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Summary

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Abbreviations

ADL	Activities of Daily Living
AJCC	American Joint Committee on Cancer
BC	Breast cancer
BCS	Breast-conserving surgery
CBCT	Cone Beam Computed Tomography
CT	Computed Tomography
CTV	Clinical Target Volume
CTCAE	Common Terminology Criteria for Adverse Events
DFS	Disease Free Survival
DIBH	Deep Inspiration Breath Hold
EFS	Event Free Survival
EORTC	European Organisation for Research and Treatment of Cancer
ER	Estrogen Receptor
FU	Follow-up
GTV	Gross Tumor Volume
Gy	Gray
HER2	Human Epidermal Growth Factor Receptor 2
HR	Hormone receptors
IBR	Immediate Breast Reconstruction
IHC	Immunohistochemical
LABC	Locally Advanced Breast Cancer
LN(s)	Lymph node(s)
MU	Monitor Unit
NAC	Neoadjuvant chemotherapy
NGS	Next Generation Sequencing
OS	Overall Survival
pCR	Pathological Complete Response
PR	Progesterone Receptor
PST	Preoperative Systemic Treatment
PTV	Planning Target Volume
QoL	Quality of Life
RCB	Residual Cancer Burden
RFS	Recurrence Free Survival
RT	Radiotherapy
SBRT	Stereotactic Body Radiation Therapy

SIB	Simultaneously Integrated Boost
TME	Tumor microenvironment
TILs	Stromal Tumor-infiltrating lymphocytes
TN	Triple Negative
VMAT	Volumetric Modulated Arc Therapy
WBI	Whole Breast Irradiation

Introduction

Breast cancer (BC) is a significant health concern worldwide, constituting a major portion of cancer diagnoses among women. The optimal management of BC consists of a multidisciplinary approach, integrating surgery, radiation therapy, and systemic treatments. Over the years, advancements in radiotherapy (RT) techniques have shown promising improvements in treatment outcomes while minimising the impact of treatment on patients' quality of life.

The standard treatment paradigm for BC often involves a combination of surgery, RT, and systemic therapies, such as chemotherapy, endocrine therapy, and targeted therapy. Surgery, as breast-conserving surgery (BCS) or mastectomy, is a cornerstone of treatment. Adjuvant RT is typically administered postoperatively to reduce the risk of local recurrence and improve overall survival (OS). However, the use of postoperative RT may be associated with late toxicities and impaired cosmetic outcomes.

Rationale For Preoperative Radiotherapy in BC

The sequence of treatment in the multimodality management of BC is mainly based on historical and empirical experience. The original “surgery-first” paradigm has been challenged in recent years introducing primary systemic therapy (PST) to improve clinical outcomes and provide information for personalising subsequent treatments based on the pathologically assessed response of the primary tumor.

Even though preoperative RT is routinely used for several types of cancer (rectal, oesophageal, sarcomas), in BC cancer it always failed in the past to become clinical practice, especially because it would have delayed surgery and the incidence of postoperative side effects was expected to be higher. So, historically, preoperative RT has been used to treat locally advanced BC refractory to PST, both to convert inoperable to operable disease or to permit less demolitive surgery (i.e., convert mastectomy to BCS).

However, nowadays, most of the limitations and concerns from the past seem to be circumventable or have become less relevant, making several relevant potential advantages of preoperative RT being identified, which are applicable in different settings, depending on the indication for RT (Figure 1**Errore. L'origine riferimento non è stata trovata.**):

In the early stage, low-risk BC, eligible for partial breast irradiation, preoperative RT would allow better target volume definition and delineation, with smaller volumes, and reduced margins compared to the postoperative situation, thanks to reduced uncertainties. In this subset of patients, pre-operative RT could also allow for de-escalation of treatment intensity, by possibly selecting patients who can forgo surgery.

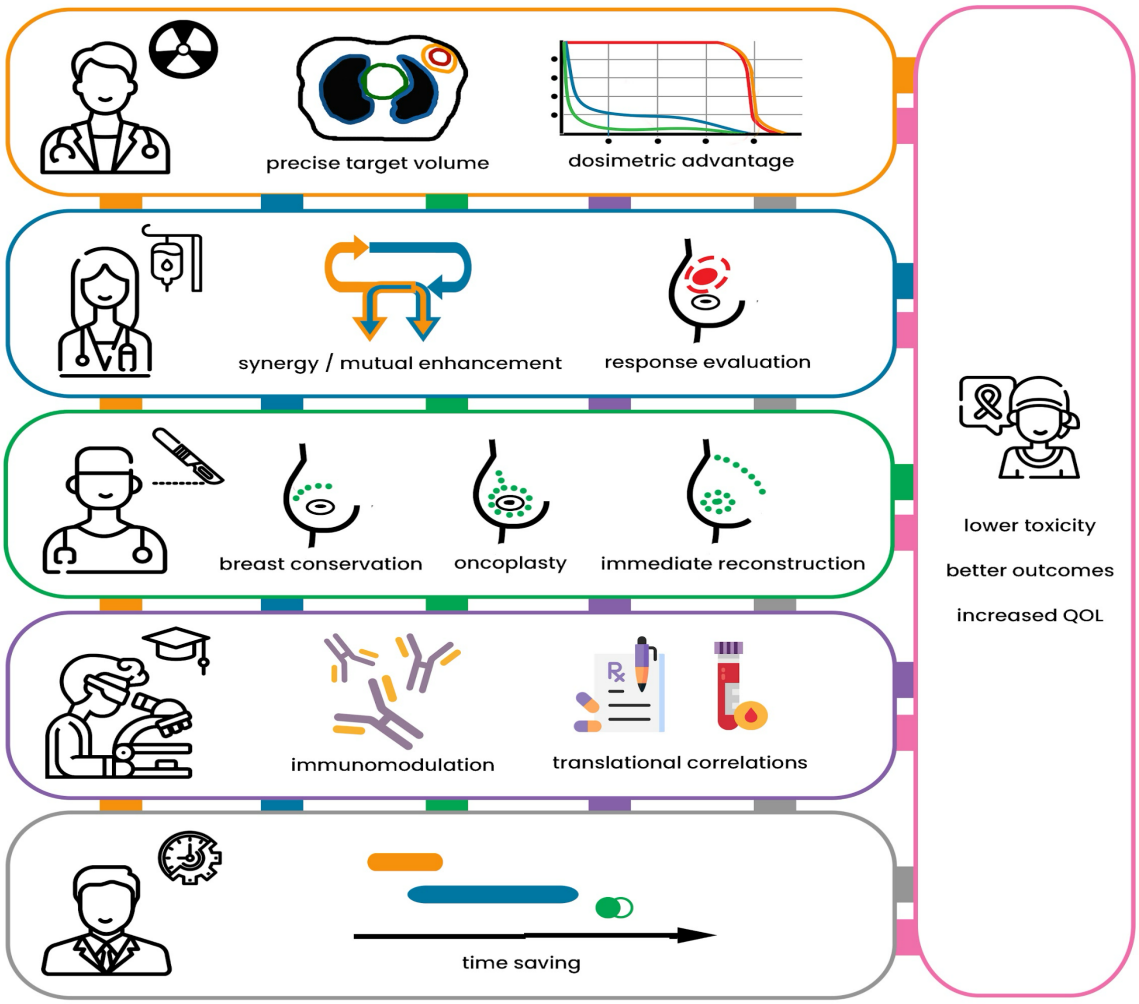
As mentioned before, in the locally advanced stages pre-operative RT could lead to a downstage, increasing the rates of breast conservative surgery (i.e., quadrantectomy vs mastectomy and sentinel LN biopsy vs axillary LN dissection).
When mastectomy and breast reconstructive surgery are planned, the use of pre-operative RT could also improve cosmetic results compared to post-mastectomy RT, which is associated with relatively high rates of unfair cosmetic outcomes.

