IRE ISTITUTO NAZIONALE TUMORI REGINA ELENA ISG ISTITUTO DERMATOLOGICO SAN GALLICANO
ISTITUTI DI RICOVERO E CURA A CARATTERE SCIENTIFICO

UOC Acquisizione Beni e Servizi

Il dirigente della UOC Acquisizione Beni e Servizi

in virtù della delega conferita con deliberazione N°327/2025

HA ASSUNTO LA PRESENTE DETERMINAZIONE

N. 564 del 17/06/2025

OGGETTO: Autorizzazione alla liquidazione delle fatture per la pubblicazione di articoli scientifici a diverse società: Elsevier B.V. (invoice nr. OAD0000580786), MDPI AG (invoice nr. 3574830), Elsevier B.V. (invoice nr. OAD0000583697), MDPI AG (invoice nr. 32993994) e Springer Nature Group (invoice nr. 2936453526). Fondo Ricerca Corrente IRE 2025 CUP H53C25000130001, responsabile il Direttore Scientifico IRE f.f, Fondo A.I.R.C. cod. IFO 25/30/R/02 CUP H53C24001670007 responsabile Dr. Giovanni Blandino, Fondo ARCO cod. IFO 21/09/R/24 NOCUP responsabile Dr. Paolo Basili.

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L'estensore

Daniela Kolziu

Il Responsabile del Procedimento

Barbara Filipponi

Il Dirigente della UOC Acquisizione Beni e Servizi

Giuseppe Navanteri

La presente determinazione si compone di n° 6 pagine e dei seguenti allegati che ne formano parte integrante e sostanziale:
Allegati nr. 37; note protocollate, copia fattura e articolo scientifico pubblicato.

Il Dirigente della UOC Acquisizione Beni e Servizi

Visto il Decreto Legislativo 30 dicembre 1992, n. 502 e ss.mm.ii.;

Visto il Decreto Legislativo 16 ottobre 2003, n. 288 e ss.mm.ii.;

Vista la Legge Regionale 23 gennaio 2006, n. 2;

Visto il Decreto Legislativo 31 marzo 2023, n. 36 ed integrato e modificato con Decreto Legislativo del 31 dicembre 2024, n.209;

Visto l'Atto Aziendale adottato con deliberazione IFO n.153 del 19.02.2019, ed approvato dalla Regione Lazio con DCA n. U00248 del 02.07.2019, modificato e integrato con deliberazioni n. 1254 del 02.12.2020, n. 46 del 21/01/2021 e n. 380 del 25.03.2021, approvate dalla Direzione Salute ed Integrazione Sociosanitaria della Regione Lazio, con Determinazione n. G03488 del 30.03.2021;

Visto il Decreto del Presidente della Regione Lazio n. T00015 del 12 febbraio 2025 avente ad oggetto "Nomina del Direttore Generale dell'Azienda Sanitaria Locale dell'IRCCS Istituti Fisioterapici Ospitalieri (Art. 8, comma 7 bis, della legge regionale 16 giugno 1994, n. 18 e s.m.i.)";

Vista la deliberazione n. 160 del 18.02.2025 di presa d'atto dell'insediamento del Direttore Generale dell'IRCCS Istituti Fisioterapici Ospitalieri Dott. Livio De Angelis;

Vista la deliberazione n. 293 del 31.03.2025 con la quale il Dott. Massimo Armitari è stato nominato Direttore Amministrativo degli Istituti Fisioterapici Ospitalieri (IFO);

Vista la deliberazione n. 367 del 23 aprile 2024 con la quale la Dott.ssa Costanza Cavuto è stata nominata Direttore Sanitario f.f. degli Istituti Fisioterapici Ospitalieri;

Visto il D.M. del Ministero della Salute del 20 giugno 2024 di conferma del riconoscimento del carattere scientifico dell'IRCCS di diritto pubblico a Istituti Fisioterapici Ospitalieri (IFO) relativamente alla disciplina di "oncologia" per l'Istituto Nazionale Tumori Regina Elena (IRE) e alla disciplina di "dermatologia" per l'Istituto Santa Maria e San Gallicano (ISG);

Vista la deliberazione n.877 del 29.10.2024 ad oggetto: "Nomina della Prof.ssa Maria Concetta Fargnoli quale Direttore Scientifico dell'Istituto Santa Maria e San Gallicano (ISG).";

Vista la deliberazione n.171 del 28.02.2025 avente ad oggetto: "Nomina del Prof. Giovanni Blandino, Direttore della UOC Ricerca Traslazionale Oncologica, quale Direttore Scientifico IRE facente funzioni, a decorrere dal 01.03.2025.";

Vista la deliberazione n. 327 del 3 aprile 2025 di attribuzione delle deleghe ai Dirigenti del Ruolo Professionale, Tecnico e Amministrativo da parte del Direttore Generale degli IFO;

Premesso che in esecuzione alla deliberazione n. 428 del 9/04/2021, è stato accettato il contributo da parte di ATS – Doxea S.r.l., Beps Engineering S.r.l. e Haedapp S.r.l., per lo svolgimento del progetto di Ricerca dal titolo: "ARCO Assistenza remota ai caregiver oncologici", cod. IFO 21/09/R/24, responsabile Dr. Paolo Basili;

con deliberazione n.27 del 02.01.2025, è stato accettato il finanziamento disposto dall'Associazione Italiana per la Ricerca sul Cancro (A.I.R.C.) a favore dell'Istituto Regina Elena per lo svolgimento del progetto dal titolo: "Deciphering the cotribution of mutant p53/yap axis to the resistance to therapy of relapsing head and neck cancers" cod. IFO 25/30/R/02, responsabile Dr. G. Blandino;

con nota protocollo n.930 del 21 gennaio 2025 del Direttore Scientifico IRE e Nulla Osta del Commissario Straordinario IFO e successivamente nominato Direttore Generale, è stato autorizzato l'appostamento della Ricerca Corrente 2025 IRE per un importo pari a € 3.462.944,05 responsabile Direttore Scientifico IRE f.f.;

Considerato che - il dr. Giovanni Blandino con nota protocollo 7567 del 20/05/2025 ha chiesto la liquidazione della seguente fattura:

-Fattura nr. OAD0000580786 del 15/05/2025 di € 5.916,00 Iva compresa della
società Elsevier B.V. relativa alla pubblicazione del manoscritto dal titolo "MicroRNA-mediated PTEN downregulation as a novel non-genetic mechanism of acquired resistance to PI3Ka inhibitors of head&neck squamous cell carcinoma"alla Rivista Scientifica: Drug Resistence Updates;

- il dr. Paolo Basili con nota protocollo n. 7678 del 22/05/2025, ha richiesto la liquidazione della seguente fattura:

- Fattura nr. 3574830 del 20/05/2025 di € 2.115,37 Iva compresa della società MDPI AG relativa alla pubblicazione del manoscritto dal titolo "The Effectiveness of patients education interventions to oncological entero-urostomy patients and caregivers: a small sample size pilot study" sulla Rivista Scientifica: Diseases;

-il dr. Eriseld Kasniqi con nota protocollo n. 8246 del 03/06/2025, ha richiesto la liquidazione della seguente fattura:

- Fattura nr. 32993994 del 05/12/2024 di € 3.803,72 Iva compresa della società MDPI AG relativa alla pubblicazione del manoscritto dal titolo "Tolerability and Preliminary outcomes of adjuvant T-DM1 in HER2-positive breast cncer after neoadjuvant therapy: the ATD study" sulla Rivista Scientifica: Cancers;

-la Dr.ssa Maria Rizzo con nota protocollo n.8223 del 03/06/2025, ha richiesto la liquidazione della seguente fattura:

-Fattura nr. 2936453526 del 22/05/2025 di € 2.982,90 Iva compresa della società Springer Nature Group relativa alla pubblicazione del manoscritto dal titolo: "Androgen receptor inhibition sensitiezes glioblastoma stem cell to temozolomide by the miR-1/miR-26a-1/miR-487b signature mediated WT1 and FOXA1 silencing" sulla Rivista Scientifica: Cell Death Discovery;

- la Dr.ssa Fabiana Cacciatoricon nota protocollo nr. 7863 del 26/05/2025, ha richiesto la liquidazione della seguente fattura:

-Fattura nr. OAD0000583697 del 23/05/2025 di € 4.013,80 Iva compresa alla societa Elsevier B.V. relativa alla pubblicazione del manoscritto dal titolo: "Induction of cell death by the CXCR2 antagonist SB225002 in colorectal cancer and stromal cells"

Acquisito il parere favorevole del Direttore Scientifico f.f. dell'Istituto Regina Elena, apposto in calce alle richieste sopra citate;

Accertata la disponibilità sui Fondi citati in premessa;

Esperiti i controlli sulle richieste presentate dai responsabili dei progetti;

Attestato che il presente provvedimento, a seguito dell'istruttoria effettuata, nella forma e nella sostanza è totalmente legittimo e utile per il servizio pubblico, ai sensi dell'art.1 della legge 20/94 e successive modifiche, nonché alla stregua dei criteri di economicità e di efficacia di cui all'art.1, primo comma, della legge 241/90, come modificata dalla legge 15/2005;

