Association between endocrine adjuvant therapy intake timing and disease-free survival in patients with high-risk early breast cancer: results of a sub-study of the UCBG- UNIRAD trial

Sylvie Giacchetti,[a,b,*] Enora Laas,[c,s] Thomas Bachelot,[d] Jérome Lemonnier,[e] Fabrice André,[f] David Cameron,[g] Judith Bliss,[h] Sylvie Chabaud,[i] Anne-Claire Hardy- Bessard,[j] Magali Lacroix-Triki,[f] Jean-Luc Canon,[k] Marc Debled,[l] Mario Campone,[m] Paul Cottu,[n] Florence Dalenc,[o] Annabelle Ballesta,[p] Frederique Penault-Llorca,[q] Bernard Asselain,[r] Elise Dumas,[s,t] Fabien Reyal,[c,s] Paul Gougis,[s] Francis Lévi,[b] and Anne-Sophie Hamy[n,s]

[a]AP-HP, Hôpital Saint Louis, Senologie, Paris and Chronotherapy, Cancers, and Transplantation Unit, Faculty of Medicine, Paris-Saclay University, France
[b]Chronotherapy, Cancers and Transplatation Unit, Faculty of Medicine, Paris-Saclay, Villejuif, France
[c]Surgery Department, Institut Curie, Universite Paris Cite, Paris, France
[d]Medical Oncology, Centre Leon Berard, Lyon, France
[e]R&D Unicancer, Paris, France
[f]Gustave Roussy, Villejuif, France
[g]ICR-CTSU, Division of Clinical Studies, The Institute of Cancer Research, London, United Kingdom
[h]Medical Oncology, Western General Hospital, Edinburgh, United Kingdom
[i]Department of Clinical Research and Innovation, Centre Leon Berard, Lyon, France
[j]Medical Oncology, CARIO, Plerin, France
[k]Medical Oncology, Grand Hopital de Charleroi, Charleroi, Belgium
[l]Medical Oncology, Institut Bergonie, Bordeaux, France
[m]Medical Oncology, Institut Cancerologie de l'Ouest, Saint Herblain, France
[n]Medical Oncology, Institut Curie, Universite Paris Cite, Paris, France
[o]Medical Oncology, Oncopole Claudius Regaud, IUCT, Toulouse, France
[p]U900, Institut Curie, Saint Cloud, France
[q]Centre Jean Perrin, Université Clermont Auvergne, INSERM, U1240 Imagerie Moléculaire et Stratégies Théranostiques, Clermont Ferrand, France
[r]Arcagy-Gineco, Paris, France
[s]RT2Lab, Residual Tumor and Response to Treatment, U932 "Immunity and Cancer", Universite Paris Cité, Paris, France
[t]Department of Mathematics, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; [t"]"Chronotherapy, Cancers, and Transplantation" Unit, Faculty of Medicine, Paris-Saclay University, Villejuif, France

eBioMedicine
2024;104: 105141
Published Online xxx
https://doi.org/10.1016/j.ebiom.2024.105141

Summary

Background Circadian rhythms regulate cellular physiology and could influence the efficacy of endocrine therapy (ET) in breast cancer (BC). We prospectively tested this hypothesis within the UNIRAD adjuvant phase III trial (NCT01805271).

Methods 1278 patients with high-risk hormonal receptor positive (HR+)/*HER2* negative (HER2-) primary BC were randomly assigned to adjuvant ET with placebo or everolimus. Patients prospectively reported in a diary the daily timing of ET intake among four 6-h slots (06:00–11:59 (morning), 12:00–17:59 (afternoon), 18:00–23:59 (evening), or 24:00–05:59 (nighttime). The association between ET timing and disease-free survival (DFS) was a prespecified secondary endpoint of the trial and the results of this observational study are reported here.

Findings ET timing was recorded by 855 patients (67.2%). Patients declaring morning (n = 465, 54.4%) or afternoon (n = 45, 5.4%) ET intake were older than those declaring evening (n = 339, 39.6%) or nighttime (n = 5, 0.6%) intake. With a median follow-up of 46.7 months, 118 patients had a local (n = 30) or metastasis relapse (n = 84), and 41 patients died. ET intake timing was not associated with DFS in the whole population (HR = 0.77, 95% CI [0.53–1.12]). The association between ET intake timing and DFS according to the stratification factors revealed interactions with ET agent (tamoxifen *versus* Aromatase inhibitors (AI) with an increased DFS in the group of evening/nighttime *versus* morning/afternoon tamoxifen intake (HR = 0.43, 95% CI [0.22–0.85]), while no association was found for AI intake (HR = 1.07, 95% CI [0.68–1.69]). The interaction between ET intake timing and ET agent remained in multivariable analysis (HR = 0.38 [0.16–0.91]).

*Corresponding author.
E-mail address: sylvie.giacchetti@aphp.fr (S. Giacchetti).

Interpretation Tamoxifen intake in the evening/nighttime could be recommended in patients with high-risk HR+/HER2- BC while awaiting for results from further ET timing studies.

Funding UNIRAD was Supported by a grant from the French Ministry of Health PHRC 2012 and received funding from La Ligue contre le Cancer, Cancer Research-UK, Myriad Genetics, and Novartis.

Keywords: Breast cancer; Endocrine therapy; Tamoxifen; Aromatase inhibitors; Circadian rhythm; Intake timing; Chronotherapy

Research in context

Evidence before this study

'Endocrine therapy (ET) has remained the cornerstone for the treatment of patients with HR + BC since the 80's.[1] In the adjuvant setting, nearly 20% of the patients relapse despite the daily oral intake of tamoxifen or aromatase inhibitors.[2] The timing of medications can largely moderate tolerability and efficacy, as shown for cancer chemotherapy, radiotherapy and immunotherapy.[3] However, the possible relevance of ET daily timing for efficacy is largely unknown, despite the timing of ET intake is a frequent question asked to their physicians by the patients with breast cancer.