Moreover, pre-operative RT could increase pathological complete response (pCR) rates in high-risk tumors with potentially improved clinical outcomes, especially in tumors that are less sensitive to PST. This would also allow the stratification of patients based on pCR. The combination with PST could enhance this effect, which could be mediated by the radiosensitiser role of chemotherapy agents, or by the immunomodulating effect of RT associated with immunotherapy drugs. Other novel drugs used for BC could elicit different synergistic mechanisms when associated with RT.

Finally, pre-operative RT could enhance translational research by identifying interactions between radiation and tumor cells, microenvironment, and healthy tissues.

On the other hand, some possible drawbacks and disadvantages need to be taken into account:
- the possible increased risks of surgical complications after RT, although it would probably be less relevant than in the past thanks to more conformal techniques and gentler RT doses;
- the possible risk of under- or over-treatment in case of inadequate/incomplete baseline staging or unexpected findings at the anatomopathological examination;
- the eventual missing information about tumor response (as usually obtained following PST) in the anatomopathological report to drive adjuvant treatment choices.

Figure 1. Possible advantages of pre-operative BC



Literature Review

Preoperative RT – Until 2000

The concept of preoperative RT in BC is not a novelty in BC treatments. Several studies (1–5) investigating this scenario since the 1980s have been reported in Table 1.

Historically, preoperative chemotherapy was also introduced in clinical practice to make locally advanced tumors operable. This approach was subsequently extended to encompass less advanced lesions to facilitate breast preservation.

The reason for combining both approaches (chemotherapy and RT) preoperatively is not only that any surviving tumor cell after chemotherapy is then sterilized by additional irradiation, but also the possibility of enhancing a synergistic effect by using chemotherapy as a radiosensitizer. Semiglazov et al. observed a notable improvement in treatment outcomes with the integration of chemotherapy and pre-mastectomy RT compared to local therapy alone, followed by RT and mastectomy. Moreover, combining RT and chemotherapy in the neoadjuvant setting could select patients with locally advanced stages for conservative treatment without excessive additional toxicity (2,3,6).

However, Colleoni et al. reported that the preliminary findings from their study do not currently advocate for the inclusion of RT alongside preoperative chemotherapy due to significant postsurgical side effects. They observed frequent postoperative complications, including grade II and III infections necessitating extended antibiotic therapy and wound dehiscence. Skinner et al. (5) also observed a significant postoperative morbidity rate, despite the well-tolerated combination of paclitaxel and RT provided an effective pathological response.

Hence, despite initial promising outcomes on tumor response, the study of this integrated treatment approach remained limited and provided evidence of low level, primarily based on case series with small patient cohorts. Consequently, these findings have not significantly influenced clinical practice.

Table 1 Studies of preoperative chemo-radiotherapy published before the 2000s.

Author, year of publication	Number of patients	Type of study	Tumor characteristics	Preoperative RT	Primary Systemic Treatment (PST)	Surgery	pCR (%)	Outcome
Semiglazov et al., 1994 (1)	271	Prospective	Stage IIB-IIIA	271 pts (group I)→ 60Gy (2Gy) WBI, 40Gy (2Gy) RNI	127 pts (group II)→ TMF	Mastectomy, 3-4 weeks	Group I: 29.1% Group II: 19.4%	Group I: 5y OS: 86.1%; 5y DFS: 81.0% Group II: 5y OS: 78.3%; 5y DFS: 71.6%
Touboul et al., 1996 (2)	97	Prospective	Stage IIIA-IV	45Gy/23fx 3 weeks after chemo	Sequential CAF-V x4	Mastectomy or BCS	40%	10y OS: 66%
Skinner et al., 1997 (3)	36	Prospective	IIB-IV	50Gy (2Gy)	5-FU	MRM	17%	2y DFS: 83%
Colleoni et al., 1998 (4)	32	Prospective	T2-T4	50Gy (2Gy) +boost of 10Gy to tumor nodule, 3-4 weeks after chemo	Doxorubicin+Cyclophosphamide x3	BCS or mastectomy	16%	NR