DETERMINA

per i motivi di cui in narrativa che si intendono integralmente confermati di:

1-autorizzare il pagamento della seguente fatture:

-Fattura nr. OAD0000580786 del 15/05/2025 di € 5.916,00 Iva compresa della società Elsevier B.V. relativa alla pubblicazione del manoscritto dal titolo "MicroRNA-mediated PTEN downregulation as a novel non-genetic mechanism of acquired resistance to PI3Ka inhibitors of head&neck squamous cell carcinoma"alla Rivista Scientifica: Drug Resistence Updates;

- Fattura nr. 3574830 del 20/05/2025 di € 2.115,37 Iva compresa della società MDPI AG relativa alla pubblicazione del manoscritto dal titolo "The Effectiveness of patients education interventions to oncological entero-urostomy patients and caregivers: a small sample size pilot study" sulla Rivista Scientifica: Diseases;

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2 - far gravare la spesa complessiva di € 18.831,77 sui Fondi: Fondo Ricerca Corrente IRE 2025 per € 10.800,40, responsabile il Direttore Scientifico IRE f.f, Fondo A.I.R.C. cod. IFO 25/30/R/02 per € 5.916,00 responsabile Dr. Giovanni Blandino, Fondo ARCO cod. IFO 21/09/R/24 per € 2.115,37 responsabile Dr. Paolo Basili che presentato la necessaria disponibilità.

Cod. IFO 25/30/R/02

- assegnato:	€	132.000,00
- utilizzato:	€	83.329,69
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Cod. IFO 21/09/R/24

- assegnato:	€	75.000,00
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- presente atto:	€	2.115,37
- residuo:	€	11.803,08

Ricerca Corrente IRE 2025

- assegnato:	€	3.462.944,05

- utilizzato: € 1.031.312,55
- presente atto: € 10.800,40
- residuo: € 2.420.831.10

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La UOC Acquisizione Beni e Servizi curerà tutti gli adempimenti per l'esecuzione della presente determinazione.

Il Dirigente della UOC Acquisizione Beni e Servizi

Giuseppe Navanteri

Documento firmato digitalmente ai sensi del D.Lgs 82/2005 s.m.i. e norme collegate

IRE
ISTITUTO NAZIONALE TUMORI
REGINA ELENA
ISTITUTO DI RICOVERO E CURA A CARATTERE SCIENTIFICO

Roma, 20/05/2025

Alla c.a. del Direttore Scientifico IRE

In relazione all'accettazione della pubblicazione del lavoro scientifico dal titolo: "*The Effectiveness of Patient Education Interventions to Oncological Entero-Urostomy Patients and Caregivers: A Small Sample Size Pilot Study*"

con autori: *Alessandro Spano, Fabrizio Petrone, Emanuele Di Simone, Aurora De Leo, Paolo Basili*, Irene Terrenato, Maria Antonietta Picano, Marco Piergentili, Albina Paterniani, Laura Iacorossi and Nicolò Panattoni*,

con principale affiliazione: *IRCCS Regina Elena National Cancer Institute, Rome, Italy*,

sulla rivista "*Diseases* (ISSN 2079-9721)" - IF: 2.9, avvenuta in data 20/5/2023 (come da certificato allegato),

si richiede il pagameto dell'invoice numero 3574830 (allegata alla presente) entro e non oltre il 30 maggio 2025 per un totale di **1733,91€** attraverso il Fondo ATS (Doxea S.r.l., Beps Engineering S.r.l., and Haedapp S.r.l.) grant number: ARCO 2109R24 - DELIBERAZIONE N. 428 DEL 09/04/2021—IRCCS Istituti Fisioterapici Ospitalieri, responsabile scientifico Dr. Paolo Basili.

Cordiali saluti
Paolo Basili

IL DIRETTORE SCIENTIFICO
Istituto Nazionale Tumori "Regina Elena"

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Article

The Effectiveness of Patient Education Interventions to Oncological Entero-Urostomy Patients and Caregivers: A Small Sample Size Pilot Study

Alessandro Spano [1,†], Fabrizio Petrone [2,†], Emanuele Di Simone [3], Aurora De Leo [2], Paolo Basili [2,*], Irene Terrenato [4], Maria Antonietta Picano [2], Marco Piergentili [2], Albina Paterniani [5], Laura Iacorossi [6] and Nicolò Panattoni [2]

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† The authors contributed equally to this work.

Abstract: Patient Education (PE) is an integral part of the treatment from taking charge to the care, assistance, and rehabilitation of the patient, and consists of structured, organised actions, the orientation of which is aimed at finding solutions supported by scientific evidence. This prospective, descriptive, exploratory, single-centre pilot study aimed to evaluate the effectiveness of a the PE intervention for oncological patients with entero-urostomies and their caregivers, through the measurement of the quality of life, perceived needs, and caregiver burden. This study was conducted in a National Cancer Institute between 22 December 2022, and 31 March 2023, and it was organised into three specific therapeutic education events days relative to the real needs measureds by the patients and caregivers, before it. Our results seem to suggest that the PE intervention in entero-urostomy patients improves their quality of life levels, while caregivers' perceived emotional burden levels are reduced. Targeted and individualised PE interventions positively affect self-care and quality of life in patients with an entero-urostomy and the emotional burden perceived by caregivers

Keywords: patient education; cancer patient; caregiver; entero-urostomy; pilot study

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1. Introduction

Patient education (PE) is a process of learning and support provided to patients to manage their health condition better [1]. It is a personalised educational approach that provides information, skills, and practical strategies to address the disease, improve quality of life (QoL), and promote patient autonomy. Specifically, for an the ostomy patient, it

Commented [M1]: Please carefully check the accuracy of names and affiliations.

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is a fundamental path to help the patient and caregiver understand and adapt to their new condition and image. A structured stoma PE training program using an interactive educational approach to the patient and caregiver can further improve outcomes such as stoma self-management and post-ostomy QoL [2–4].

PE can be carried out by various professionals with specific scientific and pedagogical skills, which are learned through dedicated training courses [5]. Furthermore, it involves all phases of the helping relationship, starting from ~~the~~ communication of the diagnosis, through a targeted methodology, up to the ability to dynamically cope with the difficulties that the disease brings with it [6]. Specifically, the nurse plays a preponderant role in the patient's educational process, so much so that PE is considered a fundamental component of nursing [7,8].

Ostomy cancer patients and their caregivers have special needs that require personalised and continuous care [6]. Stoma patients and their caregivers experience physical, psychological, and emotional stress, and an economic burden. A new body image, difficulties in social relationships, anxiety, depression, embarrassment, and sexual problems can affect this specific population [3,7]. For these reasons, PE interventions must be implemented in oncology centers, which can overcome the misinformation of patients and caregivers and all the barriers that may in some way hinder their ability to receive, process, and put the information into practice. When implementing a PE process, it is essential that the health information provided, in addition to being of high quality, is easily interpretable by patients. In the literature, we can find various tools to support the educational methodology, always and in any case built with an easily understandable language, for example, by integrating diagrams, images, or multimedia supports [10,11].

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The educational approach in PE can be practical or theoretical and can make use of multiple expository (academic lessons), operational (learning by doing), and investigative (problem-based learning) methodologies.

The Stoma care Nurse Specialist~~'s~~ (ScNS) is the point of reference for ostomy patients, providing them with specialis~~z~~ed support and care. These professionals plays a key role in implementing interventions aimed at the prevention, treatment, rehabilitation, and education of ostomy patients. Working closely with patients, the ScNS provides detailed information on ~~the~~ daily management of the ostomy, including ~~the~~ care of the stomal area, ~~the~~ application of collection bags, and ~~the~~ adoption of a proper lifestyle. It also enables the ScNS to assess the patient's specific needs, provide individualis~~z~~ed support, and prepare an appropriate care plan in preparation for surgery and the postoperative period [6]. The presence of an ScNS in the care pathway contributes significantly to improving QoL and promoting appropriate treatment adherence by ensuring ongoing support and effective ostomy management [12]. Our PE project originated as a nursing reflection on personalis~~z~~ed patient care. PE is also a quality requirement from the OECI (Organization European Cancer Institute) accreditation system for Comprehensive Cancer Centers, such as the Istituto Ricovero e Cura a Carattere Scientifico (IRCCS) National Cancer Institute "Regina Elena" in Rome, Italy.

Thus, PE is an integral part of ~~the~~ treatment, from taking charge to the care, assistance, and rehabilitation of the patient, and consists of structured, organis~~z~~ed actions, the orientation of which is aimed at finding solutions supported by scientific evidence [6,13]. Promoting learning processes is a stimulating objective as it puts the operator in a strong relationship with the patient and caregiver. The "educational" approach aims to provide knowledge and ensure understanding of health problems in such a way as to encourage ~~the~~ autonomous analysis of the patient's behaviours and habits and to make informed decisions for their health [13]. Indeed, further research seems necessary to underline the effectiveness of ~~P~~patient ~~E~~education interventions for oncological entero-urostomy patients and caregivers.