Added value of this study

Considering the scarcity of data on the influence of timing of ET intake as adjuvant treatment for patients with HR+/HER2- breast cancer, we investigated this issue through a pre-planned ancillary ET intake timing study within a large prospective randomized controlled UNIRAD trial (NCT01805271).[4] Between June 2013 and March 2020, 1278 patients with HR+/HER2-high-risk early BC patients were randomized to receive ET with placebo or everolimus, a mammalian target of rapamycin (mTOR) inhibitor. The patients were asked to prospectively record the timing of ET intakes in a daily diary during their expected 2-year participation in the trial. The UNIRAD study did not demonstrate any therapeutic benefit of the combination of Everolimus with ET. Of the 855 patients who participated in this preplanned sub study, only 1% of the patients changed ET intake timing during the planned 2 years of their participation. Strikingly, tamoxifen intake in the evening or at night was independently associated with a significant prolongation of Disease-Free Survival as compared to morning or afternoon intake with a Hazard Ratio of 0.43 [95% CI, 0.22–0.85]. In contrast, no timing effect was found for aromatase inhibitors efficacy.

Our results for tamoxifen are consistent with the larger metastatic potential of circulating breast cancer cells at night,[5] and the frequent dampening of circadian rhythms in older women.[6]

Implications of all the available evidence

In the absence of any current guidelines, tamoxifen oral intake could be recommended in the evening for patients with HR+/HER2– breast cancer, especially in the younger women. This prospective and hypothesis-generating timing study stresses the need for further chronotherapeutic trials testing endocrine therapies in patients with breast cancer and for elucidating the circadian mechanisms at work.

Introduction

Breast cancer is a prevalent global health issue, affecting approximately 2.3 million women annually.[7] Nearly 70% of cases are categorized as hormone receptor-positive (HR-positive) and human epidermal growth factor receptor 2-negative (*HER2*-negative) BC. The standard treatment for patients with HR-positive BC is endocrine therapy (ET), which encompasses the use of tamoxifen and aromatase inhibitors (AI), either as monotherapy or in combination with other agents.[8,9] Despite the widespread use of ET, some patients still experience recurrence - either locally or in distant organs–indicating the need for improved treatment strategies.[8] Though CDK4/6 inhibitors such as abemaciclib and ribociclib are progressively being integrated in the therapeutic arsenal of early BC with high risk of recurrence, ET remains the cornerstone for the treatment of HR+/HER2– BC.

Compliance to ET is known to be suboptimal, since rates reportedly range between 50% and 89% of the patients.[10–12] Studies have shown that the survival of patients with HR-positive BC is strongly linked to adherence to ET, particularly in the adjuvant setting. In the French CANTO cohort, serum assessment of tamoxifen identified 16.0% of patients (n = 188) below the set adherence threshold. Patients who were biochemically nonadherent had significantly shorter distant recurrence or death (hazard ratio, 2.31; $p = 0.036$).[13] Poor adherence to ET is influenced by multiple factors and particularly the occurrence of side effects or to the fear of experiencing them.[14] Lifestyle modifications, such as increased physical activity, and pharmacologic interventions have been used to attempt improving compliance through the mitigation of ET side effects. Nevertheless, data investigating whether the timing of

ET oral intake could influence the compliance and tolerance of ET and thus the outcomes of these patients remains scarce.

Endogenous circadian rhythms control the cellular and molecular processes that determine absorption, distribution, metabolism, and elimination of medications over the 24 h.[15,16] Chrono pharmacology is important for anticancer drugs, whose adverse events need to be minimized, whilst enhancing efficacy. Circadian rhythms are approximately 24-h oscillations that moderate cellular and organismic physiology,[16] and are generated within each individual cell by three transcription/posttranscription feedback loops that involve fifteen clock genes.[17] Such molecular clocks within cells are coordinated by the hypothalamic suprachiasmatic nuclei (SCN). This central circadian pace maker generates an array of circadian signals, including cortisol and melatonin secretions, rest-activity and feeding patterns, body temperature, and sympathetic/parasympathetic tones, that reset and coordinate the cellular clocks over the 24 h.[16] The circadian timing system is synchronized by environmental cycles such as the alternation of light and darkness over the 24 h, as well as socio-professional time cues. Molecular clocks further rhythmically regulate cellular metabolism, proliferation, apoptosis, autophagy, and drug responses over the 24 h.[17,18]

Considering the scarcity of data on the influence of timing of ET intake in patients with HR+/HER2– BC,[19,20] we included a pre-planned ancillary study on the association between ET intake timing and treatment efficacy as part of a large prospective randomized controlled trial.[4] Between June 2013 and March 2020, 1278 patients with HR-positive, *HER2*-negative high-risk early BC participated in the UNIRAD clinical trial (NCT01805271), testing the effect of adding everolimus, a m-TOR inhibitor, to ET in the adjuvant setting. During their 2-year participation in the trial, patients were asked to prospectively record the timing of ET intakes in a daily diary. Here, we present the findings of this pre-planned analysis regarding the association between ET intake timing and disease-free survival (DFS).

Methods

Patients and study design

Between June 2013 and March 2020, 1278 patients with high-risk HR+/*HER2*– primary breast cancer were randomly assigned to receive everolimus or placebo in addition to adjuvant ET. Patients were randomly assigned in a 1:1 ratio to receive 2 years of placebo or 2 years of everolimus, added to ongoing ET. Patients were assigned to one of two treatment arms on the basis of a dynamic randomization method by minimization according to Pocock and Simon algorithm. Eligible patients were women aged 18 years or older, with estrogen receptor (ER)-positive *HER2*-negative early breast cancer at a high risk of relapse, defined as ≥ 4 positive lymph nodes at primary surgery; or ≥1 positive lymph node if surgery was performed after neoadjuvant chemotherapy; or 1–3 positive lymph nodes at primary surgery and an EndoPredict (EPclin) score ≥3.3. Only patients without any distant metastasis at diagnosis, and with at least one ET intake timing diary field filled were included in the current study.

ET intake timing

Patients were requested to record in a daily diary the timing of ET intake categorized into four 6-h time slots, defined as follows: 06:00–11:59 (morning), 12:00–17:59 (afternoon), 18:00–23:59 (evening), or 24:00–05:59 (nighttime). Each ET intake timing change noted in the diary was reported in the case report form (CRF). Patients were considered to have changed ET intake timing if at least two different time slots for ET for a duration >7 days within the trial duration were declared. Descriptive analyses of patients and tumor characteristics were provided according to the four time slots and were then binned into two categories (morning/afternoon or evening/nighttime) for the analyses on DFS because of the low number of patients declaring ET intake in the afternoon (n = 49, 5% of the patients) or at nighttime (n = 5, 1%). The study included all patients who reported their intake timing, ensuring that the analysis encompassed the available data from the study cohort.