Skinner et al., 2000 (5)	29	Prospective	IIB-III	45Gy (1.8Gy)	Paclitaxel x8 weeks	MRM within 4-6 weeks	26%	NR
Aryus et al., 2000 (6)	56	Prospective	T>3cm or <3cm (when an initial breast-preserving approach was impossible due to an unfavourable ratio of tumor/breast or anatomic difficulties)	50Gy (2Gy) + boost (6 to 11 Gy)	Sequential: CMF or EC	61%: breast-preserving procedures; 11%: IBR; 28%: MRM	43%	NR

Abbreviations: BCS: breast conservative surgery; CAF-V: cyclophosphamide-adriamycin-5FU-vincristine; CMF: Cyclophosphamide+Methotrexate+5FU; DFS: disease free survival; IBR: immediate breast reconstruction; MRM: modified radical mastectomy; NR: not reported; OS: overall survival; RNI: regional node irradiation; TMF: thiotepa-methotrexate; WBI: whole breast irradiation; 5FU: 5Fluoracil

Preoperative RT – 2000-Today

In recent years, advancements in RT techniques have significantly changed the landscape of BC treatment. The emergence of these modern RT modalities has led to a paradigm shift, offering a broader spectrum of possibilities in the field of preoperative RT for BC.

In the next chapters, various contexts of preoperative RT use, distinguished by risk and disease stage, will be explored to provide a comprehensive understanding of its potential applications.

LOW-RISK BC: Use of Preoperative RT To Facilitate Irradiation, Especially After Oncoplastic Surgery.

Preoperative Partial Breast Irradiation (PBI)

In selected low-risk patients, PBI is an alternative to standard whole breast irradiation (WBI), enabling a better target definition and reduced treatment volumes, resulting in decreased toxicity and better cosmesis (7).

Oncoplastic procedures represent a challenge in accurately delineating the postoperative tumor bed due to the potential dispersion of surgical clips within the breast. The expected tumor bed based on preoperative imaging might significantly differ from the actual target volume location. Implementing preoperative RT in this setting may mitigate the risk of a geographic miss. Moreover, tissues exposed to the highest radiation doses are excised during surgery following preoperative partial breast RT (8).

Preoperative single-dose RT to intact breast tumors seems well-tolerated, resulting in limited fibrosis in a small volume and with excellent cosmetic outcomes (9,10).

Preoperative RT appears to be a feasible treatment for select early-stage BC patients, also demonstrating the potential for achieving a pCR rate of 15% (11), 42% (12) and 9% (13), maintaining an acceptable toxicity profile.

Guidolin et al. (9), in their pilot study, revealing no significant toxicity and excellent cosmetic and quality-of-life outcomes, further support the promising potential of preoperative RT. Additionally, this approach demonstrates a low postoperative complication rate and offers favourable results in terms of limited fibrosis and good to excellent cosmetic outcomes, as reported by Bosma et al. (14).

This preoperative RT setting potentially allows delay or even omission of surgery in selected patients if pCR can be accurately predicted. However, it is not yet routine practice due to a few disadvantages compared to standard treatments. These include the need for a multidisciplinary evaluation of the patient starting from the early phases of diagnostic workflow, which is not always feasible in clinical routine practice, a potentially high interobserver variability in target definition and the need for a careful review of diagnostic imaging.

Table 2 summarises the main studies regarding this setting.

Table 2. Summary of key clinical studies and trials investigating preoperative RT in low-risk BC

Author, year of publication	Number of patients	Type of study	Inclusion criteria	Preoperative RT	Surgery	pCR (%)	Outcome
Horton et al., 2015 (DUKE study) (Horton et al., 2015b)	32	Prospective	Age>55 years; cT1N0; G1-G2 CDIS ≤2cm; ER+, PR, HER-	15-21Gy/1fx	Within 10 days after RT	NR	0% recurrences; good or excellent cosmetic outcomes
Van der Leii et al, 2015 (15)	70	Prospective	Age≥60 years; cT1–2 (T size ≤3cm); pN0; invasive, unifocal, non-lobular	40Gy/10fx	6 weeks after RT	NR	2 ipsilateral breast tumor recurrence
Nichols et al., 2017 (11)	27	Prospective	T1-T2 (<3 cm), cN0; invasive, unifocal	38.5Gy/10fx	>21 days after RT	15%	Grade3 seromas; good or excellent cosmetic outcomes
Guidolin et al., 2019 (SIGNAL study) (9)	27	Prospective	T<3 cm, ER+, cN0 invasive, unifocal carcinoma with tumors at least 2 cm away from skin and chest wall	21Gy/1fx	1 week after RT	NR	Toxicity, patient- and physician-rated cosmesis, and quality of life were not significantly different from baseline

Vasmel et al., 2020 (ABLATIVE study) (12)	36	Prospective	Age≥50, unifocal, non-lobular, T<30mm, cN0, pN0 (SLNB)	20Gy to the PTV_{GTV}+15Gy to the PTV_{CTV} /1fx	6-8 months after RT	42%	No grade>2 acute toxicity; 17% grade 2 late toxicity
Tiberi et al., 2020 (16)	10	Prospective	Age≥65, luminal A, cT1N0, G1-G2	20Gy/1fx	3 months after RT	0%	NR
Bosma et al., 2021 (PAPBI) (14)	133	Prospective	Age>60 years, invasive, unifocal, non-lobular carcinoma	40Gy/10fx in 2 weeks (2010-2013); 30Gy/5fx in 1 week (after 2013)	6 weeks after RT	NR	Excellent or good cosmetic outcome; 5y LR rate: 3%
Weinfurtner et al., 2022 (SABR study) (17)	19	Prospective	Age≥50 years, unifocal, invasive adenocarcinoma, cT1-2, ER+, PR+, HER2-, cN0	28.5Gy/3fx	5-6 weeks after RT	0%	NR
Meattini et al., 2022 (ROCK study) (13)	70	Prospective	Age≥50 years, HR+/HER2-, T<25mm	21Gy/1fx	2 weeks after RT	9%	No grade>2 toxicity

Abbreviations: LR: local relapse; NR: not reported; RT: radiotherapy; SLNB: sentinel lymph node biopsy.

