified version of the Pan-Tompkins method was used to detect an R peak in the ECG signal; the accompanying RR series of interbeat intervals was then calculated as the difference between subse-

quent R peaks (26, 27). For the HRV assessment, both time-domain features (average HR, standard deviation of RR intervals (SDNN), and Root Mean Square of Successive Differences of the RR, RMSSD) and frequency-domain features (power in low, LF: 0.04-0.15 Hz, and high, HF: 0.15-0.40 Hz, frequency bands as well as the LF/HF ratio representing the sympatho-vagal balance) were analyzed.

Therefore, selected EDA and HRV indices were chosen because they represent well-established markers of sympathetic (EDA, LF) and parasympathetic (HF, RMSSD) function (26, 27). Tonic SCL reflects baseline sympathetic arousal, whereas phasic SCR amplitude, integral, and peak frequency capture transient sudomotor responses to stimuli. HRV parameters were included as they reflect cardiac autonomic modulation and have been associated with chronic pain and stress reactivity, and the LF/HF ratio is useful for addressing the dynamic sympatho-vagal equilibrium. Processing was carried out in Python language using the Spyder software version 4.1.5, while ECG processing was carried out in MATLAB v2023a.

Pain Assessment

Pain intensity was assessed using the 0–10 Numeric Rating Scale (NRS), a validated unidimensional tool widely used in cancer pain assessment, where 0 = “no pain” and 10 = “worst imaginable pain”. Scores 0–3 are generally considered mild, 4–6 moderate, and ≥ 7 severe pain (28). For the analyses, in line with previous research on cancer pain, patients were categorized into two groups: NRS < 6 vs NRS ≥ 6 (moderate vs severe pain phenotypes) (28).

Statistical Analysis

Analyses were stratified according to pain intensity (0–10 numeric rating scale, NRS) in two independent groups based on an empirically established threshold (NRS = 6) for identifying a “high pain” category and a “low pain” category (NRS < 6 and NRS ≥ 6). After grouping the patients, Shapiro-Wilk test was adopted to check the normality of the data distributions in the two groups and, given the non-normality of the distributions ($p < 0.05$), a Mann Whitney U test was carried out as an alternative to the Student’s t test for independent samples in order to assess statistical differences in the median values of the biosignals’ signatures across the two groups. Level of significance alpha was set to 0.05 (Confidence Level 95%) for all the statistical analyses performed in this study. All statistical tests were carried out in Python language using the Spyder software version 4.1.5.

RESULTS

The analysis included data from 45 patients enrolled between May and August 2025. No participants were excluded after enrollment (**Table 1**).

Table 1. Demographic characteristics (n = 45).

VARIABLE	N (%) OR MEAN \pm SD
Age (years)	64.5 \pm 14
Gender	Male: 22 (48.9%); Female: 23 (51.1%)
BMI (kg/m ²)	27.1 \pm 5.3
Cancer type	Pancreatic 11 (24.4%) Breast 8 (17.8%) Lung 6 (13.3%) Colorectal 6 (13.3%) Prostate 3 (6.7%) Gastric 2 (4.4%) Others 9 (20%)
Metastasis	Yes: 32 (71.1%) No: 13 (28.9%)
Bone metastasis	Yes: 17 (37.8%) No: 28 (62.2%)
ECOG-PS	0: 8 (17.8%) 1: 11 (24.4%) ≥ 2 : 26 (57.8%)
Pain intensity (NRS)	5.8 \pm 2.7
Pain type	Nociceptive: 8 (17.8%) Neuropathic: 8 (17.8%) Mixed: 29 (64.4%)

Across all Stroop phases, patients in the low-pain group (NRS < 6) showed higher median SCL values (e.g., Stroop I: median 0.67 μ S vs 0.41 μ S; Stroop II: 0.72 μ S vs 0.45 μ S; Stroop III: 0.80 μ S vs 0.48 μ S). In this subgroup, a stronger phasic SCR component in the Stroop phases was found compared to patients with NRS ≥ 6 . Significant differences were observed in SCL mean ($p \leq 0.05$ across phases), SCR mean ($p \leq 0.01$), and SCR integral ($p \leq 0.01$), indicating a more pronounced autonomic response in the low-pain group. In contrast, rise time and recovery time did not significantly differ between groups, suggesting that magnitude rather than temporal dynamics of sudomotor responses distinguished between the two groups. The frequency of SCR peaks normalized by phase duration showed only modest differences between groups and was significant in phases I and III, indicating that pain intensity may influence the strength rather than the frequency of sympathetic reactivity during cognitive interference (**Figure 1**).

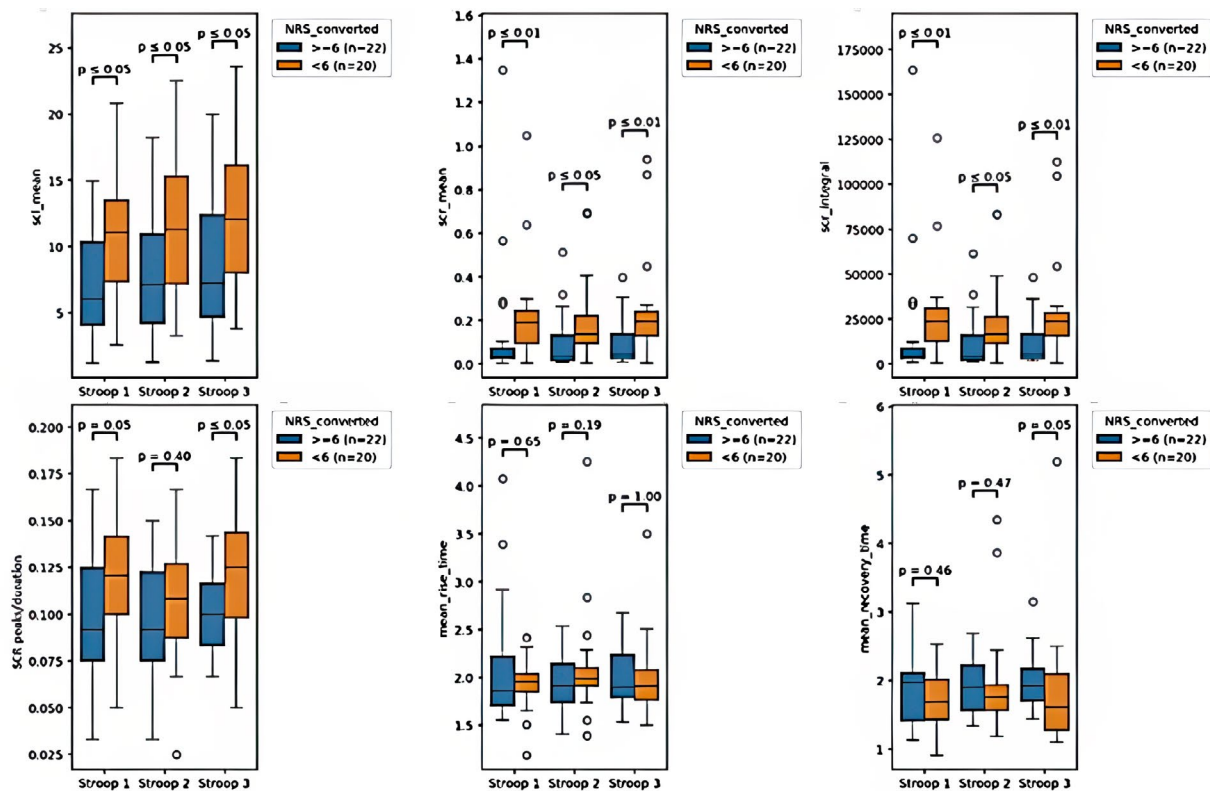


Figure 1. Electrodermal activity (EDA) features across Stroop test phases in patients with different pain intensity levels. Boxplots show the distribution of EDA-derived features during the three phases of the Stroop task (Stroop 1 to 3) for patients with high pain intensity (NRS ≥ 6 , $n = 22$) and low pain intensity (NRS < 6 , $n = 20$). EDA parameters (from the top): mean skin conductance level (scl_mean), mean skin conductance response amplitude (scr_mean), integrated SCR signal (scr_integral), ratio between the number of SCR peaks and phase duration (num_peaks/timing), mean SCR rise time, and mean SCR recovery time. Horizontal bars indicate statistically significant differences between groups (Mann–Whitney U tests), with p -values reported above each comparison. Patients with mild to moderate pain (NRS < 6) showed more pronounced tonic and phasic EDA activity. Response times (peaks) did not differ, but the amplitude of the response was more evident.