Study endpoint

The primary endpoint of the UNIRAD study was disease-free survival (DFS) according to the allocated treatment as previously reported.[4] DFS was measured from the date of random assignment, and DFS events were defined as invasive local, regional, or metastatic relapse, contralateral breast cancer, or death from any cause. Assessment of the association between ET timing and DFS was a prespecified secondary endpoint. Results on overall survival (OS) were also reported. Stratification factors in the trial included ET agent, receipt of neoadjuvant *versus* adjuvant chemotherapy, progesterone receptor status, duration of ET before random assignment and lymph node involvement and were considered in the analyses.

Statistical analysis

Qualitative variables were compared using Fisher's exact test when any expected frequency was less than five in a category. In cases where all expected frequencies were five or greater, the Chi-squared test was employed. Quantitative variables were assessed for normality using graphical methods, specifically Q–Q plots, and statistically with the Shapiro–Wilk test. Where normality was confirmed, comparisons were made using Welch's t-test; for distributions not meeting the normality assumption, the Wilcoxon rank-sum test was employed. A significance threshold of 5% was applied. The date of

randomization was used as the origin date for survival analysis. Survival probabilities were estimated by the Kaplan–Meier method, and survival curves were compared in log-rank tests. Hazard ratios and their 95% confidence intervals were calculated with the Cox proportional hazards model.

Univariate analyses on DFS were performed for ET intake timing and for the following confounding factors: age, performance status, menopausal status, ET duration at random assignment, clinical T and N stage, pathological T stage, lymph nodes involvement, SBR grade, PR status, arm of treatment (placebo *versus* everolimus), and ET agent (tamoxifen *versus* AI). Confounding factors were identified following the disjunctive cause criterion proposed elsewhere.[21] Confounding factors with a *p*-value for the likelihood ratio test equal to 0.05 or lower in univariate analysis were selected for inclusion in the multivariable analysis. We tested the proportional hazards assumption of the Cox models by assessing the correlation between the Schoenfeld residuals and time for each covariate. The association between temporary or definitive stop of ET and the outcome was further analyzed, but the variable was not considered a confounder because it was assessed after the timing of ET intake.

We also tested the hypothesis of potentially different effects of the ET intake timing according to the stratification factors of the study. The assessment of heterogeneity was assessed as a multiplicative interaction (using product term in the cox model). The results as an additive interaction were also presented as relative excess risk due to interaction (RERI). Due to the lack of statistical power for analyzing interactions,[22] a *p*-value of 0.10 or lower was considered statistically significant. The interaction contrast was presented with 95% confidence interval (CI) along with *p*-value as recommended.[23]

We selected variables to be included in the multivariable analysis using a stepwise backward selection procedure, starting with a model that included ET intake timing, confounding factors significantly associated with outcome in univariable analyses, and interaction terms between ET intake timing and a trial stratification factor significantly associated with outcome in univariable analyses. Analyses were performed with R software, version 4.2.3.[24]

Ethics

The study was conducted in accordance with Good Clinical Practice principles, the Declaration of Helsinki, and all local regulations. All patients provided written informed consent. The study was approved by the French medicine's agency (ANSM— Agence Nationale de Sécurité'du Médicament et des produits de santé), by an ethics committee (Comite' de Protection des Personnes Sud-Est IV—Lyon) in September 2012, and by institutional review boards of each participating center. A steering committee supervised the study, and an independent data monitoring committee met every year and was responsible for monitoring safety and efficacy in the trial participants. Unirad study was approved by the ethics committee SUD-EST IV, reference number 12/077.

This study is registered online in clinicalTrials.gov, ID = NCT01805271.

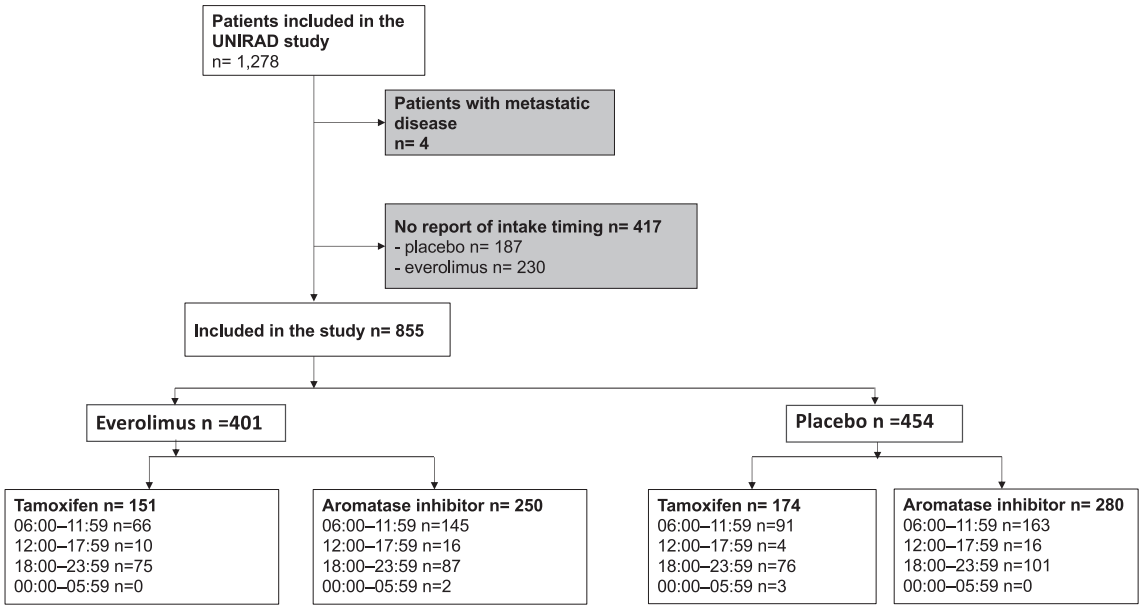

Fig. 1: Consort diagram of the study. The four 6-h slots were binned as follows: 06:00–11:59 (morning), 12:00–17:59 (afternoon), 18:00–23:59 (evening), or 24:00–05:59 (nighttime).