Preoperative RT Associated with PST (Endocrine Therapy)

The administration of neoadjuvant anastrozole in conjunction with RT represents a promising strategy for achieving a robust clinical response in postmenopausal BC patients with hormone-receptor-positive tumors. This approach maintains comparable adverse effects to the adjuvant setting, although its efficacy in achieving histological response is somewhat limited (18). We report details on this study in Table 3.

Table 3. Preoperative RT associated with endocrine therapy.

Author, year of publication	**Number of patients**	**Type of study**	**Inclusion criteria**	**Preoperative RT + PST**	**Surgery**	**pCR (%)**	**Outcome**
Ishitobi et al., 2012 (18)	25	Prospective	Postmenopausal women, T≥3cm, N0-N2, ER+ and/or PR+	50Gy/2fx + Anastrozole	7-8 weeks	0% of 92% objective response rate	NR

HIGH-RISK BC: Preoperative RT To Enhance pCR

Preoperative RT Associated with PST (Chemotherapy, Targeted Therapy, Immunotherapy)

In most studies, preoperative RT combined with PST was used in patients eligible for BCS to achieve tumor downstaging and improve pCR. A summary of clinical trials analysing this setting is reported in Table 4.

The RT regimens followed a conventional fractionation schedule of 1.8-2 Gy administered daily, occasionally including a tumor bed boost. In a prospective study by Ciérvide et al. (19), a moderate hypofractionation approach was implemented, delivering a total dose of 40.5 Gy (2.7 Gy/day). Various chemotherapy schedules have been explored, either administered sequentially before or concurrently with preoperative RT. In a few studies, targeted therapies have also been incorporated into the treatment regimen (19–21).

Combining preoperative RT with PST appears to be a feasible and effective primary treatment approach for locally advanced BC (LABC). Studies have consistently shown the effectiveness and good tolerance of neoadjuvant paclitaxel associated with RT, emphasizing its efficacy in achieving both pCR and enabling breast conservation (22–25).

Additionally, administering neoadjuvant RT concurrently with radio-sensitizing chemotherapy significantly improves the rate of pCR (22.6% *versus* 14.9% in the chemotherapy group, p: 0.019). However, it's important to note that this combined approach was associated with non-neglectable toxicities, including grade 3 pneumonitis in 25% of patients and dermatitis in another 25% (26).

Notably, in women achieving pCR through this combined approach, favourable long-term survival outcomes (10 y OS: 69.5%) can be anticipated (27).

However, existing research has already demonstrated the lower likelihood of achieving a pCR with preoperative treatment in luminal B tumors (28).

Cièrvide et al. (19), in their studies, observed higher pCR rates for triple negative (TN) compared to Human Epidermal Growth Factor Receptor 2 (HER2) positive tumors. Considering the separate analysis of the HER2+ component, pCR rates differed between pure HER2+/hormone receptor (HR) negative tumors and HER2+/HR+ (luminal-Her2 enriched tumor) tumors, reaching 63.6% and 47.6%, respectively.

Combining preoperative highly conformed RT with tailored systemic therapies driven by molecular subtypes (TN and HER2+) is feasible and well tolerated, resulting in notable tumor response rates.

Vincent et al. (21) conducted a randomized pilot trial investigating the feasibility of accelerated preoperative RT delivered in 5 fractions with a simultaneously integrated boost (SIB). The results demonstrated its viability, allowing for a shorter overall treatment time without any significant increase in acute toxicity.

Table 4. Clinical studies of preoperative RT combined with PST.

Author, year of publication	Number of patients	Type of study	Tumor characteristics	Preoperative RT	Primary Systemic Treatment	Surgery	pCR (%)	Outcome
Formenti et al., 2003 (22)	44	Prospective	Stage IIB-IIIB	45Gy (1.8Gy); 14Gy boost	Paclitaxel	MRM 2 weeks after RT	16%	NR
Lerouge et al., 2004 (29)	120	Prospective	Stage IIIIA-IIIB-IIIC	45Gy (1.8Gy)	Scheme I: Doxorubicina+Vincristine+5FU+cyclophosphamide x4 cycles every 28 days Scheme II: theprubicin+Vindesine+5FU+cyclophosphamide Scheme III: Epirubicin+5FU+cyclophosphamide	BCS (59%); mastectomy (41%)	35%	10y MDFS: 61%
Chakravarthy et al., 2006 (23)	38	Prospective	Stage IIA-IIIB	46.80Gy/28fx WBI; 5Gy/25fx RNI	Induction and concomitant paclitaxel	BCS (43%) or mastectomy (57%) 3-4 or 5-7 weeks after CRT	34%	NR

Bollet et al., 2006-2012 (24,25)	60	Prospective	T2-T3; N0-N1	50Gy (2Gy); 46Gy RNI	5FU+Vinorelbine	BCS (69%) or mastectomy (31%)	27%	5y OS: 88%; 5y DFS: 83%
Shanta et al., 2008 (30)	1117	Retrospective	Stage IIB-IIIB	40Gy/20fx	Regimen I: CMF Regimen II: Cyclophosphamide+5FU+Adriamycin or epirubicin	3 weeks after RT	45.1%	5y OS: 75.6%; 10y OS: 63.9%; 15y OS: 58.4% 5y DFS: 64.5%; 10y DFS 52.6%; 15y DFS: 41.4%
Alvarado-Miranda et al., 2009 (31)	112	Retrospective	Stage IIB-IIIB	50Gy (2Gy)	Regimen I: 5FU+Doxorubicin+Cyclophosphamide Regimen II: Doxorubicin+Cyclophosphamide	MRM 6-8 weeks after RT	29.5%	5y OS: 84.2% 5y DFS: 76.9%
Adams et al., 2010 (20)	105	Prospective	Stage IIB-IIIC	45Gy (1.8Gy) WBI; 14Gy (2Gy) boost	Paclitaxel+Trastuzumab (patients enrolled after 2006 with HER2+)	BCS or mastectomy 4 weeks after RT	34%	5y OS: 71.6% 5y DFS: 61.4%