To this end, we hypothesised that performing PE on patients with an entero-urostomy will improve their levels of QoL, while their levels of perceived emotional burden will be reduced for caregivers.

Therefore, this study aimed to evaluate the effectiveness of the PE intervention for oncological patients with entero-urostomies and their caregivers, through the measurement of the QoL, perceived needs, and caregiver burden.

2. Materials and Methods

2.1. Study Design

A prospective, descriptive, exploratory, single-centre pilot study [14] was conducted at the IRCCS National Cancer Institute "Regina Elena" in Rome, Italy between 22 December 2022, and 31 March 2023. The study was conducted following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [15] (Table S1).

2.2. Patient Education Intervention Methodology

According to Patient Education Guidelines [16], the PE intervention was organised into three specific therapeutic education events days, in which the educational contents were calibrated based on the real needs measureds by the patients and caregivers, assessed before it with the Needs Evaluation Questionnaire (NEQ) (detailed below).

Each PE day was conducted by the a staff of three3 ScNSs, previously trained in PE, with a specific training course [5].

The learning areas were divided into three for each PE event day [16]: PE event day one based on ostomy theoretical knowledge (a cognitive learning area relating to knowledge, concepts, procedures, and principles); PE event day two based on gesturality knowledge (a gestural learning area relating to operational and manual skills); and the third and last PE event day based on socio-relational knowledge (a relational learning area related to social, family, and work relationships).

On all days, there was a mix of face-to-face knowledge transmission with interactive reinforcement questions (a participatory lesson, where an expert "explains" and the "learners" listen and interact through an exchange of information/opinions), during which patients and caregivers could comment extemporaneously but anonymously through a digital platform; the questions were that was read aloud to the group, forming the basis of discussion on which to build new knowledge to be transferred.

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Finally, each PE event day lasted approximately 2 h, and the patient and caregivers' recruitment wereas free of a fee and voluntary.

At the end of the last PE event day, Conversation Maps, specially constructed during the events, were left for patients as a reference guide for home self-management.

In particular, Conversation Maps areis an educational and didactic tool. TheyIt consists of quick ly-consultation image guides set through which people are engaged in discussion, and shared doubts and experiences about their lives to reinforce the theoretical concept acquired [17].

2.3. Patient Recruitment

To achieve our aim, a consecutive sample of 10 participants (five5 patients and five5 caregivers) was enrolled. All these participants had an oncological entero-urostomy clinical pathway at the IRCCS National Cancer Institute "Regina Elena" in Rome, Italy. Specifically, we considered all patients who accessed the Stoma Care Nurse Specialist's Outpatient clinic. The Eenrollment in the study was based on specific inclusion criteria, as follows: (i) aged > 18 years; (ii) patient having a histologically proven diagnosis of cancer; (iii) patient with having entero-urostomies; (iv) caregiver formal or informal caregiver of

a patient ~~with~~ having entero-urostomies; (v) availability to provide written informed consent to study participation; and (vi) willingness to participate in ~~the~~ several PE meetings both for patients and caregivers.

The patients without a histologically proven cancer diagnosis or with cognitive deficits or pathological conditions that may be an obstacle to active participation in the study were excluded.

2.4. Data Collection and Assessment Tools

All patients recruited into the study were invited to complete questionnaires at three different time-points: before the PE event (t0), at the end of the training event (t1), and 30 days after the event itself (t2). We collected the following data: (1) ~~s~~Sociodemographic (age, gender, education, marital status, work activity, and family members) and clinical data (pathology, date of diagnosis, previous therapy, and ongoing therapy); (2) Needs Evaluation Questionnaire (NEQ) [18]: a validated tool created for ~~the~~ analysis of the needs of cancer patients, which has proven useful in obtaining a systematic and undistorted vision of patients' needs; (3) Short-Form Health Survey 36, SF-36 [19]: this instrument aims to explore your concept of well-being related to the dimensions of physical, psychological, and emotional well-being to assess ~~the~~ QoL overall; and (4) Ostomy Self-Care Index (OSCI) [20]: a validated tool that measures self-care in people with an ostomy and is composed of 32 items and four~~4~~ sections (A, B, C, and D) ~~which~~ that respectively evaluate: self-care maintenance, self-care monitoring, self-care management, and self-care confidence. The score has a theoretical range between 31 (worst possible self-care) and 165 (optimal self-care).

Moreover, all caregivers recruited in the study were~~ill be~~ invited to complete specific questionnaires at the same time-points t0, t1, and t2. We collected the following information: (1) ~~s~~Sociodemographic data (age, gender, education, marital status, work activity, and family members); (2) Caregiver Need Assessment (CNA) [21]: a tool with good internal consistency that measures the needs perceived by the caregiver relating to the care of their family member. It is made up of 17 items (total score: 0–51), which refer to needs that can be grouped into two factors: need for emotional and social support ($\alpha = 0.765$), and information and communication needs ($\alpha = 0.742$). For each item, there is a Likert scale with a score between zero~~0~~ and three~~3~~ (not at all, a little, quite a lot, and a lot): a higher score corresponds to a greater intensity of the perceived need; (3) Zarit Burden Interview [22]: it is a validated scale aimed at ascertaining the subjective tension overload (burden) to which a caregiver is subjected. The original version of the scale and the one validated in Italian are made up of 22 items. Conceived in the field of diseases of the nervous system and psychiatry, it has also been applied in other fields, such as oncology.

Finally, all patients and caregivers will be given a satisfaction questionnaire regarding the PE event at the end of it based on a five-point Likert scale.

2.5. Statistical Analysis

Following the study design [14], descriptive statistics were used to summari~~s~~ze relevant information about the study and presented as mean ± standard deviation (SD) or frequencies and percentage values. Statistical analyses were performed with SPSS 29.0 software (SPSS, Chicago, IL, USA).

2.6. Ethical Considerations

This study was conducted according to the Declaration of Helsinki [22] and approved by the Health Direction of IRCCS National Cancer Institute "Regina Elena" and the Local Ethics Committee IRCCS Lazio (No. 1808/22 of 13 December 2022). The data collected were analysed in aggregate and anonymously and confidentiality coded, to guarantee

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participants' privacy. The recruitment was free and voluntary, and after written informed consent was signed, and at any time, the participants enrolled could leave the study without consequences in the clinical pathway. All the nurses involved in the study completed a PE training course.

3. Results

A total of 10 participants (five oncological entero-urostomy patients and five caregivers) were included in the study. The sociodemographic and clinical characteristics are summarised in Table 1. The sample was mainly composed of predominantly male patients and predominantly female caregivers, both 60%, and all married to each other (100%). Furthermore, patients from 60% to 80% were undergoing chemotherapy or radiotherapy treatment.

Table 1. Sample sociodemographic and clinical characteristics.

	Patients N (%)	Caregivers N (%)
Total	5 (100%)	5 (100%)
Gender		
Male	3 (60%)	2 (40%)
Female	1 (20%)	3 (60%)
Unknown	1 (20%)	0
Education		
Primary	1 (20%)	0
Secondary	1 (20%)	3 (60%)
Higher	2 (40%)	2 (40%)
Degree	1 (20%)	0
Marital status		
Married	5 (100%)	5 (100%)
Unmarried	0	0
Job		
Worker	1 (20%)	1 (20%)
Housewife	1 (20%)	2 (40%)
Retired	2 (40%)	2 (40%)
Undeclared	1 (20%)	0
Living alone		
Yes	0	0
No	5 (100%)	5 (100%)
Children		
Yes	4 (80%)	5 (100%)
No	1 (20%)	0
Surgery		
Yes	5 (100%)	/
No	0	/
Chemotherapy		
Yes	4 (80%)	/
No	1 (20%)	/
Radiotherapy		
Yes	3 (60%)	/
No	2 (40%)	/

Regarding patient surveys, the NEQ's results showed an overall increase in patients' perceived needs at t2 compared with the baseline survey.

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Similarly, Table 2 describes the results for the OSCI showing the average increase in ~~the~~ several dimensions of self-care. The average trend of the OSCI score shows how for all the self-care dimensions explored (self-care maintenance, self-care monitoring, self-care management, and self-care confidence), we have a decline at t1, and then an increase at t2 tending to optimal self-care.

Table 2. OSCI results.

	T0	T1	T2
Pz 1	134	135	147
Pz 2	153	123	145
Pz 3	143	136	152
Pz 4	142	126	144
Pz 5	151	128	148
	Mean (SD)	*Mean (SD)*	*Mean (SD)*
Overall	144.6 (±0.4)	129.6(±0.1)	147.7 (±0.1)

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Figure 1 graphically shows the trend in QoL related to the dimensions of physical, psychological, and emotional well-being from t0 to t2. All the dimensions explored except for "role limitations due to physical health", "pain", and "general health", show a trend of improvement at t2.