Concerning HRV parameters, median average HR values were slightly higher in the high-pain group throughout the task (e.g., Stroop I: median 83 bpm vs 78 bpm), although these differences did not reach significance. Time-domain HRV markers (SDNN and RMSSD) also showed a consistent trend toward reduced variability in the high-pain group (e.g., RMSSD Stroop I: median 16 ms vs 22 ms; Stroop III: 14 ms vs 20 ms), without significant divergence across phases. Frequency-domain indices displayed comparable patterns in phases I and II, whereas during the incongruent block (phase III), HF power was significantly higher in the low-pain group (e.g., median 402 ms^2 vs 255 ms^2 , Mann–Whitney $p < 0.05$), indicating more preserved parasympathetic regulation under maximal cognitive interference. LF power and LF/HF ratio did not show meaningful between-group differences in any phase. No other statistically significant differences were found between the high- and low-pain groups across Stroop phases (**Figure 2**).

DISCUSSION

This study explored autonomic responses during a cognitive interference task in cancer patients with chronic pain. The main finding of this study was the clear differential sensitivity between EDA-derived features and HRV indices in detecting pain-related autonomic alterations during cognitive interference. Specifically, while tonic and phasic EDA components (SCL, SCR amplitude, and SCR integral) consistently distinguished between pain groups across all Stroop phases, HRV separation was modest and limited to a single phase (HF power during the incongruent block, phase III). Therefore, the sympathetic-sudomotor reactivity during cognitive interference may better reflect chronic pain phenotypes than cardiac variability alone in this context. Notably, a feasibility work in ambulatory cancer patients reported that machine-learning models trained on physiological signals achieve approximately 70% accuracy for pain state discrimination (9). Our EDA-forward pattern is

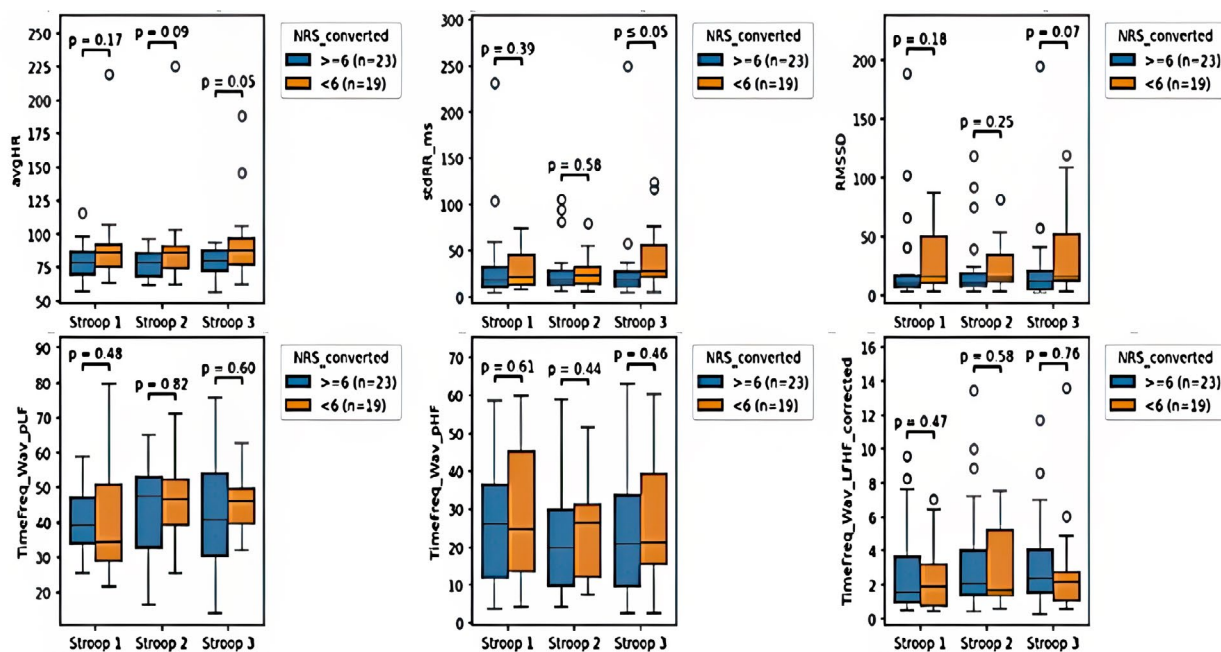


Figure 2. Heart rate and heart rate variability (HRV) features across Stroop test phases in patients with different pain intensity levels. Boxplots display the distribution of cardiovascular features recorded during the three Stroop task phases (Stroop 1 to 3) for patients with high pain intensity (NRS ≥ 6 , $n = 23$) and low pain intensity (NRS < 6 , $n = 19$). HRV parameters (from the top): average heart rate (avgHR), standard deviation of RR intervals (stdRR_ms), root mean square of successive differences (RMSSD), low-frequency power (TimeFreq_Wav_pLF), high-frequency power (TimeFreq_Wav_pHF), and LF/HF ratio corrected (TimeFreq_Wav_LFHF_corrected). Horizontal bars indicate statistically significant differences between groups (Mann-Whitney U tests), with p -values shown above each comparison.

consistent with those observations and extends them with phase-resolved Stroop probing. This approach can better highlight the relationship between pain and its impact on cognitive functions. Although the finding requires confirmation, it can have meaningful implications for APA development and for capturing pain-related autonomic signatures, particularly when contextualized within cognitive or emotional load. Autonomic dysfunction has been documented in patients with advanced cancer, where it is associated with fatigue, worse quality of life, and reduced survival. For example, Stone *et al.* (29) found that autonomic dysregulation is highly prevalent in advanced cancer, suggesting that cancer itself or its treatment may injure autonomic pathways. Furthermore, Ben-David *et al.* (30) observed that cancer patients exhibit lower HRV compared to healthy controls. The prognostic importance of autonomic measures has also been demonstrated. For example, reduced HRV seems to be associated with shorter survival (31). Probably, cardiac autonomic dysfunction is an underrecognized feature in oncology (32). In our analysis, the EEG-derived parameters showed less pronounced results compared to EDA. It emerged that HR increased and HRV decreased with pain; nevertheless, effect sizes vary across tasks, diagnoses,

and sensors, explaining why our HRV separation was limited outside the most demanding Stroop block. Methodological syntheses further emphasize wearable-first, multimodal, and interpretable designs, again consonant with our findings that EDA is an informative anchor modality but benefits from fusion. The relationship between ANS evaluations and pain intensity is particularly interesting because it can allow for to identification of the variables to be investigated within a multimodal model. In patients with bone metastases, an initial study aimed to define an HRV-based objective method for assessing metastatic pain, reporting promising associations between HRV measures and pain status (33). From the perspective of pain modulation, there is evidence that lower parasympathetic function may be linked to heightened pain sensitivity. For example, in patients suffering from chemotherapy-induced polyneuropathy, Nahman-Averbuch *et al.* (34) demonstrated that lower Valsalva ratios and diminished HRV, indicative of reduced parasympathetic tone, correlated with higher ratings of experimental pain stimuli. Interestingly, we found that in chronic oncologic pain, the analysis of the ANS in relation to cognitive tasks highlights different patterns depending on pain intensity. Patients with lower pain levels exhibit an active and functional system.

However, in subjects reporting severe pain, the system appears less active and less functional.