Daveau et al., 2011 (32)	165	Retrospective	T2-T3	45Gy (1.8Gy)	Regimen I: Adriamycin or Epirubicin + Cyclophosphamide + 5FU Regimen II: thioTEPA	BCS (82%) or mastectomy (18%)	40% (39% of patients treated with breast surgery+ RT, 61% with RT alone)	5y OS: 91%; 10y OS: 77%; 5y DFS: 65%; 10y DFS: 52% in the no-surgery group 5y OS: 82; 10y OS: 79%; 5y DFS: 72%; 10y DFS: 61% in the surgery group
Brackstone et al., 2017 (26)	32	Prospective	T3-T4, N2-N3	45Gy/25fx +/- 5.4Gy/3fx to 9Gy/5fx to the gross disease	FEC x3 + Docetaxel x9weeks (concurrent to RT)	MRM 5 weeks after chemo	22.6% in the CTRT group (vs 14.9% in the chemo group) p: 0.019)	3y OS: 89% vs 74% (in the chemo group) 3y DFS: 81% vs 69% (in the chemo group)
Haussmann et al., 2022 (27)	356	Retrospective	cT1-cT4, cN0—N+	50Gy/25fx; 10Gy/5fx boost	61% sequential EC/CMF/AC/Mitoxantrone; 36% concurrent chemo; 3% no chemo	BCS or mastectomy	31.1%	10y OS: 69.5%

Ciérvide et al., 2022 (19)	58	Prospective	HER2+ or TN BC; cT1N+ or cT2N+/-	40.5Gy (2.7Gy)	Pertuzumab-Trastuzumab-Paclitaxel+AC in HER2+; CBDCA-Paclitaxel+AC in TNBC	BCS or MRM	TN: 70.8% HER2+: 53.1% HR+: 47.6% HR-: 63.6%	DFS: 100%
Vincent et al., 2022 (POP-ART) (21)	20	Prospective	cT1-T3, N0-N1, eligible for BCS	28.5Gy/5fx WBI+ 31Gy/5fx SIB to tumor bed (intervention group)	ECx4 + Paclitaxelx12 +/- Carboplatinum +/-Trastuzumab for HER2+	BCS (90%)	60%	NR

Abbreviations: MDFS: metastatic disease-free survival; RNI: regional node irradiation; CMF: Cyclophosphamide+Methotrexate+5FU; MRM: modified radical mastectomy; AC: Adriamycin-cyclophosphamide; EC: epirubicin-cyclophosphamide; FEC:5FU-epirubicin-cyclophosphamide; CBDCA: carboplatin; TNBC: triple-negative breast cancer

HIGH-RISK BC: Preoperative RT to Allow BCS Or Facilitate Breast Reconstruction

Preoperative RT And PST to Facilitate Breast Reconstruction After Mastectomy

The optimal sequence for mastectomy combined with immediate breast reconstruction (IBR) and RT in treating LABC is actually an object of debate. While most guidelines typically endorse administering RT before reconstruction, this approach involves two separate surgical procedures, impacting postoperative healing and resulting in a subsequent delay in the reconstructive phase. Furthermore, the incidence of postoperative complications tends to increase when RT follows mastectomy (33) .

In this setting, neoadjuvant RT aims to enhance aesthetic outcomes and optimise the reconstructive process, especially in patients eligible for skin-sparing mastectomy (SSM) and autologous reconstruction. Additionally, opting for immediate post-mastectomy reconstruction holds the potential to improve patient satisfaction and overall quality of life.

Several studies have already demonstrated the safety and feasibility of this novel approach, particularly concerning morbidity in IBR and its influence on disease free survival (DFS) and OS (34–36).

Zinzindohoué et al. showed the feasibility of utilizing the latissimus dorsi flap technique in SSM–IBR following neoadjuvant chemotherapy (NAC) and RT. This approach showed an acceptable necrosis rate within a carefully selected patient cohort. Reconstruction was successfully achieved in all cases, emphasizing the efficacy of this strategy. By mitigating potential complications linked to adjuvant irradiation, this strategic sequence offers an additional viable option for IBR, especially in scenarios where the standard approach to reconstruction is not feasible.

Recent findings from the PRADA study have demonstrated low incidences of complications such as open wounds, mastectomy skin necrosis, fat necrosis, and unplanned returns to the operating theatre. Notably, there were no reported failures of DIEP flap procedures (37).

Table 5 reports more details regarding studies on this setting.

Table 5. Studies about preoperative RT and PST to facilitate breast reconstruction after mastectomy.

Author, year of publication	Number of patients	Type of study	Tumor characteristics	Preoperative RT	Primary Systemic Treatment (PST)	Surgery	pCR (%)	Outcome
Monrigal et al., 2011 (34)	210	Retrospective	BC with IBR	50Gy (2Gy); 10Gy (2Gy) boost	Concurrent Antracyclin-based chemo+/- Taxanes+/- Trastuzumab	6-8 weeks after RT, IBR	35.2%	5y OS: 86.7%; 5y DFS: 75.6%
Ho et al., 2012 (35)	30	Retrospective	LABC	60%: 50Gy (2Gy) 40%: 42.5Gy (2.67Gy); boost	Sequential: AC; CMF; FEC; A-TAX	SSM and immediate autologous breast reconstruction	NR	5y OS: 68%; 5y DFS: 65%
Paillocher et al., 2016 (38)	111	Retrospective	BC with IBR	50Gy (2Gy)	FEC+Taxotere	SSM	NR	5y OS: 98.3%; 5y DFS: 93.2%
Zinzindohoué et al., 2016 (39)	83	Prospective	BC with IBR	50Gy (2Gy)	Sequential: Antthracyclines+taxanes	SSM+IBR 6-8 weeks after RT	36%	5y DFS: 68%
Pazos et al., 2017 (36)	22	Retrospective	LABC	50.5Gy (1.8Gy)	ECx4 +Paclitaxelx12	SSM + IBR	55%	2y OS: 89.3%; 2y DFS: 79.8%; LRFS: 95.2%