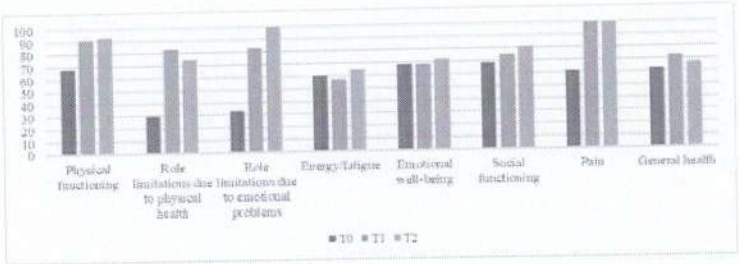

Figure 1. SF-36 results.

From a caregiver's point of view, the CNA results show an overall increase from t0 to t2, reporting an increase in the needs perceived by the caregiver relating to the care of their family member (Table 3), while the overall Zarit Burden Interview results are decreasing across follow-ups, reflecting a decrease in the subjective tension overload (burden) to which the caregiver is subjected (Table 4).

The levels of satisfaction for each PE event are encouraging, as they are high, very close to the maximum value of 5 (4.81 on average for patients and 4.71 for caregivers).

Table 3. CNA results.

Question n°	T0	T1	T2
	Mean (SD)	Mean (SD)	Mean (SD)
1	3.20 (±1.30)	2.60 (±1.14)	3.60 (±0.55)
2	2.80 (±1.64)	2.80 (±1.10)	3.20 (±0.45)
3	2.80 (±1.64)	2.20 (±1.30)	3.60 (±0.55)
4	1.80 (±1.30)	2.00 (±1.22)	1.80 (±1.30)
5	2.20 (±1.10)	2.40 (±0.55)	3.20 (±0.45)
6	2.20 (±1.64)	1.60 (±0.55)	1.40 (±0.55)
7	2.00 (±1.00)	3.40 (±0.55)	3.80 (±0.45)

8	2.20 (±1.64)	2.60 (±0.55)	3.40 (±0.55)
9	2.40 (±1.34)	1.60 (±0.89)	1.60 (±0.89)
10	2.80 (±1.30)	2.60 (±1.14)	3.40 (±0.55)
11	2.00 (±1.00)	1.60 (±0.55)	1.40 (±0.55)
12	2.20 (±1.10)	3.40 (±0.89)	3.80 (±0.45)
13	1.80 (±0.84)	2.60 (±1.14)	3.40 (±0.55)
14	1.00 (±0)	1.60 (±1.34)	1.80 (±1.30)
15	1.40 (±0.55)	2.80 (±1.30)	3.00 (±1.00)
16	1.00 (±0)	1.00 (±0)	2.20 (±0.84)
17	1.00 (±0)	1.40 (±0.55)	1.40 (±0.55)

Note: Range 1–4 (1. Never, 2. Sometimes, 3. Often, 4. Always).

Table 4. Zarit Burden Interview results. ~~The text continues here.~~

Question N°	T0	T1	T2
	Mean (SD)	*Mean (SD)*	*Mean (SD)*
1	0.20 (±0.45)	0.60 (±0.55)	0 (±0)
2	1.00 (±0.71)	0.60 (±0.55)	1.20 (±0.84)
3	1.60 (±0.89)	0 (±0)	0 (±0)
4	0.20 (±0.45)	0 (±0)	0 (±0)
5	0 (±0)	0 (±0)	0 (±0)
6	0.80 (±0.84)	0 (±0)	0 (±0)
7	1.80 (±1.10)	1.40 (±0.55)	2.20 (±0.84)
8	0.60 (±0.89)	0 (±0)	0.60 (±0.89)
9	1.20 (±1.10)	0.40 (±0.55)	0.40 (±0.55)
10	0.80 (±0.84)	0.40 (±0.55)	0.80 (±0.84)
11	0 (±0)	0 (±0)	0 (±0)
12	1.40 (±1.67)	0.40 (±0.55)	0.40 (±0.55)
13	0.60 (±0.55)	0 (±0)	0 (±0)
14	0.80 (±1.30)	0 (±0)	0.40 (±0.55)
15	0.40 (±0.89)	0.60 (±0.55)	0.60 (±0.55)
16	0 (±0)	0.40 (±0.55)	0.20 (±0.45)
17	0.40 (±0.55)	0.60 (±0.55)	0.40 (±0.55)
18	0 (±0)	0 (±0)	0 (±0)
19	0.40 (±0.55)	0.60 (±0.55)	0 (±0)
20	1.40 (±1.14)	1.00 (±1.00)	0.40 (±0.55)
21	1.00 (±1.41)	0 (±0)	0 (±0)
22	1.40 (±0.55)	0 (±0)	0 (±0)

Note: Range 0–4 (0. Never, 1. Rarely, 2. Sometimes, 3. Often, 4. Always).

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4. Discussion

The results achieved by this prospective, descriptive, exploratory, single-centre pilot study made it possible to achieve the study aim, which was aimed at evaluating the effectiveness of the PE intervention for patients with entero-urostomies and their caregivers, through the measurement of ~~the~~ QoL and perceived needs before the events (t0), at the end of the PE intervention (t1), and 30 days after the PE intervention (t2).

Further, we can state that these results seem to suggest that performing a PE intervention on patients with an entero-urostomy could improve their levels of QoL, while their levels of perceived emotional burden will be reduced for caregivers.

Specifically, regarding the actual needs detected with the patient ~~facing~~ NEQ, the results show an apparent increase in need from t0 to t1, which returns to generally

adequate levels at t2. For example, needs such as "more information" or "involvement in treatment choices," "more attention from health care staff", or "need for reassurance", "feeling more useful in the family", or "being less left to one's own devices," show an increasing trend at t1 that tends to improve after the last day of PE.

In parallel, the caregiver's perceived needs related to their family member's care overlap. In particular, needs such as "need to be informed", "be empowered to deal with the care needs of the family member", "be prepared to deal with changes in the family", and "need to compare myself with family members of other patients" show an increasing trend across PE days.

This trend can be accounted for by an increase in theoretical awareness of an ostomy and its practical management, which initially increased difficulties in the middle phase of the study, growing doubts in both patients and caregivers.

Scores related to self-care, in all its explored dimensions, also show the same trend. Moreover, strongly supporting the initial hypothesis are the results related to caregiver burden, which show substantial decreases in all items except the one inherent in the question, "Do you fear what the future holds for your family member?", and the results related to QoL show substantial improvements in all dimensions explored. We believe that this decline can be justified by the oncological disease and not by the stoma.

Our results are in line with other similar educational events reported in the literature, which show high levels of patient satisfaction in participating in such events with an important contribution to improving self-care and QoL levels [24–26]. Carrying out PE training interventions enables the patient to manage their treatment process (empowering patients), as many people wish to play an active role in protecting their health: when they are fit, they want to know how to protect and improve their condition; and when they are sick, they ask about treatment options and the chances of success. So, in addition to seeking quick and effective advice when they need it, people want to know what they can do to help themselves [27]. Furthermore, the training carried out by the ScNS may also have positively influenced the participants' satisfaction with the training event. Studies underline how essential it is to be trained by specialized reference figures for both patients and caregivers [28], throughout the treatment process. This presence influences the perception of empathy, honesty, and emotional support [29], and on the best possibility of communicating and sharing information in the decision-making process [30]. The use of Conversation Maps may also have contributed to organizing, synthesizing, and deepening new knowledge, and, therefore, may have influenced the levels of satisfaction of patients and caregivers; studies report, in fact, their important support as a PE tool [31].

However, the literature agrees on the fact that the feasibility of patient PE programs requires the creation of dedicated tools and an effort to both empower patients and support healthcare professionals [24,32], with positive long-term impacts on healthcare costs [33].

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4.1. Limitations

The authors are aware of the finding's limitations. The limited sample size and the pilot design of the study did not allow us to carry out inferential statistical analyses and to generalize the results. The research aims to test the effectiveness of a targeted PE pathway on a small oncological population based on the specific needs detected [14]. Thus, this study aimed to test PE methodology and our effectiveness hypothesis before starting with more complex research (biggest sample, longest timing observation, other tools, etc.). Further studies will be needed to conduct the PE model on a larger population and in multicentric studies to obtain generalizable results. The improvement trend in outcomes for both patients and caregivers suggests the feasibility of such PE events. A future

qualitative study dedicated to exploring the experiences of patients, carers, and healthcare professionals will address the shortcomings of the present descriptive study.

5. Conclusions

Despite the limitations mentioned above, the objective of the study was achieved. Targeted and individualised interventions on PE have positive effects on self-care and QoL in cancer patients with an entero-urostomy and on the emotional burden perceived by caregivers.

Our results could suggest the usefulness of specific PE training for healthcare professionals and encourage the construction of methodologically appropriate and individualised PE events on patients with a stoma, who expressed high levels of satisfaction in our attempt.

To conclude, future research in this field should be conducted on a larger population and from a multicentre perspective, in contexts other than oncology, considering our results as a valid reference basis.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/doi/s1: Table S1: STROBE Statement—Checklist of items that should be included in reports of cohort studies.