LIMITATIONS AND PERSPECTIVES

This analysis is exploratory, single-center, and modest in size ($n = 45$), limiting power for subgroup analyses by tumor type, therapy line, or comorbidities. Medication effects (opioids, adjuvants), metastasis distribution (including bone), performance status, and mood/sleep could confound autonomic readouts. Moreover, in cancer patients, the autonomic effects of systemic inflammation, metabolic derangements, treatment toxicity (e.g., anthracyclines, taxanes), and microvascular injury may further distort baseline autonomic tone and responsiveness (29-34). We acknowledge that, given the small sample size, dividing participants into two groups impaired statistical power. Moreover, adopting the NRS as a continuous variable within a single group would be more appropriate. Nevertheless, we followed the methodology of prior cancer-pain research in treating the NRS as a categorical variable rather than a continuous one (35). Specifically, several studies in oncology pain categorize NRS scores into mild, moderate, and severe pain bands (for example, ≤ 3 , 4-6, ≥ 7) because these thresholds are strictly linked to clinical decision-making and analgesic escalation protocols (28, 36). Therefore, we used the cut-off of NRS ≥ 6 to separate two categories of pain (moderate to severe pain). Concerning methods for sympatho-vagal investigations, while we focused on EDA and HRV, additional modalities such as EEG, EMG, PPG, skin temperature, and accelerometry were not assessed here. Furthermore, the temporal dynamics of autonomic change in response to pain or cognitive stress may be subtle and transient, requiring high-resolution recording and careful signal processing (time-, frequency-, and nonlinear domains). Finally, we did not perform external validation or causal modeling, as multiple comparisons across features and phases also raise a risk of type I error. Future work should integrate these findings into multimodal APA models. Importantly, machine learning techniques can be implemented to classify pain levels based on combined physiological, clinical, and behavioral features. In this direction, feature fusion, temporal modelling (e.g., using recurrent deep learning architectures or transformers), and explainability methods may enhance performance and clinical trust. From an ethical standpoint, the development

of APA systems must ensure transparency, patient autonomy, and equitable access. Therefore, future APA frameworks must be developed to support safe clinical decision-making and to safeguard patient rights and well-being (37).

CONCLUSIONS

Biosignal can be implemented for phenotyping chronic cancer pain. Nevertheless, further research is needed for developing multimodal APA systems in oncology. Prospective, interpretable, and context-aware models should integrate EDA and other selected biosignals with clinical data. This step is mandatory for improving assessment and, ultimately, personalizing pain management in cancer patients.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' contributions

FS, AMP, OP and MC initiated and supervised the project. VS and CG collected the data. MR, DE and MPB performed the data analysis. FA, LS, RD, VC, SP and OP interpreted the experimental data and prepared figures. MC wrote the manuscript with input from all Authors. All Authors have been involved in the Manuscript's revisions.

Availability of data and materials

The data underlying this article are available in the public domain.

Publications ethics

Plagiarism

The article provides a comprehensive review of the latest studies in the field, with accurate citations.

Data falsification and fabrication

The writing and contents of the article are entirely original and were developed entirely by the authors. The article provides a comprehensive review of the latest studies in the field, with accurate citations. The writing and contents of the article are entirely original and were developed entirely by the authors.

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ORIGINAL ARTICLE

SHOULD ITERATIVE CYTOREDUCTIVE SURGERY AND HYPERTHERMIC INTRAPERITONEAL CHEMOTHERAPY BE CONSIDERED THE BEST TREATMENT OF RECURRENT PSEUDOMYXOMA PERITONEI (PMP)?

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ABSTRACT: *Background and objectives.* After CRS-HIPEC, approximately 25-45% of patients with pseudomyxoma peritonei (PMP) experience recurrence even after optimal treatment. Treatment of recurrent PMP is controversial and based mainly on surgeon and center experience. The aim of this study was to assess the feasibility, safety, and oncological benefit of iterative CRS-HIPEC (i-CRS-HIPEC) in patients with recurrent PMP.

Methods. Consecutive PMP patients treated according to an institutionally standardized protocol of CRS-HIPEC were retrospectively analyzed for postoperative and long-term oncological outcomes.

Results. Between January 2010 and May 2023, 76 patients with PMP were treated with CRS and HIPEC. Of these, 21 patients underwent i-CRS-HIPEC for recurrent PMP and were compared with those who underwent primary surgery (p-CRS-HIPEC). Peritoneal Cancer Index (PCI), cytoreduction grade (CC), and histological grade (acellular mucin, low-grade, and high-grade PMP) didn't differ significantly from primary CRS-HIPEC. Postoperative outcomes and complications were similar between the groups. After a median follow-up of 24.5 months (IQR 18.89-30.18), there was no difference between groups in the 5-year OS and DFS.

Conclusions. i-CRS-HIPEC can be performed safely and is associated with the same oncological outcome in terms of local disease control and should be considered the first choice for recurrent PMP after appropriate patient selection.

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IMPACT STATEMENT: The treatment of recurrent pseudomyxoma peritonei PMP is controversial and based mainly on surgeon and center experience. In this study, we have assessed the impact of iterative CRS-HIPEC (i-CRS-HIPEC) in terms feasibility, safety, and oncological benefit in recurrent PMP.

Key words: peritoneal neoplasms; cytoreductive surgery; HIPEC, appendiceal tumors.

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BACKGROUND

Pseudomyxoma peritonei (PMP) is a rare malignant clinical syndrome with an estimated incidence of 2-4 cases per million people per year (1-3) and is clinically

characterized by implantation of neoplastic cells on peritoneal surfaces with progressive mucin production (mucinous ascites) throughout the abdominal cavity. PMP was first described by Werth in 1884 as the peritoneal spread of an ovarian neoplasm (4);

however, recent evidence has shown that PMP most commonly results from the spread of mucin-producing cells from an appendiceal neoplasm or, in a minority of cases, from mucinous extra-appendiceal neoplasms (5-7).

Cytoreductive surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC) has significantly improved oncological outcomes in selected patients with PMP. The rationale of CRS-HIPEC is to remove all macroscopic peritoneal implants by multiple peritonectomies and surgical resections and to treat microscopic residual tumors with hyperthermic intraperitoneal chemotherapy (8). CRS-HIPEC has been included in several international and national guidelines as the standard of care for PMP (9) and is the only treatment with a potential chance of cure and long-term disease control for affected patients (10-11).

Although management and overall survival have recently improved, approximately 25-45% of patients with PMP experience recurrence even after receiving optimal combination treatment (12-15). The options available range from repeated surgery with or without HIPEC to palliative systemic chemotherapy, and the clinical management of recurrence is not yet standardized (16).

The main aim of this study was to assess the feasibility, safety, and oncological benefit of i-CRS-HIPEC in terms of local control and survival in patients with recurrent PMP.

METHODS

Study design and data collection

This study is a retrospective and comparative analysis of patients with primary or recurrent PMP who underwent CRS with HIPEC between January 2010 and May 2023 at the Surgical Oncology department of the Veneto Institute of Oncology IOV-IRCCS. After written consensus, all patients were selected and treated according to an institutionally standardized protocol; prior to surgery, eligibility for CRS and HIPEC was reviewed by our multidisciplinary tumor board, considering clinical and pathological features and imaging results (CT scan, PET-CT scan or abdominal MRI in doubtful cases). The study was approved by the institute's ethics committee (BIOPMP CET ANV: 2024-08) and in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Patients

Patient records were extracted from our institutional electronic health record software and prospectively collected in an electronic database. All patients were informed of the nature of the procedure and signed an informed consent form. Demographic and preoperative data included age, sex, body mass index (BMI), ECOG performance status, comorbidities according to the ASA physical status classification system, and systemic chemotherapy before or after CRS-HIPEC.