Thiruchelvam et al., 2022 (PRADA) (37)	33	Prospective	LABC, suitable for DIEP flap reconstruction	40Gy/15fx or 42.72Gy/16fx	91% of patients	SSM+DIEP flap reconstruction 2-6 weeks after RT	21%	OS: 93.9%; DFS: 84.8%

Abbreviations: AC: Adriamycin-cyclophosphamide; A-TAX: Adriamycin-paclitaxel; CMF: cyclophosphamide-methotrexate-5FU; DIEP: deep inferior epigastric perforator; FEC: SSM: skin-sparing mastectomy; LABC: locally advanced breast cancer; LRFS: local-recurrence-free survival

Preoperative RT (With PST) In Unresectable LABC

The combination of preoperative RT and PST has demonstrated a significant increase in achieving complete remission rates compared to NAC alone. Specifically, when cytotoxic drugs are combined with RT in a preoperative setting, the rate of histopathological complete response significantly elevates (42% versus 3%). This suggests a potential synergy between chemotherapy and RT, improving treatment outcomes in the neoadjuvant setting (40).

Moreover, various studies have highlighted the feasibility, tolerability, and efficacy of combining RT with capecitabine as a second-line neoadjuvant treatment for patients with LABC refractory to initial anthracycline-based therapy (41,42).

In a retrospective study, Roth et al. (43) revealed a statistically significant improvement of 13.6% in 10-year OS when employing neoadjuvant RT combined with PST for patients with cT1 and cT2 tumors, reaching 85.76%. This was in comparison to 72.04% of patients with cT1 and cT2 tumors who received adjuvant treatment ($p = 0.0026$). Additionally, neoadjuvant PST resulted in a remarkable 29.2% pCR rate and significantly improved recurrence-free survival (RFS) and OS rates in patients with cT2-category BC.

Pathological complete response has emerged as a robust prognostic indicator for enhanced long-term survival (44). Furthermore, Matuschek et al. (45), observed that an extended time interval to surgery (>2 months) increases the probability of pCR after preoperative RT and chemotherapy.

Research has consistently shown that the combination of preoperative chemoradiotherapy improves the rate of pathological complete responses (46).

Even RT alone has been shown to facilitate BCS for tumors larger than 3 cm (47), and thus preoperative RT may represent an effective treatment to downsize LABC tumors with a minimal or no response to chemotherapy, enabling subsequent surgical resection and potentially improving OS (48).

Additionally, stereotactic body radiotherapy (SBRT) can safely be combined with preoperative chemotherapy to obtain a tumor downsizing and allow for BCS, as showed in Bondiau et al. (49), where the breast-conserving rate reached 92% with promising results also in terms of pCR rate (36%).

A summary of clinical studies on this topic is summarized in Table 6.

Table 6. Preoperative RT (with/without PST) in unresectable LABC.

Author, year of publication	Number of patients	Type of study	Tumor characteristics	Preoperative RT	Primary Systemic treatment (PST)	Surgery	pCR (%)	Outcome
Calitchi et al., 2001 (47)	75	Retrospective	Unresectable LABC	45Gy (1.8Gy); 15Gy boost to internal mammary nodes	None	100% BCS	11%	NR
Gerlach et al., 2003 (40)	198	Retrospective	<77years; T diameter>3cm or <3cm in case of an unfavourable ratio of tumor/breast volume or anatomic difficulties so that an initial breast-preserving approach was impossible	50Gy(2Gy); 6-11Gy boost	64 received only chemo; 134 sequential RTCT: ECx4+CMFx3	4-24 weeks for the NAC group 3-38 weeks for RTCT group	3% (NAC group) 42% (RTCT group)	NR
Gaui et al., 2007 (41)	28	Retrospective	Unresectable LABC refractory to first-line anthracycline-based treatment	50Gy	Capecitabine	82% of patients	4.3%	NR
Roth et al., 2010 (43)	315	Retrospective	LABC	50 Gy (2Gy)	53%: ECx4; 35.6%: Mitoxantrone; 6.7%: ACx4; 3.2%: no chemo; 0.3%: CMFx3;	50.8%: BCS	36.8% in breast (ypT0); 56% in axilla (ypN0);	10y OS: 68.59%

					0.3%: CMFx6; 0.3%: ECx6		29.2% in both (ypT0ypN0)	10y RFS: 67.95%
Matuschek et al., 2012 (45)	315	Retrospective	LABC	50Gy (2Gy); 10Gy HDR boost+/- hyperthermia	53%: ECx4; 35.6%: Mitoxantrone; 6.6%: ACx4; 0.6%CMFx3; 0.6%: CMFx6; 0.6%: ECx6	50.8%: BCS	29.2% (ypT0 ypN0)	NR
Bondiau et al., 2013 (49)	26	Prospective	Unifocal BC not suitable for BCS	Robotic SBRT: 19.5-31.5Gy/3fx	Docetaxel+FEC	Breast-conserving rate: 92%; surgery 4-8 weeks after the last chemo cycle	36%	NR
Coelho et al., 2017 (48)	57	Retrospective	Unresectable LABC	50Gy (2Gy)	98.2% chemo regimen containing anthracyclines; 26.3% taxanes+anthracyclines; 1.8% docetaxel+cyclophosphamide; 5% trastuzumab	75.4%: mastectomy	0%	5y OS: 36.4%; 5y DFS: 35.1% *vs* 5y OS: 9.7% in patients not eligible for surgery

Woodward et al., 2017 (42)	32	Prospective	Unresectable LABC in progression after PST	50-57Gy Boost (60-66Gy to gross disease<1cm; up to 72Gy to gross disease>1cm)	Capecitabine	75% of patients converted to operable; mastectomy 3-6 weeks after RT	73%	1y actuarial OS: 54%
Loap et al., 2020 (RADIOPARP) (50)	24	Prospective	Unresectable TNBC	50Gy/25fx	Olaparib	87.5% underwent surgery and adjuvant Olaparib; 12.5% unresectable	0%	NR

Abbreviations: AC: Adriamycin-cyclophosphamide; BC: breast cancer; CMF: cyclophosphamide-methotrexate-5FU; EC: Epirubicin-cyclophosphamide; FEC: 5Fluorouracil-epirubicin-cyclophosphamide; LABC: locally advanced breast cancer; RFS: relapse-free survival; RTCT: radiochemotherapy.