Variable name	level	Overall	Morning	Afternoon	Evening	Nighttime	p
	N	855	465 (54.4)	46 (5.4)	339 (39.6)	5	
Age at BC diagnosis (years)		55.1 (10.3)	56.4 (10.1)	58.4 (11.5)	53.1 (10.1)	50.8 (3.8)	0.00051
	[0–40)	65 (7.6)	28 (6.0)	3	34 (10.0)	0	0.0027
	[40–50)	237 (27.7)	111 (23.9)	10	114 (33.6)	2	
	[50–60)	276 (32.3)	158 (34.0)	12 (26.1)	103 (30.4)	3	
	60+	277 (32.4)	168 (36.1)	21 (45.7)	88 (26.0)	0	
ECOG performance status	0	764 (89.5)	412 (88.8)	38 (82.6)	310 (91.4)	4	0.14
	1	90 (10.5)	52 (11.2)	8	29 (8.6)	1	
Menopausal status	Premenopausal	254 (30.2)	114 (25.2)	14 (30.4)	124 (36.8)	2	0.0058
	Postmenopausal	586 (69.8)	338 (74.8)	32 (69.6)	213 (63.2)	3	
Clinical T stage (TNM)	T0	19	12	1	6	0	0.25
	T1	221 (30.2)	107 (26.7)	13 (35.1)	100 (34.7)	1	
	T2	348 (47.6)	210 (52.4)	17 (45.9)	118 (41.0)	3	
	T3	131 (17.9)	67 (16.7)	5	58 (20.1)	1	
	T4	12	5	1	6	0	
Clinical N stage (TNM)	N0	285 (39.0)	147 (35.9)	19 (50)	116 (41.6)	3	0.27
	N1	327 (44.8)	191 (46.7)	13 (34.2)	122 (43.7)	1	
	N2	86 (11.8)	56 (13.7)	3	27 (9.7)	0	
	N3	32 (4.4)	15	3	14	0	
Lymph node involvement	1–3N+	266 (31.1)	148 (31.8)	14 (30.4)	103 (30.4)	1	0.97
	≥4N + or ≥1N + after neoadjuvant setting	589 (68.9)	317 (68.2)	32 (69.6)	236 (69.6)	4	
SBR grade	Grade I	65 (7.8)	32 (7.1)	5	26 (7.8)	2	0.18
	Grade II	520 (62.4)	276 (61.6)	31 (67.4)	210 (62.7)	3	
	Grade III	249 (29.9)	140 (31.2)	10	99 (29.6)	0	
IHC subtypes	ER+/PR–	137 (16.1)	70 (15.2)	6	60 (17.8)	1	0.64
	ER+/PR+	712 (83.9)	390 (84.8)	40 (87.0)	278 (82.2)	4	
PR status	Negative	137 (16.1)	70 (15.2)	6	60 (17.8)	1	0.65
	Positive	712 (83.9)	390 (84.8)	40 (87.0)	278 (82.2)	4	
Pathological T stage (TNM)	pT0 or pTis	4	2	0	2	0	0.02
	pT1	230 (27.0)	104 (22.5)	15 (32.6)	110 (32.5)	1	
	pT2	428 (50.2)	254 (54.9)	25 (54.3)	147 (43.5)	2	
	pT3	172 (20.2)	92 (19.9)	4	74 (21.9)	2	
	pT4	18 (2.1)	11	2	5	0	
Arm of treatment	Everolimus	401 (46.9)	211 (45.4)	26 (56.5)	162 (47.8)	2	0.49
	Placebo	454 (53.1)	254 (54.6)	20 (43.5)	177 (52.2)	3	
ET duration at random assignment	≤3 years	726 (84.9)	389 (83.7)	37 (80.4)	296 (87.3)	4	0.29
	>3 years	129 (15.1)	76 (16.3)	9	43 (12.7)	1	
ET agent	Aromatase inhibitor	530 (62.0)	308 (66.2)	32 (69.6)	188	2	0.0079
	Tamoxifen	325 (38.0)	157 (33.8)	14 (30.4)	151 (44.5)	3	
Temporary or definitive stop of ET	No	614 (71.8)	338 (72.7)	32 (69.6)	239 (70.5)	5	0.56
	Yes	241 (28.2)	127 (27.3)	14 (30.4)	100 (29.5)	0	

Fisher exact test was used for age class, ECOG status, menopausal status, clinical T stage, clinical N stage, Lymph node involvement, SBR grade, IHC subtype, PR status, pathological T stage (TNM), arm of treatment, ET duration at random assignment, ET agent, temporary or definitive stop of ET. Missing data: ECOG performance status, n = 1; Menopausal status, n = 15; Clinical T stage (TNM), n = 124; Clinical N stage (TNM), n = 125; SBR grade, n = 21; IHC subtypes, n = 6; PR status, n = 6; Pathological T stage (TNM), n = 3. Abbreviations: ET, Endocrine therapy; EPclin, Endopredict score; IHC, Immunochemistry; pN, pathological nodal involvement; PR, Progesterone receptor; SBR, Scarff Bloom Richardson. Age is displayed as years old. In the case of categorical variables, and percentages are expressed between brackets. In the case of continuous variables, the mean value is reported, with standard deviation (SD) between brackets. In the case of nonnormal continuous variables, the median value is reported, with interquartile range between brackets [IQR]. The variable age as continuous variable follows a non-normal distribution.

Table 1: **Patients and tumor characteristics according to timing intake.**

Role of funders

UNIRAD was Supported by a grant from the French Ministry of Health PHRC 2012 and received funding from La Ligue contre le Cancer, Cancer Research-UK, Myriad Genetics, and Novartis. Funding sources had no role in the conduct or reporting of this research.

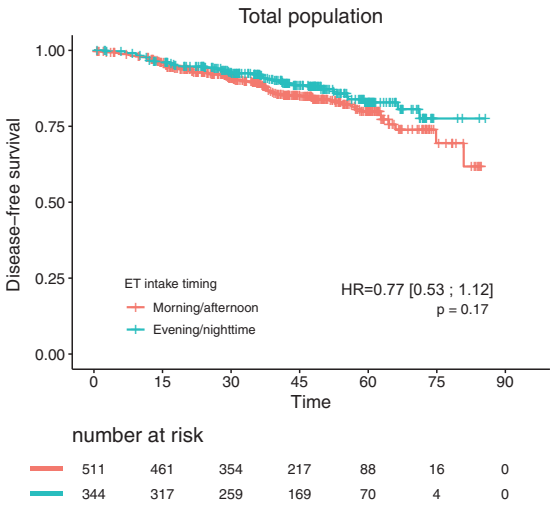

Fig. 2: Disease free survival of the whole population according to the timing of ET intake. Abreviations: ET, Endocrine therapy. *p*-value was obtained from the log-rank test.