Author Contributions: Conceptualization, F.P. and A.S.; methodology, N.P. and L.I.; statistical analysis, I.T.; investigation, M.P. and M.A.P.; writing—original draft preparation, N.P. and A.S.; writing—review and editing, E.D.S., L.I., A.D.L., N.P., and A.P.; resources, N.P.; supervision, F.P. and L.I.; project administration, F.P.; funding acquisition, P.B. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted according to the Declaration of Helsinki and approved by the Health Direction of IRCCS National Cancer Institute "Regina Elena" and the Local Ethics Committee IRCCS Lazio (No. 1808/22 of 13 December 2022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients and caregivers to publish this paper.

Data Availability Statement: The datasets generated and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request due to restrictions privacy and ethical issues.

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Conflicts of Interest: The authors declare no conflicts of interest.

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MicroRNA-mediated PTEN downregulation as a novel non-genetic mechanism of acquired resistance to PI3Kα inhibitors of head & neck squamous cell carcinoma

Claudio Pulito [a 1], Sebastiano Vaccarella [a 1], Alina Catalina Palcau [b], Federica Ganci [a], Renata Brandi [a], Carlotta Frascolla [a], Andrea Sacconi [c], Valeria Canu [a], Anna Benedetti [a], Valentina De Pascale [a], Sara Donzelli [a], Anne-Sophie Fisch [d], Valentina Manciocco [e], Renato Covello [f], Fulvia Pimpinelli [b], Aldo Morrone [g], Francesco Fazi [h], Raul Pellini [e], Paola Muti [i], Jalna Meens [j]...Giovanni Blandino [a]

Abstract

Aims

Head and neck squamous cell carcinomas (HNSCCs) frequently harbor alterations in the PI3K signalling axis and, particularly, in the PIK3CA gene. The promising rationale of using PI3K inhibitors for the treatment of HNSCC has, however, clashed with the spontaneous development of resistance over time.

Methods

To identify valuable targets for overcoming acquired resistance to PI3Kα inhibitors in HNSCC, we performed microRNA profiling on a cohort of HNSCC PDXs that were treated with alpelisib, including both responsive and resistant tumors. Using CRISPR/Cas9, siRNA, and PTEN-/- isogenic and alpelisib-resistant cell models, we examined the role of PTEN in resistance acquisition. Phospho-proteomic analysis identified PTEN-dependent phosphorylation events, while PI3Kα inhibitor-resistant organoids were used to assess PLK1 inhibitor efficacy.

Results

We identified microRNAs altered in resistant PDXs, including members of the miR-17-92 cluster. Mechanistically, we observed that the hyperactive c-Myc was recruited to MIR17HG regulatory regions in alpelisib-resistant cells, sustaining miR-17-5p, miR-19b-3p, and miR-20a-5p expression, which downregulated PTEN. PTEN knockout or depletion conferred alpelisib resistance in HNSCC cells. We identified PTEN-dependent phosphorylation events, such as p-PLK1-T210, involved in resistance. Interestingly, pharmacological inhibition of PLK1 strongly reduced the viability of PI3Kα-resistant organoids derived from HNSCC PDXs and cell line models.

Conclusion

Overall, this study unveils a novel, microRNA-driven, non-genetic mechanism contributing to acquired resistance to PI3Kα inhibitors in HNSCC. Indeed, linking hyperactive c-Myc to sustained miR-17-92 expression and consequent PTEN downregulation, we also propose that targeting PTEN-dependent downstream effectors, such as PLK1, may offer a powerful therapeutic strategy for resistant HNSCC.

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Article

Tolerability and Preliminary Outcomes of Adjuvant T-DM1 in HER2-Positive Breast Cancer After Neoadjuvant Therapy: The ATD Study

Eriseld Krasniqi [1], Lorena Filomeno [1], Teresa Arcuri [1,*], Francesca Sofia Di Lisa [2], Antonio Astone [3], Claudia Cutigni [3], Jennifer Foglietta [4], Martina Nunzi [4], Rosalinda Rossi [5], Mauro Minelli [5], Icro Meattini [6,7], Luca Visani [7], Jacopo Scialino [7], Lorenzo Livi [6,7], Luca Moscetti [8], Paolo Marchetti [9], Andrea Botticelli [10], Ida Paris [11], Francesco Pavese [11], Tatiana D'Angelo [11], Valentina Sini [12], Simonetta Stani [12], Maria Rosaria Valerio [13], Antonino Grassadonia [14], Nicola Tinari [15], Marco Mazzotta [16], Matteo Vergati [16], Giuliana D'Auria [16], Teresa Gamucci [16], Loretta D'Onofrio [17], Simona Gasparro [17], Arianna Roselli [17], Alberto Fulvi [17], Gianluigi Ferretti [17], Andrea Torchia [17,18], Monica Giordano [19], Filippo Greco [20], Francesco Pantano [21], Giuseppe Tonini [21], Agnese Fabbri [22], Emilio Bria [23,24], Giovanna Garufi [23], Elena Fiorio [25], Mimma Raffaele [26], Mirco Pistelli [27], Rossana Berardi [27], Rosa Saltarelli [2], Ramy Kayal [28], Francesca Romana Ferranti [28], Katia Cannita [29], Azzurra Irelli [29], Nicola D'Ostilio [30], Costanza De Rossi [31], Raffaella Palumbo [32], Anna Cariello [33], Giuseppe Sanguineti [34], Fabio Calabrò [17], Laura Pizzuti [17], Maddalena Barba [17], Claudio Botti [35], Fabio Pelle [35], Sonia Cappelli [35], Flavia Cavicchi [35], Ilaria Puccica [35], Amedeo Villanucci [35], Isabella Sperduti [36], Gennaro Ciliberto [37] and Patrizia Vici [1]

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25 months, 31 relapse events (7.6%) and 22 deaths (5.4%) were reported. The preliminary incidence of RFS and OS events was similar between patients who completed the T-DM1 course and those who discontinued it early. Conclusions: T-DM1 demonstrated a manageable safety profile, and the adverse events were consistent with those reported in randomized trials. The data are not yet sufficient to allow for a formal analysis of RFS and OS, and long-term follow-up is required.

Keywords: breast cancer; HER2-positive subtype; adjuvant T-DM1; adverse events; real-world evidence

1. Introduction

Human epidermal growth factor receptor 2 (HER2)-positive breast cancer (HER2$^+$BC) represents approximately 15–20% of all breast tumors and is associated with aggressive behavior and poor prognosis [1]. These tumors are characterized by HER2 protein overexpression, measured by immunohistochemistry (IHC), or *HER2* amplification, detected by in situ hybridization (ISH). Anti-HER2 targeted therapies have dramatically altered the natural history of HER2$^+$BC, improving outcomes in both early-stage and metastatic disease [2,3]. Locoregional surgery, systemic treatment, including neoadjuvant and adjuvant chemotherapy with anti-HER2 agents, endocrine therapy, and radiotherapy are all commonly employed in treating early HER2$^+$BC. Neoadjuvant treatment (NAT) is commonly administered in cases of locally advanced and operable BC, with the primary objective of achieving a pathological complete response (pCR). In the case of HER2$^+$BC, a pCR is strongly associated with significantly improved long-term outcomes [4,5], making it a key therapeutic goal. The combination of chemotherapy and trastuzumab has demonstrated significant advantages in achieving a pCR in the early HER2$^+$BC setting. This approach has been shown to reduce the risk of relapse, disease progression, and death when compared to chemotherapy alone, offering a more effective treatment strategy for this group of patients [6,7]. Numerous efforts have been undertaken to increase pCR rates and improve long-term outcomes in early HER2$^+$BC treatment. A pivotal multicenter, open-label, phase II randomized trial, NeoSphere, was the first study to demonstrate a significant improvement in pCR rates among patients receiving a combination of pertuzumab, trastuzumab, and docetaxel compared to those receiving either pertuzumab or trastuzumab plus docetaxel, or dual HER2 blockade, without chemotherapy. These findings led to the approval of pertuzumab in the neoadjuvant setting, marking a major advancement in treatment strategies for HER2$^+$BC [8]. A pooled analysis further confirmed the more favorable event-free survival in patients who achieved a pCR after neoadjuvant treatment with dual HER2 blockade compared to those treated with trastuzumab alone [9].