Intraoperative and postoperative short-term outcome variables

Intraoperative variables collected included operative time, blood loss, and number of packed red blood cells (PRBCs) transfused. Abdominal spread of the tumor was assessed intraoperatively using the Peritoneal Cancer Index (PCI), and residual disease after CRS was classified according to the Completeness of Cytoreduction (CC) score (17); surgical technique (open, video-laparoscopic, or hybrid approach) and HIPEC technique (open or closed) were recorded, as well as the number and type of peritonectomies and organ resections. The surgical procedure consisted of peritonectomy and cytoreductive surgery as described by Sugarbaker (8). The HIPEC protocol consisted of cisplatin at 90mg/m² plus mitomycin-C 12 mg/m² at a target temperature of 41.5°C maintained for 60 minutes at a target flow rate of approximately 1000 ml/min. Histology of the PMP was performed in all cases according to the PSOGI histological classification (18). All specimens obtained from outside institutions were systematically reviewed. Postoperative data included Intensive Care Unit (ICU) length of stay, hospital length of stay, 30-day readmission rate, and complications. Complications were graded according to the Clavien-Dindo grading system (19). All patients underwent an institutionally approved follow-up schedule with at least clinical examination, CT scan, and serum tumor markers every six months for the first three years and then every 12 months up to 10 years postoperatively.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Mac v.29.0.1.0 (IBM Corporation, Armonk, NY, USA). Patients were divided into two main groups: patients who underwent i-CRS-HIPEC and the control group consisting of patients who underwent primary cytoreductive surgery and HIPEC (p-CRS-HIPEC). Quantitative data are presented as median

and interquartile range (IQR), and categorical data are presented as numbers and percentages. Categorical data and quantitative data were analyzed using chi-squared or Fisher's exact test and t-test, respectively. Median overall survival (OS) and disease-free survival (DFS) were calculated using the Kaplan-Meier estimator. Statistical significance was considered when p-values were less than 0.05. DFS and OS were calculated from the day of CRS-HIPEC.

RESULTS

Between January 2010 and May 2023, 80 patients with PMP were referred to our institution; 76 patients were selected and treated with CRS and HIPEC. Two patients were excluded at presentation and underwent palliative surgery only, and two other patients were excluded at recurrence for unresect-

able disease. Three of these unresectable patients died within 12 months after diagnosis. Twenty-one patients underwent i-CRS-HIPEC, of which 15 and 6 patients underwent a second and a third i-CRS-HIPEC, respectively. A comparison of demographic and preoperative variables (**Table 1, Table 2**) showed that the BMI was significantly lower in the i-CRS-HIPEC group (20.96 vs. 25.95, $p = .012$) and that a greater percentage of i-CRS-HIPEC patients received neoadjuvant chemotherapy within six months prior to CRS-HIPEC (28.6% vs. 3.6%, $p = .005$), while there was no difference between the two groups for neoadjuvant chemotherapy treatment beyond six months prior to surgery and for adjuvant chemotherapy treatment. When comparing the intraoperative variables (**Table 3, Table 4**), PCI was higher in the p-CRS-HIPEC patients (21 vs. 15, $p = .072$), although not statistically significant. Correspondingly, the extent of surgery was lower in the i-CRS-HIPEC group, as evi-

Table 1. Comparison of demographic and preoperative variables between First CRS-HIPEC and Iterative CRS-HIPEC.

	P-CRS-HIPEC N = 56	I-CRS-HIPEC N = 21	P
Age at diagnosis (y) , median (IQR)	52 (47 - 63)	52 (44 - 65)	0.861
Gender , n (%)			
Male	18 (32.7%)	5 (23.8%)	0.580
Female	37 (67.3%)	16 (76.2 %)	
BMI (kg/m²) , median (IQR)	25.95 (22.14 - 28.73)	20.96 (19.58 - 26.97)	0.012
ASA physical status , n (%)			
ASA 1	6 (10.9%)	1 (4.8%)	
ASA 2	33 (60%)	17 (81%)	0.226
ASA 3	16 (29.1%)	3 (14.3%)	
Performance status , n (%)			
ECOG 0	50 (90.9%)	16 (76.2%)	
ECOG 1	4 (7.3 %)	5 (23.8%)	0.142
ECOG 2	1 (1.8%)	0 (0.0%)	
PMP Histology , n (%)			
Acellular Mucin	8 (14.5%)	1 (4.8%)	0.289
Low-grade PMP	35 (63.6%)	14 (66.7%)	
High-grade PMP	8 (14.5%)	6 (28.6%)	
High-grade PMP with SRC	4 (7.3%)	0 (0%)	
Systemic chemotherapy , n (%)			
SC <6 months before intervention	2 (3.6%)	6 (28.6%)	0.005
SC >6 months before intervention	6 (10.9%)	1 (4.8%)	0.666
SC after intervention	1 (1.8%)	0 (0%)	0.534
Months from last CRS-HIPEC , median (IQR)	-	22.67 (16.67 - 39.10)	-

Abbreviations. CRS, Cytoreductive Surgery; HIPEC, Hyperthermic Intraperitoneal Chemotherapy; IQR, Interquartile Range; BMI, Body Mass Index; ASA, American Society of Anesthesiologists; ECOG, Eastern Cooperative Oncology Group; PMP, Pseudomyxoma Peritonei; SRC, Signet Ring Cells; SC, Systemic Chemotherapy.

Table 2. Comparison of demographic and pre-operative variables between first i-CRS-HIPEC and second i-CRS-HIPEC.

	FIRST I-CRS-HIPEC N = 15	SECOND I-CRS-HIPEC N = 6	P
Age at diagnosis (y) , median (IQR)	52 (44 - 72)	51 (41 - 55)	0.308
Gender , n (%)			
Male	4 (26.7%)	1 (16.7%)	0.613
Female	11 (73.3%)	5 (83.3%)	
BMI (kg/m²) , median (IQR)	20.63 (19.34 - 26.01)	25.13 (19.43 - 29.36)	0.218
ASA physical status , n (%)			
ASA 1	1 (6.7%)	0 (0.0%)	0.662
ASA 2	11 (73.3%)	6 (100%)	
ASA 3	3 (20.0%)	0 (0.0%)	
Performance status , n (%)			
ECOG 0	11 (73.3%)	5 (83.3%)	0.573
ECOG 1	4 (26.7%)	1 (16.7%)	
ECOG 2	0 (0.0%)	0 (0.0%)	
PMP Histology , n (%)			
Acellular Mucin	0 (0.0%)	1 (16.7%)	0.624
Low-grade PMP	10 (66.7%)	4 (66.7%)	
High-grade PMP	5 (33.3%)	1 (16.7%)	
High-grade PMP with SRC	0 (0.0%)	0 (0.0%)	
Systemic chemotherapy , n (%)			
SC <6 months before intervention	4 (26.7%)	2 (33.3%)	0.300
SC >6 months before intervention	0 (0.0%)	1 (16.7%)	0.613
SC after intervention	0 (0.0%)	0 (0.0%)	-
Months from last CRS-HIPEC , median (IQR)	24 (16 - 37)	25 (18 - 57)	0.425

Abbreviations: CRS, Cytoreductive Surgery; HIPEC, Hyperthermic Intraperitoneal Chemotherapy; IQR, Interquartile Range; BMI, Body Mass Index; ASA, American Society of Anesthesiologists; ECOG, Eastern Cooperative Oncology Group; PMP, Pseudomyxoma Peritonei; SRC, Signet Ring Cells; SC, Systemic Chemotherapy.

denced by the shorter duration (555 vs. 605 minutes, $p = .011$), lower number of peritonectomies and visceral resections (1 vs. 3 $p = <.001$), (1 vs. 3, $p = <.001$), and lower median blood loss (20.96 vs. 25.95, cc, $p = .012$). Major complications requiring surgical intervention were observed in 4 patients (19.0%) in the i-CRS-HIPEC group compared with 12 patients (21.8%) in the p-CRS-HIPEC cohort. In the i-CRS-HIPEC group, these complications included two anastomotic leaks, one postoperative bleeding event, and one bowel perforation (**Table 5**).

The histology of PMP did not show a statistically significant difference between the two groups ($p = .289$); however, a higher percentage of acellular mucin cases were found in the first treatment group (14.5% vs. 4.8%), while a greater proportion of high-grade PMP was found in the patients with recurrence (28.6% vs. 14.5%), as expected. After a median follow-up of 24.53 months (18.89-30.18), the 5-year OS and DFS

were 94.9.0% and 44.5%, respectively. There was no significant statistical difference in 5-year overall survival (OS) and disease-free survival (DFS) between the two groups, 93.1% and 46.6% for p-CRS-HIPEC and 100% and 41.7% for i-CRS-HIPEC, respectively (**Figure 1**).