Results

Patients and tumor characteristics

Out of 1274 patients included in the trial, 855 patients (67.2%) recorded the time they chose for ET intake (everolimus arm, n = 401; placebo arm, n = 454) (Fig. 1). As compared to the patients who did not report intake timing, patients who reported ET intake timing were slightly older (55.1 *versus* 53.6 y.o.), more likely to be postmenopausal, to have smaller size tumors, and in the placebo arm rather than in the everolimus arm (Table S1). Patients and tumor characteristics were well balanced between the placebo and the everolimus group (Table S2).

Timing of ET intake

ET was mostly taken in the morning (n = 465, 54.4%), or in the evening (n = 339, 39.6%), while a minority of patients took ET in the afternoon (n = 46, 5.4%) or at nighttime (n = 5, 0.6%). Only 10 patients changed their initial choice of timing slots for ET intake throughout the trial (1.1%). Patients with morning and afternoon intake were older, with respective median ages of 56.4 y.o and 58.4 y.o, whilst patients taking ET in the evening or at nighttime were younger (53.1 y.o and 50.8 y.o. respectively).

The patients declaring taking ET in the evening or at nighttime were mostly premenopausal and mostly received tamoxifen as ET (Table 1). The timing of ET intake was not associated with the temporary or definitive stop of ET (morning (27.3%), afternoon (30.4%), evening (29.5%), nighttime 0.0% (0/5), $p = 0.47$, *p*-value was obtained from chi-square test). Due to the low

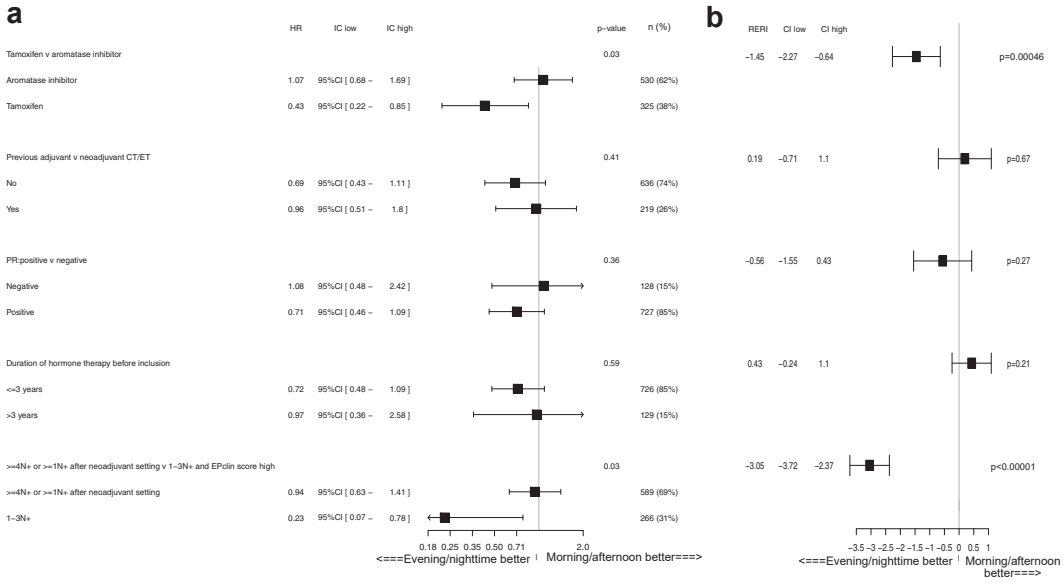

Fig. 3: Association between disease free survival and endocrine therapy intake timings according to the stratification factors of the pivotal trial. a: Analysis on a multiplicative scale. Hazard Ratios (HR) with 95% Confidence Intervals (CI) are provided for the comparison of Tamoxifen *versus* aromatase inhibitors, presence or absence of previous adjuvant/neoadjuvant chemotherapy/endocrine therapy (CT/ET), and other trial stratification factors, *p*-value was obtained from wald test; b: Analysis on an additive scale. In addition to HR and 95% CI, the Relative Excess Risk due to Interaction (RERI) is reported to assess the additive interaction between timing of endocrine therapy intake and other factors, *p*-value was obtained from an asymptotic z-test for the RERI[38] The horizontal scale at the bottom indicates the benefit direction for disease-free survival concerning evening/nighttime or morning/afternoon intake of endocrine therapy. Each stratification category includes the number of patients (n) and their percentage of the total (%).