Despite the advancements accomplished with HER2-targeted agents in the neoadjuvant setting, approximately 40–60% of HER2$^+$BC patients who undergo standard neoadjuvant therapy have residual disease at surgery. These patients face a significantly higher risk of both local and systemic relapse [8,10], underscoring the need for more effective strategies to improve long-term outcomes. Trastuzumab emtansine (T-DM1) is an antibody–drug conjugate consisting of trastuzumab linked via a non-reducible thioether bond to a cytotoxic agent, emtansine, a microtubule inhibitor [11]. T-DM1 has demonstrated significant activity in patients with pretreated advanced HER2$^+$BC [12], offering a targeted and potent therapeutic option for this population. In the early BC setting, additional post-neoadjuvant treatment with T-DM1 in patients with HER2$^+$BC who did not achieve a pCR at surgery has been shown to significantly improve both invasive disease-free survival (iDFS) and overall survival (OS). These findings were demonstrated in the KATHERINE trial [13,14], establishing T-DM1 as the current standard of care for this patient population. In the safety analysis of the KATHERINE trial, a higher incidence of grade 3 or greater AEs was observed in patients treated with adjuvant T-DM1 (25.7%) compared to those receiving adjuvant trastuzumab (15.4%). Additionally, 18.0% of patients in the T-DM1 group discontinued

Clinical data were retrospectively extracted from medical records and included age at diagnosis, menopausal status, performance status at the time of diagnosis, the type and duration of prior NAT, treatment-related toxicities, types of surgery performed, definitive histological results, and the administration of radiotherapy or hormone therapy. Tumor characteristics and molecular profile were assessed on the original pathological report, when possible. The tolerability of T-DM1 was assessed by documenting adverse events (AEs) according to the Common Terminology Criteria for Adverse Events (CTCAE, version 5). Additionally, detailed follow-up data were collected to monitor time-to-event outcomes such as relapse-free survival (RFS) and OS. Median follow-up was calculated from the pathological diagnosis of the residual tumor after surgery to the date of recurrence, death, or the last follow-up. All data were encoded and entered into a dedicated database overseen by the coordinating center, with data entry performed by the recruiting centers.

2.4. Study Endpoints

The primary objective of the ATD study was to evaluate the tolerability of adjuvant T-DM1 treatment, measured in terms of AEs according to the CTCAE, version 5, in a real-world population. The secondary objective focused on assessing the effectiveness of adjuvant T-DM1 in $HER2^{+}$BC patients who had undergone NAT and exhibited invasive residual disease at surgery in terms of RFS and OS measured from the time of pathological diagnosis of invasive residual disease.

2.5. Statistical Methods

The sample size calculation was based on the primary objective of assessing the toxicity profile and tolerability of adjuvant T-DM1, focusing on the proportion of patients experiencing grade 3 or greater adverse events. An expected grade ≥ 3 AE rate of 20% was assumed. To achieve 80% statistical power for detecting it, with a 3% margin of error and a 95% confidence level, a total of 291 patients were required (alpha 0.05). This sample size ensured sufficient power to reliably estimate the safety outcomes in the real-world population.

All variables included in the data collection forms were analyzed, with descriptive statistics provided for each: mean, median, standard deviation, range, minimum and maximum values for continuous variables, and absolute and relative frequencies for categorical variables. Associations between categorical variables were evaluated using the Chi-square test or Fisher's exact test, as appropriate. Relapse-free events were defined as either disease recurrence or death from any cause. Overall survival events were defined as death from any cause. All statistical analyses were performed independently by two authors using SPSS statistical software (v21.0) and R programming (v4.4.1).

3. Results

3.1. Patient and Tumor Characteristics

From May 2019 to January 2024, 410 patients meeting the inclusion criteria were included in the present study. All patients received at least one cycle of adjuvant T-DM1, and at the time of this analysis, 18 (4.4%) were still undergoing treatment. The patient and tumor characteristics prior to NAT are summarized in Table 1, while details regarding neoadjuvant regimens, types of definitive surgery, and residual disease characteristics are presented in Table 2.

Table 2. Neoadjuvant regimens employed, the type of definitive surgery, and residual disease characteristics.

		Absolute Count	Percentage
Neoadjuvant Treatment	Chemotherapy plus Pertuzumab–Trastuzumab	35	8.5
	Anthracycline–Taxane plus Trastuzumab	345	84.1
	Taxane plus Trastuzumab	20	4.9
	Other Chemotherapy plus Trastuzumab	1	0.2
	Unknown	9	2.2
Neoadjuvant Treatment Completion	Completed	356	86.8
	Interrupted for Toxicity	27	6.6
	Interrupted for Progression	1	0.2
	Withdrew	4	1
	Unknown	22	5.4
Type of Breast Surgery	Mastectomy	233	56.8
	Conservative	177	43.2
Type of Nodal Surgery	Sentinel Lymph Node	192	46.8
	Axillary Lymph Node Dissection	169	41.2
	None	47	11.5
	Unknown	2	0.5
Pathological T Size	0	34	8.3
	1	311	75.9
	2	55	13.4
	3	7	1.7
	4	2	0.5
	Unknown	1	0.2
Pathological N Involvement	0	238	58
	1	118	28.8
	2	36	8.8
	3	18	4.4
Grade	1	21	5.1
	2	147	35.9
	3	172	42
	Unknown	70	17.1
ER	Negative	89	21.7
	Positive	297	72.4
	Unknown	24	5.9
PgR	Negative	176	42.9
	Positive	210	51.2
	Unknown	24	5.9
HER2 IHC + score	3	213	52
	2	109	26.6
	1	29	7.1
	0	26	6.3
	Unknown	33	8

patients had residual disease in either the breast or regional lymph nodes, with 172 patients (42.0%) exhibiting residual node-positive disease. Regarding pT classification, 34 patients (8.3%) had no residual invasive disease in the breast (pT0/pTis), while the majority, 311 (75.9%), had ypT1 residual disease. Additionally, 55 patients (13.4%) had ypT2, 7 (1.7%) had ypT3, and 2 (0.5%) had ypT4 residual disease, while pT status was unspecified for 1 patient (0.2%). With respect to the baseline evaluation at biopsy, residual disease at surgery remained $HER2^+$ in 319 cases (77.8%), changed into $HER2^-$ in 18 (4.4%) cases, and was not reported for 73 (17.8%) cases. Of the 319 $HER2^+$ residual tumor samples, 213 were found to be HER2 3+ by IHC, 92 were found to be HER2 2+ by IHC and ISH-amplified, 9 were diagnosed as HER2 1+ by IHC and ISH-amplified, and 5 were diagnosed as HER2 0 by IHC and ISH-amplified. In 18 cases (4.4%), the residual disease was discordant with the baseline HER2 status, becoming HER2-negative. Among this subset, the HER2 IHC score was 2+ in 9 cases, 1+ in 5 cases, and 0 in 4 cases, with all cases showing non-amplified ISH results. HER2 status was not fully characterized in the remaining 73 cases (17.8%), as ISH results were not reported. Within this group, the HER2 IHC scores were 2+ in 8 cases, 1+ in 15 cases, 0 in 20 cases, and unspecified in 30 cases. Among the 129 patients with HER2 ISH amplification at biopsy (and IHC scores of 2+, 1+, or 0), 66 retained HER2 amplification in the residual tumor. Within this group, the IHC score at surgery was 2+ in 58 cases, 1+ in 5 cases, 0 in 2 cases, and not reported in 1 case. Conversely, 12 cases lost HER2 ISH amplification in the residual disease, with corresponding IHC scores of 2+ in 6 cases, 1+ in 4 cases, and 0 in 2 cases. For the remaining 51 patients with HER2 ISH amplification at baseline, ISH was not performed on the surgical specimen. Among these patients, the IHC score was 3+ in 25 cases, 2+ in 4 cases, 1+ in 5 cases, 0 in 11 cases, and not reported in 6 cases. Regarding HR status, residual tumors were ER-positive in 297 patients (72.4%) and PgR-positive in 210 (51.2%). Both ER and PgR were positive in 204 patients (49.8%), while both receptors were negative in 83 cases (20.2%). HR status was not reported for at least one among ER and PgR in 24 cases (5.9%). For 385 patients (94.0%), ER and PgR status was available at both baseline and at residual disease evaluation. Among these, 352 cases (85.9% of the total study population) showed a concordant ER status, while 33 (8.1%) were discordant, with 18 cases changing from ER-positive to ER-negative and 15 cases changing from ER-negative to ER-positive. Similarly, for PgR status, 279 patients (68.0%) had concordant results at baseline and surgery. However, 74 cases (18.0%) shifted from PgR-positive to PgR-negative, and 32 cases (7.8%) changed from PgR-negative to PgR-positive.

3.3. Adjuvant Treatment with T-DM1

Following surgery, all patients received adjuvant treatments. Adjuvant radiation therapy was administered to 303 patients (73.9%), with 282 receiving it prior to T-DM1 and 21 undergoing radiotherapy concurrently with T-DM1. Endocrine therapy was given to 291 patients (71.0%), delivered either concomitantly with radiotherapy and/or during T-DM1 treatment. Data on adjuvant treatments are reported in Table 3.

The median time from surgery to the initiation of T-DM1 was 2 months (range, 1–7 months), with 205 patients (50.0%) receiving their first cycle within this 2-month window. The median number of T-DM1 cycles administered was 14 (range, 1–17). Specifically, 289 patients (70.5%) received the full 14 cycles of T-DM1, while 102 patients (24.9%) received between 1 and 13 cycles. Two patients (0.5%) received, respectively, 15 and 17 cycles, and for seventeen patients (4.1%), the number of cycles was not reported. At the time of this analysis, treatment was still ongoing for 18 patients (4.4%). For a total of 41 patients (10.0%), T-DM1 was discontinued, with the reason being explicitly reported as toxicity, 5 patients (1.2%) withdrew voluntarily, and 8 patients (2.0%) experienced a relapse during T-DM1 treatment. An additional 45 (10.9%) patients interrupted T-DM1 for unspecified reasons, and for 2 (0.5%) patients, information on T-DM1 completion (and number of cycles) was not reported.