DISCUSSION

Despite recent improvements in management and survival outcomes, approximately 25-45% of patients with PMP experience recurrence even after optimal treatment (12-15). The clinical management of recurrence is not standardized and the options available can range from non-operative management, including the watch-and-wait strategy, to palliative systemic chemotherapy and iterative surgery with or without the addition of HIPEC. The potential survival bene-

Table 3. Comparison of intraoperative and postoperative variables between First CRS-HIPEC and Iterative CRS-HIPEC.

	P-CRS-HIPEC N = 56	I-CRS-HIPEC N = 21	P
Intraoperative PCI , median (IQR)	21 (13 - 28)	15 (11 - 19)	0.072
CC score , n (%)			
CC0	49 (89.1%)	15 (71.4%)	
CC1	6 (10.9%)	5 (23.8%)	0.087
CC2	0 (0.0%)	1 (4.8%)	
Surgical technique , n (%)			
Open	49 (89.1%)	20 (95.2%)	
Lap	5 (9.1%)	1 (4.8%)	0.668
Lap/Open	1 (1.8%)	0 (0%)	
HIPEC technique , n (%)			
Open technique	11 (20%)	4 (19%)	0.926
Closed technique	44 (80%)	17 (81%)	
Operation duration (min) , median (IQR)	605 (480 - 720)	555 (497 - 585)	0.011
CRS variables			
Peritonectomies, median (IQR)	3.0 (2.0 - 4.0)	1.0 (0.0 - 1.5)	<0.001
Visceral resections, median (IQR)	4.0 (3.0 - 6.0)	1.0 (1.0 - 2.5)	<0.001
Bowel resections, n (%)	30 (54.5%)	11 (52.4%)	0.866
Stoma, n (%)	4 (7.3%)	1 (4.8%)	0.693
Blood loss (mL) , median (IQR)	300 (100 - 575)	150 (100 - 375)	0.012
Blood transfusion			
Intraoperative transfusion, n (%)	17 (51%)	3 (23.1%)	0.329
Number of PRBCs, median (IQR)	0.0 (0.0 - 1.5)	0.0 (0.0 - 0.5)	0.226
Length of stay , (days), median (IQR)			
ICU length of stay	1.0 (1.0 - 2.0)	1.0 (1.0 - 2.0)	0.166
Hospital length of stay	11.0 (8.0 - 19.0)	11.0 (8.0 - 18.5)	0.726
Surgical complications* , n (%)			
Grade I-II	20 (36.4%)	7 (33.3%)	0.805
Grade III-IV	19 (34.5%)	4 (19.0%)	0.266
Reintervention , n (%)	12 (21.8%)	4 (19.0%)	0.791
Re-admission in 30 days , n (%)	2 (3.6%)	0 (0%)	0.376
90-day mortality , n (%)	0 (0%)	0 (0%)	-

*According to the Clavien-Dindo classification.

Abbreviations. CRS, Cytoreductive Surgery; HIPEC, Hyperthermic Intraperitoneal Chemotherapy; IQR, Interquartile Range; PCI, Peritoneal Cancer Index; CC, Completeness of Cytoreduction; Lap, Laparoscopy; PRBCs, Packed Red Blood Cells; ICU, Intensive Care Unit.

fits of i-CRS-HIPEC in terms of feasibility, safety and oncological outcomes are not well established (16). Our study has clearly shown that iterative CRS and HIPEC is safe and effective in patients with recurrent disease with similar survival and disease control to patients treated for primary PMP. All the postoperative outcomes parameters including morbidity, length of stay, readmission rate and mortality are similar after i-CRS-HIPEC in comparison with p-CRS-HIPEC, confirming the safety of iterative procedures in recurrent PMP. Moreover, from an onco-

logical point of view, i-CRS-HIPEC can offer durable disease control comparable to p-CRS-HIPEC. The study clearly showed that surgery should be always considered as the first line of treatment in every recurrent PMP even in patients with second recurrence. In this perspective the role of center expertise in patient selection is crucial. The preoperative multidisciplinary discussion should be focused on an accurate radiological evaluation to quantify disease burden and the possibility of achieving complete cytoreduction, with the final aim to maximize

Table 4. Comparison of intraoperative and postoperative variables between first i-CRS-HIPEC and second i-CRS-HIPEC.

	FIRST I-CRS-HIPEC N = 15	SECOND I-CRS-HIPEC N = 6	P
Intraoperative PCI , median (IQR)	18.50 (13.75 - 21.25)	6.00 (3.75 - 12.00)	0.001
CC score , n (%)			
CC0	10 (66.6%)	5 (83.3%)	0.643
CC1	4 (26.7%)	1 (16.7%)	
CC2	1 (6.7%)	0 (0%)	
Surgical technique , n (%)			
Open	15 (100%)	5 (83.3%)	0.300
Lap	0 (0%)	1 (16.7%)	
Lap/Open	0 (0%)	0 (0%)	
HIPEC technique , n (%)			
Open technique	2 (13.3%)	2 (33.3%)	0.549
Closed technique	13 (85.7%)	4 (66.7%)	
Operation duration (min) , median (IQR)	567 (532 - 632)	472 (407 - 546)	0.010
CRS variables			
Peritonectomies, median (IQR)	1.0 (0.0 - 2.2)	0.0 (0.0 - 1.0)	0.110
Visceral resections, median (IQR)	1.5 (1.0 - 3.0)	1.0 (0.0 - 2.5)	0.424
Bowel resections, n (%)	10 (71.4%)	1 (16.7%)	0.050
Stoma, n (%)	0 (0%)	1 (16.7%)	0.300
Blood loss (mL) , median (IQR)	200 (100 - 437)	125 (62 - 487)	0.922
Blood transfusion			
Intraoperative transfusion, n (%)	3 (20.0%)	0 (0%)	0.491
Number of PRBCs, median (IQR)	0.00 (0.00 - 1.75)	0.00 (0.00 - 0.00)	0.095
Length of stay , (days), median (IQR)			
ICU length of stay	1.00 (1.00 - 2.00)	1.00 (0.75 - 2.50)	0.882
Hospital length of stay	10.5 (8.0 - 21.0)	12.5 (6.5 - 23.0)	0.905
Surgical complications* , n (%)			
Grade I-II	6 (40.0%)	1 (16.7%)	0.254
Grade III-IV	3 (20.0%)	1 (16.7%)	0.807
Reintervention , n (%)	3 (20.0%)	1 (16.7%)	0.807
Re-admission in 30 days , n (%)	0 (0%)	0 (0%)	-
90-day mortality , n (%)	0 (0%)	0 (0%)	-

*According to the Clavien-Dindo classification.

Abbreviations. CRS, Cytoreductive Surgery; HIPEC, Hyperthermic Intraperitoneal Chemotherapy; IQR, Interquartile Range; PCI, Peritoneal Cancer Index; CC, Completeness of Cytoreduction; Lap, Laparoscopy; PRBCs, Packed Red Blood Cells; ICU, Intensive Care Unit.

Table 5. Major postoperative complications following p-CRS-HIPEC and i-CRS-HIPEC.

	P-CRS-HIPEC N = 56	I-CRS-HIPEC N = 21
Perforation (IIIb*) , n (%)	3 (5.4%)	1 (4.8%)
Anastomotic leak (IIIb*) , n (%)	3 (5.4%)	2 (9.5%)
Hemoperitoneum (IIIb*) , n (%)	6 (10.9%)	1 (4.8%)
Bleeding (IIIa*) , n (%)	1 (1.8%)	0 (0.0%)
Abdominal fluid collection (IIIa*) , n (%)	6 (10.9%)	0 (0.0%)

*According to the Clavien-Dindo classification.