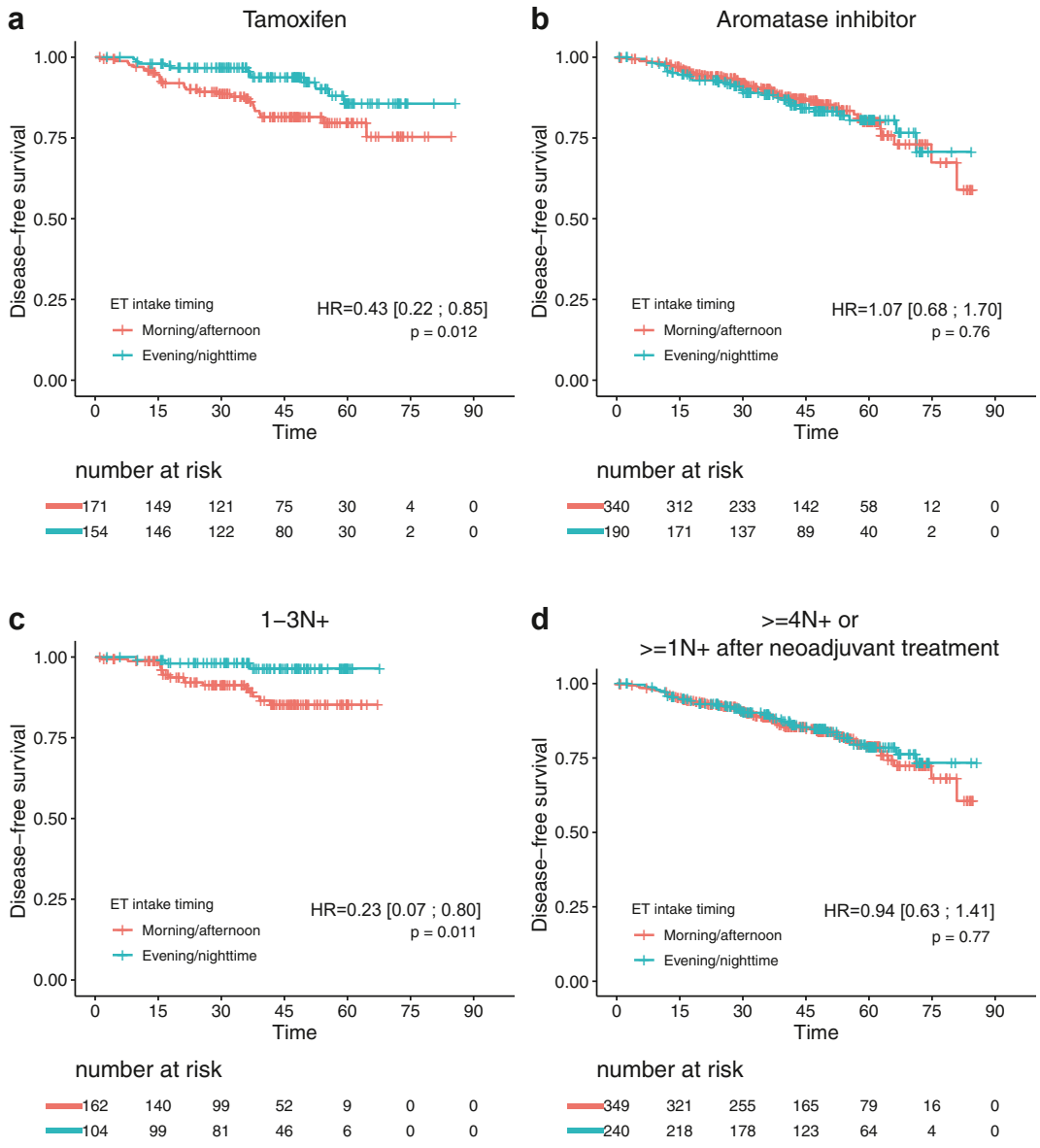

Fig. 4: Disease-free survival according to the timing of ET intake in subgroups; a: tamoxifen; b: Aromatase inhibitors; c: 1–3 positive nodes; d: ≥4 positive nodes or ≥1 node involved after neoadjuvant chemotherapy. Abreviations: ET, Endocrine therapy *p*-value was obtained from the log-rank test.

numbers of patients in the afternoon or in the nighttime intake groups (n = 49 and n = 5 respectively), timings of ET intakes were binned into two categories for the subsequent analyses (morning or afternoon, n = 511; evening or nighttime, n = 344, Table S3).

Oncological outcomes

Among the 855 patients of this sub-study, and with a median follow-up of 46.7 months (IQR 44.2–47.6, censoring proportion: 52.9%), 118 patients experienced an event (local recurrence, n = 30, distant metastases n = 84, death without recurrence n = 4, death with recurrence n = 37). In the whole population, ET intake timing was not associated with DFS (HR = 0.77, 95% CI [0.53–1.12]) (Fig. 2). DFS between patients who reported endocrine therapy intake timing (n = 855) and those who did not (n = 417) was similar (HR = 1.01, 95% CI [0.70–1.41]).

The association between ET intake timing and DFS was analyzed according to the stratification factors of the trial. There were interactions between ET intake timing and ET agent and between ET intake timing and nodal

Characteristics	Class		Univariable					Multivariable		
		n	Ev	HR	95% CI	p	p*	HR	95% CI	p
Age at BC diagnosis (year)	[0–40)	65	14	1			0.01	1	–	
	[40–50)	237	23	0.4	[0.21–0.78]	0.007		0.36	0.17, 0.74	0.01
	[50–60)	276	34	0.49	[0.26–0.91]	0.02		0.49	0.23, 1.03	0.06
	60+	277	47	0.73	[0.4–1.33]	0.3		0.81	0.37, 1.75	0.6
ECOG performance status	0	764	106	1			0.76			
	1	90	12	1.1	[0.6–1.99]	0.76				
Menopausal status	Premenopausal	254	33	1			0.99			
	Postmenopausal	586	85	1	[0.67–1.5]	0.99				
Clinical T stage (TNM)	T0-T1	240	23	1			0.001	1	–	
	T2	348	43	1.35	[0.81–2.23]	0.24		1.24	0.74, 2.07	0.42
	T3-T4	143	35	2.55	[1.51–4.31]	0.00049		2.32	1.35, 3.98	0.0013
Clinical N stage (TNM)	N0	285	40	1			0.74			
	N1	327	51	1.25	[0.83–1.9]	0.28				
	N2	86	12	1.06	[0.56–2.02]	0.85				
	N3	32	5	1.23	[0.49–3.12]	0.66				
Lymph node involvement	1–3N+	266	20	1		0.01	0.02	1	–	
	≥4N + or ≥1N + after neoadjuvant setting	589	98	1.78	[1.09–2.89]			1.7	1.00, 2.90	0.05
SBR grade	Grade I	65	5	1			0.12			
	Grade II	520	69	1.61	[0.65–3.98]	0.3				
	Grade III	249	42	2.17	[0.86–5.5]	0.1				
IHC subtypes	ER+/PR-	137	26	1			0.09			
	ER+/PR+	712	92	0.69	[0.45–1.07]	0.09				
PR status	Negative	137	26	1			0.09			
	Positive	712	92	0.69	[0.45–1.07]	0.09				
Pathological T stage (TNM)	pT0 or pTis	4	1	1			0.04			
	pT1	230	20	0.18	[0.02–1.33]	0.09				
	pT2	428	62	0.3	[0.04–2.17]	0.23				
	pT3	172	32	0.4	[0.05–2.9]	0.36				
	pT4	18	2	0.22	[0.02–2.46]	0.22				
Arm of treatment	Everolimus	401	51	1			0.64			
	Placebo	454	67	1.09	[0.76–1.57]	0.64				
ET duration at random assignment	≤3 years	726	100	1			0.17			
	>3 years	129	18	0.7	[0.42–1.17]	0.17				
ET intake timing	morning/afternoon	511	76	1			0.17	a		
	evening/nighttime	344	42	0.77	[0.53–1.12]	0.17				
ET agent	Aromatase inhibitor	530	78	1			0.31	a		
	Tamoxifen	325	40	0.82	[0.56–1.2]	0.31				

Abbreviations: ET, Endocrine therapy; EPclin: Endopredict score; IHC, Immunochemistry; pN, pathological nodal involvement; PR, Progesterone receptor; SBR, Scarff Bloom Richardson. Age is displayed as years old. *p** is the *p*-value for the global test, and *p* represents the test of a given class *versus* the reference class. [a]The association between DFS and ET intake timing is modified according to ET agent, and results in these four groups are displayed in Table S4.