Table 4. *Cont.*

		Absolute Count	Percentage
Neutropenia grade			
	1	11	2.7
	2	10	2.4
	3	1	0.2
	4	1	0.2
	None/Unknown	387	94.4
Thrombocytopenia			
	Yes	72	17.6
	No	338	82.4
Thrombocytopenia grade			
	1	45	11
	2	18	4.4
	3	5	1.2
	4	1	0.2
	None/Unknown	341	83.2
Gastrointestinal toxicity			
	Yes	54	13.2
	No	356	86.8
Gastrointestinal toxicity grade			
	1	38	9.3
	2	14	3.4
	3	1	0.2
	None/Unknown	357	87.1
Neurotoxity			
	Yes	26	6.3
	No	384	93.7
Neurotoxicity grade			
	1	13	3.2
	2	11	2.7
	3	1	0.2
	None/Unknown	385	93.9
Hepatotoxicity			
	Yes	76	18.5
	No	334	81.5
Hepatotoxicity grade			
	1	55	13.4
	2	17	4.1
	3	3	0.7
	None/Unknown	335	81.7
Other toxicities			
	Yes	55	7.3
	No	355	86.6
Other toxicity type			
	Fatigue	7	1.7
	Mucositis	5	1.2
	Nausea	3	0.7
	Anemia	2	0.5
	Conjunctivitis	2	0.5
	Pneumonitis	2	0.5
	Allergy	1	0.2
	Fatigue	1	0.2
	Fever	1	0.2
	Unspecified	31	7.6
	No	355	86.6

explicitly linked to toxicity, 95.1% (31/41) experienced at least one AE of any grade, with 11 cases involving grade 3 or higher toxicities. The most common AEs in this group were thrombocytopenia (34.1% of any grade, with 4.9% grade 3), hepatotoxicity (34.1% of any grade, with 4.9% grade 3, including one case with concurrent grade 3 thrombocytopenia), gastrointestinal toxicity (26.8% of any grade, with no grade 3 or higher events), and neurotoxicity (9.9% of any grade, with 2.4% grade 3).

3.5. *Preliminary Outcomes of Relapse and Survival*

At the time of this analysis, the median follow-up from the pathological diagnosis of the residual tumor post-surgery was 25 months (range, 1–55 months). During this follow-up period, 31 relapse events (7.6% of patients) were recorded. Of these, 4 relapses occurred in the ipsilateral breast, 1 in the contralateral breast, and 22 at distant sites; in 4 cases, the secondary site was not specified. A total of 22 deaths (5.4%) from any cause were also reported. The follow-up period, however, was not sufficient, and the number of events was too limited to allow for formal survival analyses of either time-to-relapse or time-to-death outcomes. Nonetheless, we performed non-parametric comparisons using Fisher's exact test to evaluate the distribution of events across relevant patient groups. Patients who were still undergoing T-DM1 treatment (n = 18) or those who experienced relapse during T-DM1 treatment were excluded from the analysis, as these conditions represented competing factors that could confound the potential impact of incomplete T-DM1 administration on relapse and mortality rates. Additionally, two patients for whom data on T-DM1 completion and the number of administered cycles were both missing were also excluded.

After these exclusions, the incidence of relapse was 4.4% (4/91) in patients who received fewer than 14 cycles of T-DM1, compared to 6.2% (18/291) in those who completed at least 14 cycles. The odds ratio for relapse between patients who discontinued T-DM1 and those who completed treatment was 0.70 ($p = 0.616$), suggesting no significant difference. Similarly, the incidence of death from any cause was 3.3% (3/91) in the group that discontinued treatment, compared to 4.5% (13/291) in those who completed the full course of T-DM1, with an odds ratio of 0.73 ($p = 0.771$), again indicating no statistically significant difference.

4. Discussion

In this multicenter, observational, retrospective study, we evaluated the tolerability of adjuvant T-DM1 in a real-world population of 410 patients with HER2$^+$BC who had residual invasive disease after NAT. Our primary objective was to assess the safety profile of T-DM1 in this setting, while the secondary objective was to provide data on its effectiveness in terms of RFS and OS.

Our findings demonstrate that adjuvant T-DM1 is generally well tolerated in routine clinical practice. We observed that 55.6% of patients experienced at least one AE associated with T-DM1. Grade 3 or 4 treatment-related AEs occurred in 4.9% of patients, and only one grade 5 AE (pneumonitis) was reported. Lung toxicity, including pneumonitis, interstitial lung disease, and acute respiratory distress syndrome, is a relatively rare event associated with T-DM1 treatment. An integrated safety analysis of phase III trials reported an incidence of 1.1% of such toxicity, with death occurring in approximately 0.1% of cases [17]. Our findings are consistent with these reports, with a total of two cases of lung toxicity observed, including the grade 5 pneumonitis case mentioned and an additional case of grade 3 pneumonitis. Interstitial lung disease is of particular concern for the class of antibody–drug conjugates, which T-DM1 belongs to. However, this incidence is significantly higher with the use of other compounds of the same category such as trastruzumab deruxtecan (around 15%) and trastuzumab duocarmazine (around 7%) [18]. The most frequent AEs (incidence $\geq$ 5%) in this study were hepatotoxicity (18.5%), thrombocytopenia (17.6%), gastrointestinal toxicity (13.2%), neurotoxicity (6.3%), and neutropenia (5.6%). This profile also aligns with findings from the previously mentioned integrated safety

management of subsequent T-DM1 therapy, although further investigation is needed to elucidate this relationship.

Our study has several strengths. It represents one of the largest real-world cohorts examining the safety of adjuvant T-DM1 in patients with HER2$^+$BC and residual disease after NAT. Its multicenter nature and the inclusion of multiple cancer centers across Italy enhance the generalizability of our findings. Additionally, the high rate of completion of the planned 14 cycles of T-DM1 underscores the feasibility of administering this therapy in routine practice. However, there are limitations to consider. The retrospective design may introduce biases, including underreporting of AEs and incomplete data capture. Our data model for toxicity reporting prioritized key adverse event categories to streamline data collection and enhance compliance across centers, but the inclusion of an open-ended 'Other toxicities' field, while useful for capturing rare events, may have contributed to underreporting due to the frequent lack of specification and the limitation of recording only one such event per patient. Furthermore, the multicenter nature of the study, while enhancing its generalizability, may have introduced variability into the data collection and reporting practices used across the participating centers. These factors could have impacted the consistency and completeness of the dataset, representing potential sources of error. Our study was adequately powered to evaluate the primary endpoint of tolerability, with the sample size exceeding the requirements for detecting AEs at a 20% rate. However, the relatively short median follow-up of 25 months and the limited number of relapse events preclude definitive conclusions regarding the effectiveness of T-DM1 in this setting. Our secondary and exploratory analyses are preliminary, and longer follow-up is necessary to assess long-term outcomes such as RFS and OS. Finally, the absence of routinely collected data on ethnicity in Italian clinical practice limits the ability to explore potential differences in outcomes across diverse racial groups, although the majority of patients are presumed to be Caucasian based on national demographics.

5. Conclusions

In conclusion, our study suggests that adjuvant T-DM1 is well tolerated in a real-world population of patients with HER2$^+$BC and residual invasive disease after neoadjuvant treatment. The safety profile observed is consistent with that reported in previous studies, including the prospective randomized KATHERINE trial, which compared T-DM1 to trastuzumab, and the KARMA study, which reported data on the efficacy and safety of T-DM1 in a real-world setting. These findings support the use of T-DM1 as a standard adjuvant treatment in this patient population, although continued monitoring and further research are necessary to fully understand its long-term impact. An updated analysis with extended follow-up will be important to validate our preliminary findings on the effectiveness of adjuvant T-DM1. As more events accrue over time, we will be able to perform robust survival analyses and potentially identify factors influencing outcomes. Further research could also explore the impact of variables such as hormone receptor status and HER2 expression changes and specific toxicity profiles on the tolerability and effectiveness of T-DM1.

Author Contributions: Conceptualization: P.V.; data curation: L.F., T.A. and F.S.D.L.; formal analysis: E.K. and I.S.; investigation: E.K., C.C., M.N., R.R., L.V., J.S., F.P. (Francesco Pavese), T.D., V.S., N.T., M.M. (Marco Mazzotta), M.V., G.D., L.D., S.G., A.R., A.F. (Alberto Fulvi), G.F., A.T., F.P. (Francesco Pantano), G.G., M.P., R.K., F.R.F., A.I., F.P. (Fabio Pelle), S.C., F.C. (Flavia Cavicchi), I.P. (Ilaria Puccica) and A.V. project administration: P.V.; supervision: P.V.; validation: E.K.; writing—original draft: E.K.; writing—review and editing: A.A., J.F., M.M. (Mauro Minelli), I.M., L.L., L.M., P.M., A.B., I.P. (Ida Paris), S.S., M.R.V., A.G., T.G., M.G., F.G., G.T., A.F. (Agnese Fabbri), E.B., E.F., M.R., R.B., R.S., K.C., N.D., C.D.R., R.P., A.C., G.S., F.C. (Fabio Calabrò), L.P., M.B., C.B., G.C. and P.V. All authors have read and agreed to the published version of the manuscript.