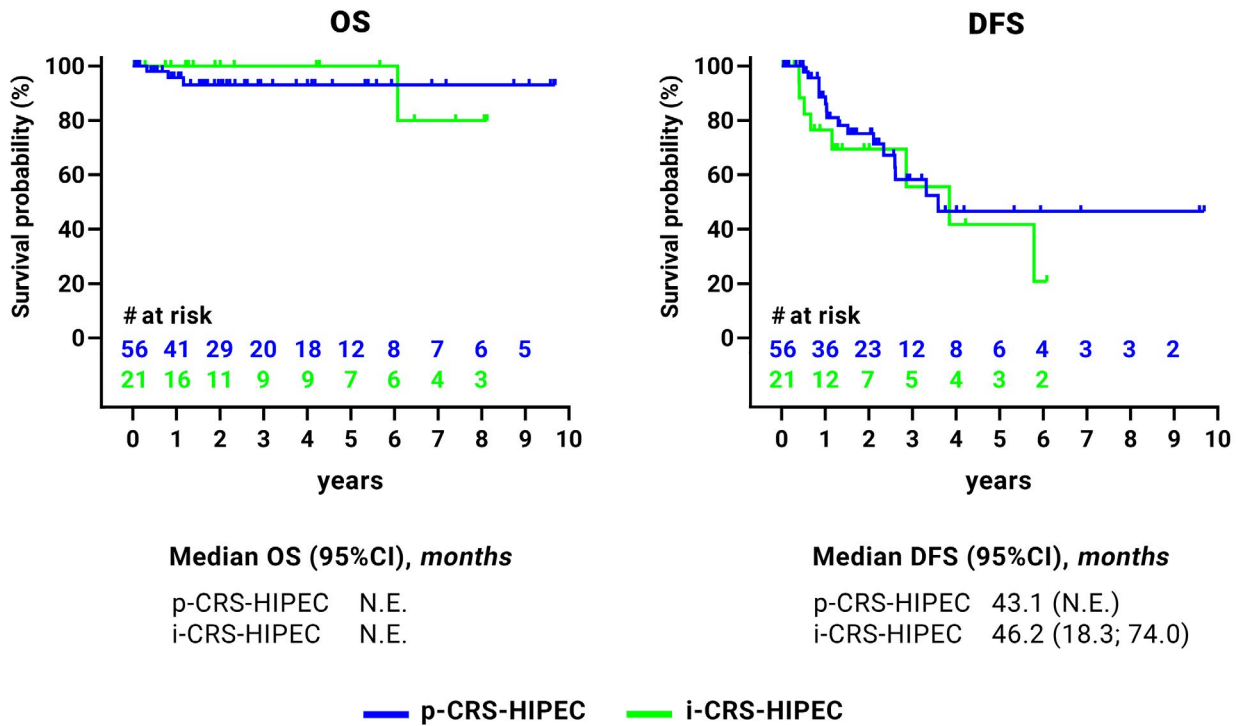


Figure 1. Overall survival (OS) and disease-free survival (DFS) analysis.

the benefit of surgery and reduce the risk of morbidity and mortality.

In our study the morbidity profile of CRS-HIPEC is lower to that reported in the literature for the surgical management of PMP (16). In the largest worldwide series, accounting for 1548 PMP patients treated with CRS-HIPEC, the rate of grade 3-5 complications, re-intervention and 90-day mortality is 32.7%, 9.2% and 3.1% respectively (9). We found comparable rates of severe complications, re-intervention, 30-day readmission and 90-day mortality rate between the i-CRS-HIPEC and p-CRS-HIPEC groups, confirming the safety and feasibility of i-CRS-HIPEC in patients with recurrent PMP (**Table 2**). I-CRS-HIPEC appears to be less surgically demanding, as evidenced by a significantly shorter operative time compared to p-CRS-HIPECs ($p = .011$). This result may be partly due to a lower disease burden in recurrent PMP, expressed by a lower median PCI (15 vs. 21) and a reduced need for peritonectomy and visceral resection. Although i-CRS-HIPEC appears to be less invasive, the grade 3-4 complication rate does not differ significantly from that of primary surgery ($p = 0.266$) with no 30-day mortality in both groups. This may be due, at least in part, to the fact that these patients had already undergone extensive surgery during p-CRS-HIPEC, which may have

added complexity due to more adhesions and previous resections.

Our study further confirms that the comprehensive strategy of cytoreductive surgery and HIPEC provides good disease control of PMP both in the upfront treatment and recurrence setting, with excellent 5-year overall survival (OS) results. Specifically, only 4 out of 76 (5.26%) patients who underwent CRS and HIPEC for PMP at our institution died from causes unrelated to the oncological disease. In addition, patients who underwent i-CRS-HIPEC for recurrent PMP had a similar overall survival (OS) and disease-free survival (DFS) at 5 years as patients who underwent p-CRS-HIPEC (**Figure 1**). I-CRS-HIPEC guarantees the same disease control as p-CRS-HIPEC, confirming the favorable long-term survival outcomes observed in other studies of patients with PMP recurrence treated with i-CRS-HIPEC (14, 16, 24, 25).

The histological grading of PMP is considered an important piece of information in the selection process for evaluating all available treatment options. Our survival outcomes support the findings of previous studies regarding the more aggressive nature of high-grade PMP, but also the equivocal behavior of some cases of low-grade PMP with a certain tendency to recur (20-22). The appropriate identi-

fication of low-grade PMP patients at risk of recurrence may be improved in the future by using the Ki67 proliferation index or NGS analysis, and further studies are needed to validate this approach (23). PMP histologic grade (low/grade) does not appear to influence our clinical decision to treat recurrent patients with surgery. As expected, high-grade PMP showed a higher tendency to recur as shown by the higher percentage of high-grade PMPs seen in the i-CRS-HIPEC group (28.6% vs. 14.5%). Conversely, a slightly higher percentage of i-CRS-HIPEC patients received neoadjuvant chemotherapy in the six months prior to surgery ($p = .005$). This data confirms that the main selection criteria adopted for selecting the patient for i-CRS-HIPEC was the possibility to achieve a complete cytoreduction regardless of the histologic grade. Indeed, almost all (95%) of patients selected for i-CRS-HIPEC had a completeness of cytoreduction (CC) of 0-1, confirming the value of our patient selection process. Previous studies have confirmed our results in recurrent PMP, with a reported median progression free survival (PFS) in low-grade and high-grade PMP of 174.1 and 42.0 months respectively (24, 25). Recurrent high grade PMP with signet ring cells (SRC) is associated with a bad prognosis after i-CRS-HIPEC, with a reported PFS of 15 months only (25). In our study no PMP patient with high-grade SRC histology has been selected for i-CRS-HIPEC and should be therefore considered a relative contraindication for treatment.

The study has some limitations. In a retrospective analysis, the decision-making process and the selection criteria (clinical, radiological and histological) which might have driven the decision for i-CRS-HIPEC are difficult to identify. Early recurrence (within 12 months after p-CRS-HIPEC), symptomatic patients and unfavorable tumor biology (adenocarcinoma/signet ring histology) have been reported as factors associated with worse overall survival (26). The agreement on when and which recurrent patients are to select for i-CRS-HIPEC remains still controversial and there is no clear evidence supporting the decision. In our series the only criteria adopted was the possibility to achieve a complete or near complete cytoreduction and this decision to perform i-CRS-HIPEC was probably mainly based on this key factor. Moreover, the number of recurrent patients treated with i-CRS-HIPEC is too small for further sub analysis, such as investigate whether preoperative chemotherapy or histological grade could increase postoperative morbidity.

Another limit is the number of cases in the iterative group ($n = 21$), which may have limited some statistical analyses. In this perspective a multicentre data collection would in the next future strengthen the results of our study. In addition, it was not possible to retrospectively analyze postoperative pain and the impact of i-CRS-HIPEC on quality of life due to the lack of standardized recording in medical records. Finally, the potential role of biomolecular markers for prognostic stratification was not investigated and should be better defined in the next future.