Future Perspectives

Several trials on preoperative RT are ongoing, and their number is constantly increasing: NeoAPBI01, NEORAD, Neo-CheckRay (NCT03875573), P-RAD, CBCV, BreastVAX, IBISCO, KORTUC, and others (PRADA-2, ABLATIVE-2).

Currently, various ongoing studies are examining the potential synergies of combining immunotherapies with different preoperative approaches. One study (NCT03366844) primarily investigates the viability of preoperative pembrolizumab associated with RT and its impact on Stromal Tumor-infiltrating lymphocytes (TILs) for TN and high-risk HR+, HER2- BC. Another study (NCT04443348) focuses on determining the optimal preoperative RT dose to the breast when combined with pembrolizumab and chemotherapy. Additionally, this study explores different boost doses (low-9 Gy and high-24 Gy) and their effects. Conversely, an ongoing trial (NCT04454528) evaluates the efficacy of preoperative single-dose RT in combination with pembrolizumab alone, investigating different timing strategies (pembrolizumab alone and before or after RT). In a separate ongoing study (NCT04807192), researchers are exploring preoperative SBRT as a standalone treatment or in combination with CMP-001, a toll-like receptor 9 agonist, to evaluate the increase in TILs within the tumor microenvironment (TME) (51).

Breast Cancer Immunogenicity

The interaction between RT and the immune system is mediated by two main effects: the bystander effect and the abscopal effect. The first one is related to the interaction of tumor cells directly damaged by RT and the cells next to them, while the second one is associated with the immunogenic tumor cell death induced by the former (rather than by the cytocide effect of RT), which determines the release of tumor-associated antigens and the subsequent activation of the host immune system. This immune modulation can elicit a local effect on the irradiated tumor but is basically a systemic effect, thus could theoretically be effective against distant lesions (regional LNs, metastases) (52).

However strong evidence about this interaction is lacking, and this is probably related to some setting pitfalls (53):

- Conventional RT fractionation is not useful for immunostimulation, since the balance of all the interactions with the host immune system seems to be in favour of an immunosuppressive net effect
- Irradiation of regional LNs could lead to immunosuppression as well
- The cellular alterations elicited by RT are temporally dynamic, suggesting that the treatment schedule is a major determinant of the immunomodulation effect of RT

Moreover, it seems difficult to obtain a clinically relevant immunomodulation effect with an RT treatment alone, so the combination with systemic treatment (particularly immunotherapy drugs) seems to be an essential step to enhance an immunologic response to the tumor.

All these aspects prevented this setting from being studied in BC, given that the RT for this tumor is historically post-operative and BC itself is a “cold” (i.e., “non-immunogenic”) cancer.

However, different subtypes of BC have different immunosuppressive or immune-activating behaviours (54). This is related to some intrinsic features such as the genomically upregulated pathways, the tumor mutational board, the expression of PD-L1, the downregulation of MHC class I. These differences determine a gradient from HR+ to TN tumors in their capability of immunostimulating the host system, with HER2+ tumor having intermediate behaviour. Therefore, the TME is different when comparing HR+, HER2+ and TN tumor, having HR+ is an immunotolerant TME characterized mainly by immunosuppressive tumor-associated macrophages, decreased T cell infiltration and non-activated cancer-associated fibroblasts. In HER2+ BC, there are increased TILs proportion compared to HR+ BC as well as increased recruitment of NK cells and, in TN, a higher level of TILs compared to both HR+ and HER2+ BC is often found, along with tertiary lymphoid structures and different types of cancer-associated fibroblasts, possibly leading to an immune stimulating T cell balance in the TME.

These differences explain why finding a unique approach for enhancing immune response in BC is difficult and why immunotherapy has been of limited interest for years. Nowadays, a lot of research is being carry on this topic, but the majority of trials are still phase I-II studies in the metastatic setting, more frequently in TN BC, and the most studied drugs are the immune checkpoint inhibitors, usually

combined with chemotherapy. Less than 10% of all ongoing trials evaluate the use of RT as an immunomodulating strategy (55,56).

Of note, in this scenario, preoperative RT is the setting much more relevant, since it allows irradiation of the tumor on-site, it allows the use of RT doses very different from the standard for WBI, specifically those proven in preclinical trials to have the highest immune activating effect (few fractions, doses up to 10 Gy/fr).

Background For IBISCO Trial Development

The most recent evidence in aggressive BC subtypes shows that pCR after PST is a predictor of disease outcomes and survival at a patient level.
Cortazar and colleagues in 2014 (44) evaluated the results of 11 trials including more than 11000 patients, demonstrating that pCR was higher in patients with TN and HER2+ BC treated with trastuzumab (33.6% and 50.3%, respectively), and lower in Luminal BC, although the frequency was more than doubled in high-grade HR+ subgroup compared to low-grade HR+ (16.2% versus 7.5%).

The pCR rate was associated with overall better event free survival (EFS) and OS. More in detail, pCR was positively associated with EFS (HR 0.49) and OS (HR 0.43) in the overall HR+ population, with a higher correlation in the high-risk group, both for EFS (HR 0.27) and OS (HR 0.29).
In TN and HER2+ tumors, the positive association between long-term outcome and pCR was even stronger: for EFS, HR was 0.24 and 0.15, and for OS HR was 0.16 and 0.08 in TN and HER2+ BC respectively (44).

Haque and colleagues (57) as well, in 2018, analyzed the data from the National Cancer Database and found that molecular subtype was a powerful independent predictor of pCR after NAC, with Luminal A BC achieving 0.3% pCR rates and Luminal B, TN and HER2+ achieving 8.3%, 23.2% and 38.7% pCR rates, respectively. In Luminal B BC patients, 5-year OS was 81.1% in patients achieving any clinical response, but it increased to 93.0% in patients achieving pCR. These results were similar to those obtained for HER2+ and TN disease, where 5-year OS for any clinical response were 84.0% and 73.3%, respectively and 5-year OS after pCR were 94.2% and 90.6%, respectively.
The differences between Cortazar and colleagues and Haque and colleagues on pCR rates can be related to the definition of pCR (defined as ypT0 or ypTis in the first and ypT0 in the second) and, namely for Luminal BC patients also for the inclusion of intermediate–grade disease in Luminal A or Luminal B group, respectively.