***Table 2*: Univariate and multivariable analysis on factors associated with disease-free survival (DFS).**

involvement both at the multiplicative (Fig. 3a) and at the additive scale (Fig. 3b).

Patients taking tamoxifen in the evening/nighttime had a lower likelihood of relapse than patients taking tamoxifen in the morning/afternoon (HR = 0.43, 95% CI [0.22–0.85]) while no such association was seen in patients on AI (HR = 1.07, 95% CI [0.68–1.7]) (Fig. 4 a-b respectively). Of note, this association was marked in premenopausal patients (HR = 0.32, 95% CI [0.14–0.74]); but such protective effect was not evidenced in postmenopausal patients (HR = 0.65, 95% CI [2.17–0.5]) (Fig. S1), though no interaction was evidenced. Patients with a mild nodal involvement taking ET in the evening/nighttime had a lower likelihood of relapse than patients taking ET in the morning/afternoon (HR = 0.23, 95% CI [0.07–0.77]) while no such association was seen in patients with a large nodal involvement (HR = 0.95, 95% CI [0.63–1.43]) (Fig. 4c and d respectively).

After multivariable analysis, only age, clinical tumor stage (T2 *versus* T3, T4), lymph nodes involvement

(1–3N + *versus* ≥ 4N + or ≥1N + after neoadjuvant) and the interaction term between ET agent and timing of ET intake remained independently associated with DFS (Table 2 and Table S4), while the interaction term between ET intake timing and lymph nodes involvement was not associated with DFS. After multivariable analysis in the group of patients taking tamoxifen, ET intake timing was the only variable that was associated with DFS (Table S5). After multivariable analysis in the group of patients taking AI, initial clinical tumor stage and lymph node involvement at surgery but not ET intake timing were associated with DFS (Table S6).

No association between ET intake timing and overall survival was found, possibly due to a very low number of events (n = 41).

Discussion

In this ancillary study of the UNIRAD prospective randomized trial evaluating the association between ET intake timing and DFS, we found that tamoxifen intake timing in the evening or at nighttime was independently associated with a better DFS compared to morning or afternoon intakes. Although ET has been proven effective in reducing relapse and mortality in BC[2,25] and has been prescribed to millions of women for decades, few studies investigated the timing of ET intake on treatment efficacy in patients with BC.[19] A pragmatic randomized trial comparing morning *versus* evening dosing of endocrine therapy for early breast cancer (REaCT-CHRONO Study) was presented at San Antonio breast cancer symposium in 2023 and found no difference in the effects of ET timings on ET intake compliance or quality of life.[26]

The timing of tamoxifen intake could theoretically affect patient compliance and adherence to treatment. This hypothesis seems unprobeable to explain the protective association of evening/nighttime intake of tamoxifen, as two studies previously demonstrated that morning administration of medication increased the likelihood of correct drug intake.[27,28] Furthermore, in our study timing of ET intake was not associated with the temporary or definitive stop of tamoxifen. Therefore, the impact of tamoxifen timing on its pharmacologic antitumor effects needs further scrutiny.

Tamoxifen is a selective estrogen receptor (ER) modulator that inhibits estrogen enhancement of mammary epithelium proliferation. Tamoxifen is a prodrug, following its absorption in the gut, it undergoes bioactivation into endoxifen and 4-hydroxy-tamoxifen through CYP2D6 and CYP2C19 enzymatic activities with competitive metabolism through CYP3A4. Both metabolites display nearly 100-fold higher affinity for ER as compared to tamoxifen. Endoxifen is ultimately metabolized by conjugation and excreted via bile and urine. The following chronopharmacologic and tumor chronobiology data could contribute to explain a higher efficacy of tamoxifen when taken in the evening as compared to the morning hours.

First, circadian rhythms are known to regulate drug absorption, distribution, metabolism and elimination, as well as toxicities and efficacy of many medications.[29] In 27 patients with breast cancer, tamoxifen absorption was significantly slower after evening intake, resulting in longer $T_{max,}$ lower C_{max}, and lower $AUC_{0–8\ h}$, as compared to morning intake.[30] Similarly, endoxifen C_{max}, $AUC_{0–8\ h}$ were 23% less ($p < 0.001$) and $AUC_{0–24\ h}$ 15% less ($p < 0.001$) after evening versus morning, supporting reduced exposure to endoxifen in those patients taking tamoxifen in the evening rather than in the morning.[30] Interestingly also, women reported changes in the frequency and intensity of hot flashes that seemed to relate to the higher C_{max} following morning intake, although the sample size was limited to draw firm conclusions.

Data from the Genotype-Tissue Expression (GTEx) project were combined with an algorithm that assigned circadian phases in 16,000 mRNA expressions in 46 tissues from 914 dead donors.[6] Despite clock transcripts showed conserved timing relationships and tight synchrony across the body, sex dimorphism was identified in mRNA rhythms, that usually damped over aging. More specifically, large amplitude circadian rhythms characterized the mRNA expressions of CYP2D6, CYP2C19 and CYP3A4 in female human livers, with highest expressions in the early morning hours, i.e. between 08:00 and 09:00.[6] These findings further support a chronopharmacology of tamoxifen, whose mouse counterpart has also been established.[30] Nonetheless, the results from the single available tamoxifen chronopharmacokinetics study suggest decreased endoxifen exposure following evening *versus* morning dosing.