CONCLUSIONS

Our study showed that i-CRS-HIPEC can be safely performed in recurrent PMP with proper patient selection and is associated with the same oncological outcome in terms of local disease control compared to primary treatment. Further strategies with new drugs and better patient selection for surgery are warranted in recurrent PMP in the coming years.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials

The data underlying this article are available in the public domain.

Publications ethics

Plagiarism

The article provides a comprehensive review of the latest studies in the field, with accurate citations.

Data falsification and fabrication

The writing and contents of the article are entirely original and were developed entirely by the authors.

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MEETING REPORT

SBUR 2025 ANNUAL MEETING REPORT

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This report summarizes the scientific presentations from the 33rd Annual Meeting of the Society for Basic Urological Research (SBUR), held from November 13 to 16, 2025, in Orlando, Florida, USA. This year's meeting gathered researchers, clinicians, and trainees from the United States and other countries to discuss new discoveries that are shaping the future of urologic research. Sessions showcased advances from basic molecular mechanisms to clinical applications, focusing on prostate cancer biology, treatment resistance, metabolic weaknesses, and new treatment strategies. The presentations highlighted the need to combine basic research with various clinical approaches to improve patient outcomes and further the field of urologic oncology.

PLENARY SESSION I: INNOVATIVE MODELS AND TECHNOLOGIES IN UROLOGICAL RESEARCH

Nathan R. Tykocki, PhD (Michigan State University), presented "Get Up and Stretch: Compliance, Remodeling and the Sensation of Bladder Fullness." He explained that traditional methods for measuring bladder compliance ignore structural differences. His team developed a mirror-based, high-speed imag-

ing system that creates detailed 3D and 4D reconstructions of bladder shape and behavior during filling. This platform allowed the lab to uncover rapid inflammation-induced remodeling, irregular pressure events, and changed compliance partly driven by neurogenic signaling. These findings highlight the close connections between tissue mechanics, sensory pathways, and the behavior of the extracellular matrix.

Zohreh Izadifar, PhD (Boston Children's Hospital), followed with "Human Urogenital Organ Chips: Innovative *In Vitro* Models for Urological Research." She emphasized that neuro-genital organs have complex mechanics, diverse cell types, and changing biological environments that standard *in vitro* or animal models do not capture. She described organ-on-chip technologies that recreate human-like systems, showcasing cervix and vagina chips that imitate hormonal responses, mucus behavior, barrier function, and microbiome dynamics, with ongoing development of urinary tract and fertility models.

Leigh Ellis, PhD (Uniformed Services University), then discussed "Spatial Mapping of Accessible Chromatin Landscapes in Prostate Cancer." He outlined an emerging spatial-epigenomics framework to better understand prostate cancer evolution. He noted a rising incidence of the disease among Black men

and explained how tumors transition through various treatment-driven states, influenced by the loss of tumor suppressors and potentially reversible with EZH2 inhibition. His group's single-cell and spatial multi-omics analyses show shared cell-type compositions across races and distinct molecular differences, such as increased EMT programs and unique CTCF motifs, alongside significant heterogeneity within the prostate. Future integration of chromatin, transcriptional, and histological data aims to identify early resistance-related epigenetic features. Finally, Stephen A. Kaplan, MD, FACS (Icahn School of Medicine at Mount Sinai), presented "AUA – Advancing Urology Through Research and Innovation." He shared how his career in clinical practice, basic science, and entrepreneurship led to the creation of the AUA Innovation Nexus. He was motivated by funding challenges, declining physician-scientist pathways, and unmet clinical needs. He explained how turning daily clinical frustrations into practical solutions resulted in successful company launches and inspired a platform for education, mentorship, consulting, startup showcases, and future grant programs aimed at fostering ongoing urologic innovation and improving patient care.

PLENARY SESSION II: TRANSCRIPTIONAL, POST-TRANSCRIPTIONAL, AND EPIGENETIC REGULATION IN UROLOGICAL DISEASES

David P. Labbé, PhD (McGill University), presented "MYC-driven Vulnerabilities in Prostate Cancer." He emphasized that diet significantly affects prostate cancer risk, progression, and treatment response. He demonstrated that high saturated-fat intake can rapidly push early lesions towards more aggressive states, promote invasion, increase angiogenesis, cause DNA damage, and lead to immunosuppression in established tumors, while omega-3s provide protective effects. Brief dietary changes before radiotherapy can also enhance treatment sensitivity, which relies on a functional immune system, and human data support these stage-specific dietary influences.

Katherine Xu, PhD (Columbia University), then presented "Genome-Wide Association Study Across Biobanks Identifies New Susceptibility Loci for Urinary Tract Infections." This study is the largest UTI genetic analysis to date, involving over 1.8 million

individuals and identifying 36 significant loci, including a strong new signal near *PSCA*. Her team found that *PSCA* is expressed throughout the urinary tract, secreted into urine, binds directly to *E. coli*, inhibits bacterial growth, and offers protection against infection in mouse models. Protective *PSCA* variants also lower the risk of other infection-related diseases, although they showed opposite associations in some urogenital cancers.

Feng Yang, PhD (Baylor College of Medicine), delivered a talk titled "MAPK4, an Emerging Oncogenic Driver to Promote Prostate Cancer." He detailed how specific structural regions of MAPK4 are essential for activating AKT. Disruption of these regions weakens the interaction and reduces downstream signaling. He also highlighted GATA2's role as a transcription factor regulating androgen receptors linked to aggressive, treatment-resistant disease. His group discovered an enzyme that targets GATA2 for degradation, suggesting a potential therapeutic strategy to limit AR-driven tumor growth.

PLENARY SESSION III: MICROBIOME, IMMUNITY, AND IMMUNE THERAPEUTICS IN UROLOGICAL DISEASES

Di Zhao, PhD (MD Anderson Cancer Center), presented "Decipher Actionable Genetic Alterations for Personalized Immunotherapy in Advanced Prostate Cancer." He explained that tumors with *PTEN* and *TP53* loss often increase B7-H3 levels to suppress immune activity, making B7-H3 an attractive therapeutic target. While blocking B7-H3 alone offers limited benefit due to compensatory immune pathways, combining its inhibition with PD-L1 or CTLA-4 blockade results in stronger and longer-lasting anti-tumor responses. Dr. Zhao also highlighted *ASH1L* as a newly identified driver of metastasis, particularly in bone, where it promotes invasion and reshapes the microenvironment by influencing macrophages to support metastatic growth.

Nicole J. De Nisco, PhD (The University of Texas at Dallas), followed with "Microbiome After Menopause: Impact on Urinary Microbiome, Metabolome and Recurrent UTI Susceptibility." She emphasized that recurrent UTIs, especially common in postmenopausal women, result from bacterial invasion of deep bladder tissues, persistent inflammation, and changes in the urinary microbiome due to age and hormones. Her group found that premenopausal

women usually carry protective *Lactobacillus*, while postmenopausal women often display dysbiosis and lingering uropathogens linked to recurring infections. Estrogen use restored *Lactobacillus* dominance, and metabolomic profiling identified lipid-related signatures and a possible prognostic biomarker related to future UTI risk.

Conor C. Lynch, PhD (Moffitt Cancer Center), then discussed “Driving $\gamma\delta$ CAR-T Cells into the Bone Metastatic Prostate Microenvironment.” He described developing $\gamma\delta$ CAR-T cells for bone-metastatic castration-resistant prostate cancer, which responds poorly to standard immunotherapies. $\gamma\delta$ T cells naturally recognize tumors without MHC and migrate to epithelial tissues. Their activity is enhanced by zoledronate, a drug often given to these patients. In preclinical studies, $\gamma\delta$ CAR-T cells showed strong tumor-killing capabilities that improved when combined with zoledronate. Spatial transcriptomics indicated that stromal cells in the bone niche support CAR-T function. These promising findings have advanced the therapy into a first-in-human clinical trial.