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Induction of cell death by the CXCR2 antagonist SB225002 in colorectal cancer and stromal cells

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ABSTRACT

Molecular targeted therapies have had great success in cancer treatment due to their high efficacy and selectivity. Identifying specific prognostic/predictive biomarkers helps clinicians stratify patients according to individual characteristics and improves patient quality of life in terms of disease control and survival. In our previous work, we identified Interleukin-8 as an important prognostic biomarker according to specific genomic alterations of colorectal cancers, leading us to investigate the effects of its axis inhibition, by targeting the Interleukin-8 receptors CXCR1 and CXCR2. Here, we show that dual CXCR1/2 inhibition does not affect colorectal cancer cell viability, whereas CXCR2-selective inhibition by SB225002 reduces cell viability in responder colorectal cancer cell lines. More specifically, these responder cells undergo programmed cell death upon SB225002 treatment, while non responder cell lines incur in a reversible G2/M arrest. Interestingly, the same response in terms of inhibition of cell viability also occurs in the stromal compartment (normal fibroblasts): however, in this compartment, the G2/M block is non reversible, hence leading to non-apoptotic cell death. These findings suggest that SB225002 could be a potential therapeutic agent in colorectal cancer, by affecting not only cell viability, but also tumor-stroma interactions.

1. Introduction

Colorectal cancer (CRC) represents the third most frequent human cancer and the second cause of cancer-related death worldwide [1]. Significant improvements in CRC management have stemmed from early detection, optimization of available treatments, identification of different molecular subtypes, characterization of stromal/immune interactions, and precision medicine approaches [2,3]. Over time, the identification of prognostic/predictive biomarkers has led to the regulatory approval of molecularly targeted therapies (e.g., anti-EGFR monoclonal antibodies, KRAS G12C inhibitors) and immune checkpoint inhibitors (ICI) for the treatment of metastatic CRC. Nevertheless, these treatments show time-bound benefits, and, despite a relatively common initial response, resistance inevitably ensues. To improve life quality and expectancy, overcome therapy resistance, and avoid unnecessary toxicity, the goal of precision oncology is to combine or sequence specific targeted therapies, according to the clinical evolution of the disease and its molecular trajectory [4]. The recent introduction of the consensus molecular subtypes in clinical practice profoundly modified CRC classification, allowing for the planning of individualized treatment paths for advanced CRC patients [5,6].

Our group recently demonstrated that the production of interleukin-8 (IL-8) in CRC cells is dependent on their genetic/molecular landscape (i.e., *BRAF* mutational status and PTEN competency) and that CHOP-dependent transcription of the IL-8 gene is profoundly affected by molecularly targeted inhibitors interfering with such alterations [7]. In CRC patients, high circulating IL-8 levels correlate with shorter progression-free and overall survival, thereby conveying a negative prognostic impact [8]. However, the role of local IL-8 production in CRC tissue microenvironment may be more complex. Indeed, the presence of IL-8 + tumor-infiltrating immune cells in CRC samples may bear prognostically favorable consequences and is apparently driven by the presence or absence of a functional PTEN tumor suppressor in CRC cells [8,9]. Therefore, in-depth understanding of the molecular functioning of the

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For annexin V staining, the eBioscience AnnexinV-FITC Apo kit (Invitrogen, Waltham, Massachusetts, USA, Cat#BMS500FI) was used following the manufacturer's instructions. Briefly, RKO, SW480 and EA.hy926 cells were washed with 1X binding buffer and then incubated with FITC annexin V for 15 min and then stained with PI. HF cells were incubated with APC annexin V (Enzo Life Sciences, Farmingdale, NY, USA, Cat#ALX-209–252) following the same protocol described above for the other cells used.

Flow cytometric analyses were performed using BD Accuri™ C6 (BD Biosciences, San Jose, CA, USA) flow cytometer. Sample acquisition was performed by setting standardized parameters [blue laser (488 nm), FL2 filter (585/45 nm) for PI staining; red laser (640 nm), FL4 filter (675/25) for annexin V staining].

4.7. Statistical analysis

Results are expressed as average of three independent experiments or as representative experiment out of three independent experiments performed with similar results [7]. p-value < 0.05 by 2-tailed Student's t test was considered statistically significant. IC_{50} was calculated according to the Chou-Talalay method using the Calcusyn software (Biosoft, Cambridge, United Kingdom).

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CRediT authorship contribution statement

Marta Di Martile: Methodology, Formal analysis. **Chiara Bazzichetto:** Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fabiana Conciatori:** Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Michele Milella:** Writing – original draft, Data curation, Conceptualization. **Donatella Del Bufalo:** Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare no competing interests.

Data availability

All data supporting the findings of this study are available in the article along with Supplementary Information_Raw Data file. Additional information will be kindly provided without any restrictions.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2025.118203.

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Androgen receptor inhibition sensitizes glioblastoma stem cells to temozolomide by the miR-1/miR-26a-1/miR-487b signature mediated WT1 and FOXA1 silencing

Ana Belén Díaz Méndez[1,8], Marta Di Giuliani[1,8], Andrea Sacconi[2], Elisa Tremante[1], Valentina Lulli[3], Marta Di Martile[4], Giulia Vari[1,5], Francesca De Bacco[6,7], Carla Boccaccio[6,7], Giulia Regazzo[1✉] and Maria Giulia Rizzo[1✉]

Glioblastomas (GBMs) are aggressive brain tumors and challenging cancers for diagnosis and treatment. Therapeutic options include surgery followed by chemotherapy with the DNA alkylator temozolomide (TMZ) and radiotherapy. However, the patient's prognosis remains poor due to tumor heterogeneity, cell infiltration and intrinsic or acquired resistance to therapy. Understanding the resistance mechanisms together with identifying new biomarkers are crucial for developing novel therapeutic strategies. MiRNAs play an important role in the biology of gliomas, they modulate tumorigenesis and therapy response. We recently identified the diagnostic/prognostic miR-1-3p, miR-26a-1-3p and miR-487b-3p signature that displays an oncosuppressive role on several glioma biological functions. In this study, we investigated the effects of the therapeutic potential of this three-miRNA signature as a regulator of response to TMZ. We found that ectopic expression of the miRNA signature in patient-derived GBM neurospheres treated with TMZ impaired cell proliferation and viability by necroptosis induction. Moreover, we identified WT1 and FOXA1, two transcription factors specifically involved in TMZ resistance, as novel direct targets of the miRNA signature. Of note, the repression of WT1 and FOXA1, elicited by the signature, caused a downregulation of the Androgen Receptor (AR) expression, an impairment of tumor-spheroid formation and reversed cancer cell stemness. These results were recapitulated using the AR inhibitor enzalutamide, confirming the involvement of the AR pathway. Our data indicate that the miR-1-3p/miR-26a-1-3p/miR-487b-3p signature, which has an impact on treatment response and cell stemness, may pave the way for miRNA-based complementary therapies in GBM patients.

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BACKGROUND

Glioblastoma (GBM) is the most prevalent and aggressive type of glioma, accounting for half of all malignant brain tumors. Prognosis in patients with GBM remains extremely poor, with a 5-year survival of 2–10% [1]. Among the main prognostic factors there are mutations of IDH genes that are linked to a better prognosis and are essential from a diagnostic perspective as, according to the World Health Organization (WHO) 2021 classification, all IDH-wild-type gliomas allow to diagnose "glioblastoma IDH-wild-type CNS WHO grade 4" even in the absence of a glioblastoma histopathology [2]. GBMs represent a medical challenge due to their anatomical location, diffuse, infiltrative growth, the resulting impact on brain functioning and their biological complexity [3]. The clinical management of GBM includes surgical resection, followed by a combination of chemotherapy and regional fractionated ionizing radiation [4]. Temozolomide (TMZ), an alkylating agent, is the most preferred and approved drug for either first- or second-line chemotherapy in GBM patients. However, the majority of patients do not respond to therapies due to the intrinsic or acquired ability of GBM cells to develop chemoresistance, and nearly all of them ultimately experience a recurrence or a progression of the disease [4]. An important TMZ-response predictive marker is the O6-methylguanine-DNA methyltransferase (MGMT) methylation status. MGMT activity and the presence of uniquely resistant populations of glioma stem cells are the major contributors to TMZ resistance and the main reasons for treatment failure [5, 6]. Indeed, MGMT-unmethylated (MGMT-unmet) patients are resistant to TMZ and have a much shorter survival compared to those with MGMT methylation (MGMT-met) [7]. Strategies to overcome inherent and acquired resistance to TMZ in GBM have been investigated [6, 7]. However, there have been no successful treatments to render MGMT-unmet GBMs susceptible to TMZ, and therefore, there is an urgent need for novel treatment strategies, especially for this subgroup of patients [7].

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