It remains unknow how a lower plasma endoxifen exposure could achieve a higher tamoxifen efficacy in the evening in female patients with breast cancer. The lowest CYP 3A4 expression in the evening could reduce endoxifen catabolism, hence enhancing evening of nighttime exposure to this metabolite. However, we rather hypothesize that evening administration of tamoxifen is most critical for its pharmacodynamic effect, i.e. the targeting of the estrogen receptors on tumor cells, and its downstream effects, as well as its possible interaction with the estrogens role in the modulation of circadian rhythms.[31] The suprachiasmatic nucleus is regulated in part by the levels of circulating estrogens during both developmental and adult stages. Furthermore, estrogens can alter the expression of the clock genes involved in the circadian regulation of peripheral organs by the suprachiasmatic nucleus.[31] Most importantly, the pharmacodynamic effects of tamoxifen and endoxifen on the estrogen receptors and their downstream effects in tumor cells could be most pronounced in the evening or at nighttime, as supported by a recent groundbreaking study.[23]

Second, the metastasic spread of breast cancer appears to be achieved differently along the 24-h time scale, as a result of a major circadian rhythm in the haematologic dissemination of circulating tumor cells (CTCs).[5] Recently, Diamantopoulou et al.[5] have shown that CTC generation with a high proclivity to metastasize does not occur continuously. They displayed on both patients with BC and mouse models that the majority of intravasation events of spontaneous circulating tumor cells (CTCs) occurred during periods of sleep. Thus, evening tamoxifen intake could enhance antitumor efficacy through drug delivery at the proper pharmacodynamic target time, when CTCs are highly prone to metastasize, that is at the beginning of the rest-phase.

Furthermore, the investigators discovered that various key hormones that regulate circadian rhythms, such as melatonin, testosterone, and glucocorticoids, played a crucial role in CTC generation dynamics. The researchers recommended that cancer treatment approaches should be tailored to be most effective during periods of rest, and that these hypotheses should be tested in clinical trials.

Third, melatonin secretion plays an important role in the central and peripheral regulation of circadian rhythms. In patients with breast cancer, melatonin levels follow a circadian pattern,[32] with high concentrations at nighttime and low concentrations during the day.[33] Bedtime melatonin therapy improved quality of life as well as sleep quality, and duration in 32 patients with metastatic BC by enhancing their circadian rhythm,[32] possibly through its effect on estrogen receptor alpha (ERα) expression in ER + breast cancer tumors[33] and/or direct anti-tumor effect.[33] Consequently, tamoxifen could also synergize the yet controversial anti-tumor effects of melatonin.

Finally, our results showed that patients who took tamoxifen in the evening/nighttime were younger compared to those who took it in the morning/afternoon. We cannot exclude that the differences we evidenced were because younger patients have different hormonal profiles or other physiological characteristics potentially influencing the efficacy of ET. However, the age difference was relatively minor, and thus unlikely to solely account for such a significant effect. However, menopausal status potentially acting as a confounding factor remains an important limitation of our study.

In contrast, no association between daily ET intake timing and DFS was identified for aromatase inhibitors. AI's half-life is 2–4 days, thus resulting in nearly 95% inhibition of aromatase and circulating estrogens clearance with 10–20 days.[34] The deprivation of cancer cells from estrogen exposure represents the main mechanism responsible for AI antitumor efficacy, which do not directly target cancer cells. A pubmed search found not a single article reporting any chronopharmacologic investigation of AI in preclinical models or in humans. Thus, we contend that the dosing time dependency of tamoxifen efficacy mainly results from the rhythmic control of estrogen receptors susceptibility to tamoxifen by the circadian clocks in breast cancer cells.[35]

The low number of events and correspondingly large confidence intervals may indicate the presence of sparse-data bias, suggesting that the estimates of effect sizes may be unreliable.[36] Plus, we used hazard ratios to measure associations, and these may be susceptible to selection bias, as highlighted previously.[37] Despite our efforts to adjust for potential confounders, notably menopausal status, residual unmeasured confounding may still exist, which could influence the interpretation of our results.

Our study has the primacy of the prospective assessment of the ET intake timing together with efficacy endpoints within a randomized trial on a large cohort of patients with HR+/HER2–high-risk breast cancer. Randomization was not performed on the timing of ET intake, but on the allocation of patients to receive everolimus or placebo in addition to ET. Although the ancillary analysis of the ET intake timing was a prespecified secondary endpoint of the study, our study cannot be considered as a genuine randomized controlled trial but must rather be considered as hypothesis-generating. For perspective, a prospective, pragmatic, multicenter, randomized clinical trial REaCT-CHRONO completed enrollment of patients with early-stage BC (clinicaltrials.gov, NCT04864405) to determine whether morning or evening timing of ET could influence health-related quality of life and treatment adherence. The results presented at the SABCS 2023 symposium did not show difference for both endpoints between tested times of ET administration. Until the efficacy data of the current work are validated in independent study, patients with high-risk HR+/*HER2*–negative BC could be recommended evening or nighttime tamoxifen intake in the absence of any current guidelines.

Contributors

Conceptualisation: Sylvie Giacchetti, Francis Lévi, Anne Sophie Hamy.

Data collection: Sylvie Chabaud, Jérôme Lemonnier.

Data analysis: Enora Laas, Anne Sophie Hamy, Elise Dumas, Sylvie Giacchetti, Francis Lévi.

Data interpretation: Anne Sophie Hamy, Sylvie Giacchetti, Enora Laas, Francis Lévi.

The underlying data have been verified by Anne Sophie Hamy, Enora Laas, Sylvie Giacchetti and Francis Lévi.

All authors contributed to the interpretation of the findings.

Writing original draft: Sylvie Giacchetti, Anne Sophie Hamy, Enora Lass, Francis Lévi.

All authors read and approved the final version of the manuscript.

Data sharing statement

Unicancer will grant access to all study data to all requestors in the field of cancer.

Research, upon written detailed request sent to.

Unicancer will grant access to editor's reviewers to study documents and to the data

Required for independent verification of the published results. Editor guarantees that

Mandated reviewers are committed to use the data transferred for the sole aim to

Reviewing produced results, under strict conditions warranting security and

Confidentiality of the data.

Declaration of interests

The authors declared no competing interest with this study. Dr Giacchetti declared travel expenses from MSD and Novartis to SABCS 2022 meeting and ASCO 2023 meeting where these data were presented *(SABCS 2022 Abstract 1,305,036; ASCO 2023 Abstract 412,092*).

Acknowledgements

UNIRAD was Supported by a grant from the French Ministry of Health PHRC 2012 and received funding from La Ligue contre le Cancer, Cancer Research-UK, Myriad Genetics, and Novartis.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105141.

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