PLENARY SESSION IV: BIG DATA AND ARTIFICIAL INTELLIGENCE IN UROLOGY

Liang Cheng, MD, MS (Brown University), presented “Harnessing Artificial Intelligence for Prostate Cancer Care.” He discussed the increasing global burden of prostate cancer and the challenges of late diagnosis and a shrinking pathology workforce. He highlighted how AI is changing detection, biopsy interpretation, grading accuracy, and outcome prediction, achieving performance levels nearly equal to pathologists in Gleason grading, molecular prediction, and reducing errors. While AI offers improvements in efficiency, cost reduction, and better integration of histology, imaging, and clinical data, challenges remain related to generalizability, regulation, and dataset quality.

Housheng Hansen He, PhD (University of Toronto), followed with “Transcriptional and Functional Landscape of Circular RNA in Prostate Cancer.” He showed that circRNAs are widely expressed in prostate tumors and influence key pathways that drive aggressive disease, suggesting their potential as biomarkers and therapeutic targets.

Victor Jin, PhD (Medical College of Wisconsin), then presented “Learning Multi-Omics-Seq Data to Predict Pioneer Factors-Mediated Nucleosome Repo-

sitioning in Lethal Prostate Cancer.” He explained how prostate cancer evolves from androgen-dependent to castration-resistant forms through lineage plasticity driven by pioneer transcription factors like FOXA1, GATA2, and HOXB13. His team used computational tools such as ePEST and NucHMM to show that FOXA1 and GATA2 play distinct roles in chromatin remodeling. GATA2’s binding at canonical AR enhancers may drive the transition from ADPC to CRPC, highlighting it as a promising therapeutic target.

Finally, Zheng Xia, PhD (Oregon Health & Science University), presented “Single-Cell Transcriptome Atlas to Reveal Heterogeneity and Cellular Ecosystem Dynamics During Prostate Cancer Progression.” He introduced PCCAT, a detailed atlas created from hundreds of integrated single-cell datasets from normal and malignant prostate tissues. The atlas revealed significant heterogeneity among tumor, stromal, and immune cells and identified specific fibroblast and immune populations that encourage aggressive disease. It also includes an interactive portal to aid in discovering biomarkers and development strategies for advanced prostate cancer.

PLENARY SESSION V: CELLULAR HETEROGENEITY AND PLASTICITY IN UROLOGICAL BIOLOGY

David Goodrich, PhD, from Roswell Park Comprehensive Cancer Center, discussed his work on “Molecular Determinants of Prostate Cancer Lineage Plasticity.” He explained bipolar androgen therapy (BAT) as a method intended to improve differentiation. This approach alternates patients between low-androgen states and short bursts of high-dose testosterone. The goal is to limit cellular plasticity and reduce the chances of tumors becoming highly resistant forms like CRPC or NEPC. His research indicates that BAT helps maintain an androgen receptor-dependent epithelial phenotype, slows tumor growth, limits the emergence of more aggressive variants, and fosters an immune environment that helps control tumors. Overall, his findings suggest that epigenetic changes, rather than lasting genetic mutations, play the main role in prostate cancer progression. Dean Tang, PhD (Roswell Park Comprehensive Cancer Center), followed with “Epigenetic Basis and Therapeutic Targeting of Prostate Cancer Heterogeneity and Plasticity.” He talked about how prostate cancer stem cells and castration-resistant tumors arise due

to therapy-induced plasticity. He highlighted miR-34a as a strong inhibitor of PCSC activity and metastatic behavior. Although initial attempts to apply this clinically faced challenges, newer delivery methods for miR-34a using ligands and nanoparticles have shown promising preclinical activity against aggressive, treatment-resistant prostate cancer.

Jung Wook Park, PhD (Duke University), presented "Multi-Functional Neuronal Protein Drives the Growth, Differentiation, and Metastasis of Advanced Prostate Cancer." He discussed how therapeutic pressure from agents like enzalutamide can encourage neuroendocrine differentiation. He identified NPTX1 and HDAC6 as critical regulators of NEPC proliferation, differentiation, and metastatic potential. Both molecules represent promising paths for future treatment development.

Finally, Ana Aparicio, MD (MD Anderson Cancer Center), in "Dissecting the Heterogeneity within Aggressive Variant Prostate Cancers," emphasized the diverse biology of androgen-indifferent tumors. These tumors often harbor defects in *RB1*, *TP53*, and *PTEN* and respond variably to existing treatments. While some patients benefit from platinum chemotherapy or PARP inhibitors, others depend significantly on metabolic adaptations, especially arginine metabolism, to sustain progression and dodge immune responses. Current clinical efforts that target these metabolic weaknesses highlight the urgent need to better categorize aggressive variants and personalize treatments accordingly.

PLENARY SESSION VI: NOVEL AND EMERGING THERAPEUTIC APPROACHES IN UROLOGICAL RESEARCH

Isaac Kim, MD, PhD (Yale University), in "Leveraging the Surgical Trial in Metastatic Prostate Cancer Research," reflected on his career, mentorship, and the development of his department while stressing the importance of combining surgery with translational research. He discussed the challenges of treating *de novo* metastatic prostate cancer, noting that despite advancements in systemic therapy, survival improvements remain modest. Drawing on his clinical experience, he emphasized that cytoreductive radical prostatectomy can be safe and potentially beneficial for select patients, particularly those with low metastatic burden. This sometimes yields deep PSA responses or lasting disease control. Ongoing

phase I and randomized trials are assessing the impact of combining surgery with standard therapies to identify which patients benefit most. Early results suggest potential improvements in local control, quality of life, and possibly survival, although careful patient selection remains critical.

Kexin Xu, PhD (University of Virginia), in "Targeting RNA Methylation for Castration-Resistant Prostate Cancer," presented new evidence that M6A RNA modifications play a significant role in prostate cancer progression and resistance. These modifications regulate key genes related to androgen signaling and tumor growth. Manipulating this pathway in preclinical models slows cancer growth, making RNA methylation machinery a promising target for therapy.

Roberto Pili, MD (University at Buffalo), in "Integrating Dietary Interventions with the Treatment of Urological Malignancies," discussed how nutrition affects cancer risk, tumor biology, and treatment response. He shared data showing that cutting down on animal protein or using caloric or time-restricted diets changes tumor metabolism, hormone signaling, and immune responses. Clinical studies suggest that dietary changes can improve treatment tolerance, increase therapeutic effectiveness, and may enhance long-term outcomes, supporting diet as a helpful addition to standard cancer treatments.

POSTER SESSION #1 AND #2

On November 14 and 15, 2025, the conference featured evening poster sessions with a total of 88 selected presentations. Among these, Vrunda Satsiya presented our ongoing research titled "Characterization of FAM120A as a Novel Effector in the Progranulin/EphA2 Axis in Bladder Cancer." In her study, she showed that FAM120A acts as a new progranulin-dependent EphA2 interactor in bladder cancer and contributes to increased cellular motility, invasion, clonogenicity, spherogenesis, anchorage-independent growth, and *in vivo* tumor progression. Additionally, FAM120A is upregulated in bladder cancer tissues compared to normal tissues, suggesting it may serve as a biomarker for bladder cancer.

CONCLUSIONS

These presentations highlight the growing complexity of urological diseases and the rapid progress in how

they are studied and treated. Research on prostate cancer is uncovering how lineage plasticity, neuroendocrine differentiation, metabolic changes, and epigenetic alterations drive resistance. New therapeutic strategies, including immunotherapy, RNA-modifying targets, and dietary interventions, are emerging in response. Innovative technologies such as organ-on-chip systems, spatial epigenomics, AI-assisted diagnostics, and single-cell atlases are offering deeper insights into tissue biology, microenvironment interactions, and individual patient differences. Beyond cancer, new tools are enhancing our understanding of bladder mechanics and the role of the microbiome in recurrent UTIs. This collective work emphasizes the importance of interdisciplinary collaboration and tailored approaches to improve outcomes across urology.

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Author's contributions

VS attended the meeting and drafted and revised the manuscript. MNT revised the manuscript. AM provided the guidance and revised the manuscript. AG provided funds to attend the meeting and revised the manuscript.

Ethical approval

N/A.

Publication ethics

Plagiarism

Authors declare no potentially overlapping publications with the content of this manuscript and all original studies are cited as appropriate.

Data falsification and fabrication

All the data correspond to the real.



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