Given the clinically relevant results in HER+ and TN tumors, in the last years, PST has become the standard for most TN and HER2 positive BC, changing the treatment paradigm for these subtypes. In fact, pCR is used nowadays as a prognostic and predictive biomarker for these BC subtypes, and pathological response to PST (pCR versus non-pCR) is also used to tailor adjuvant treatments. (58–61)

Since pCR after NAC is still difficult to obtain in Luminal B BC compared with more aggressive subtypes (28), new strategies development is needed before changing the approach in this tumor subtype.

While RT is a well-established approach for enhancing pathological response and is routinely employed in certain contexts like rectal cancer, it hasn't become the standard for BC despite the existence of various documented experiences. (46)

The effect of RT on promoting a pathological response extends beyond its cytotoxic effects. In specific contexts, it can be utilized to enhance the host immune system's response (62), especially when combined with concurrent PST.

Systemic treatments, RT, and their synergic combination have the potential to activate the immune system. In this contest, an immune response targeting a broader spectrum of tumor antigens may be more effective against a heterogeneous cell population (63,64).

Therefore, neoadjuvant RT represents a possibility to increase pCR and immune response in Luminal B BC, and its effect on the tumor-associated inflammatory microenvironment depends on dose and fractionation. Available data (65,66) suggest that fractionated schemes such as 3x8 Gy are the most effective for determining it, and some experience on these schedules is already available (49)

The IBISCO trial aims to improve pCR in Luminal B BC by enhancing host immune response, using anticipated SBRT boost associated with NAC. The choice of focusing on the boost only and not going for WBI anticipation is related to the possibility of using the most effective dose for tumor-associated inflammatory microenvironment enhancement as a boost, whereas it would not have been possible in the context of WBI.
Moreover, data on the safety of this approach and the feasibility of subsequent surgery are reassuring (14,49,67), given both the relatively low dose delivered and the smaller volume when boost is defined with the tumor on-site (68).

Study Design and Population

IBISCO Trial is a phase II, monocentric, non-randomized trial.

Eligible patients are women with Luminal B-like (defined as estrogen receptor-positive, high-grade tumors, expressing high proliferative index at Immunohistochemical (IHC) evaluation) HER2 negative BC who will be addressed to preoperative SBRT boost associated with standard NAC.

The study also includes an observational cohort with patients fulfilling inclusion criteria who refuse enrollment in the interventional cohort and for patients where an SBRT boost appears not feasible after enrollment for technical issues.

The planned enrollment time is 24 months, and the sample size is 30 patients for the experimental cohort and 20 for the observational cohort.

Main inclusion criteria

The inclusion criteria for the interventional cohort are:

- female patients with a histological diagnosis of Luminal B-like, HER2 negative BC
- BC TNM stage: cT1-2, any N, M0
- indication for NAC after multidisciplinary team discussion
- adequate pre-operative biopsy defining tumor grade, HR and HER2 status, and Ki67 proliferative index.

Patients who meet the requirements for inclusion but decline to participate in the interventional group are asked for consent to participate in the observational cohort, as well as patients not suitable for treatment with SBRT boost for technical reasons (i.e., unfavourable anatomy or failure to identify the neoplasm in the planning computed tomography (CT) scan, inability to perform deep inspiration breath hold (DIBH), etc.).

Main exclusion criteria

The main exclusion criteria are prior RT to the ipsilateral breast, deteriorated clinical conditions, TNM stage cT3-4 or M1, pregnancy and lactation.

Objectives And Endpoints

Primary Objective

The study aims to demonstrate that adding an SBRT anticipated boost to NAC in patients with Luminal B BC can improve pCR rates. The study is powered to demonstrate an increase from 15% (as reported in the literature) to 35% (33,49).

The pathological response to neoadjuvant treatment is defined as a pCR (absence of invasive residual tumor in the surgical sample) or as a partial pathological response according to Residual Cancer Burden (RCB) (69) and the American Joint Committee on Cancer post-neoadjuvant staging (yAJCC) (70)

RCB index classifies the pathological response into:

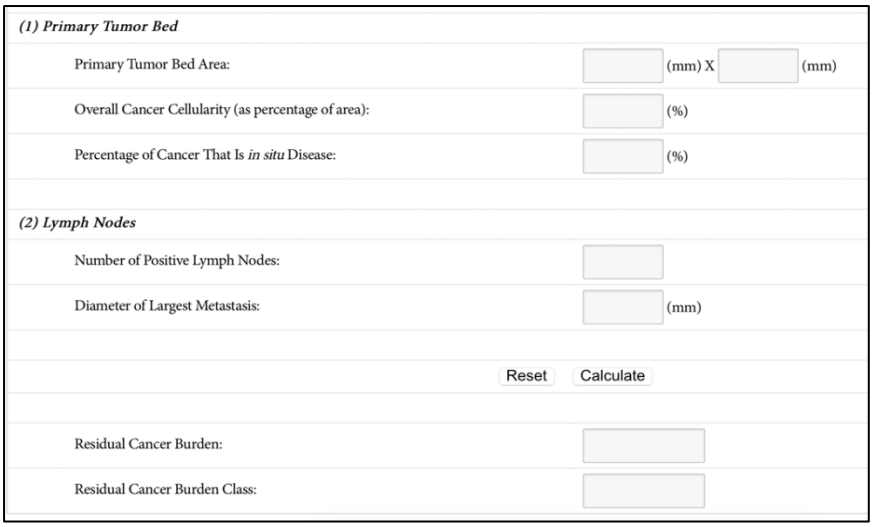


Figure 2 RCB Online tool calculator (Residual Cancer Burden Calculator, no date)

- RCB-0 (pCR)
- RCB-I (minimal burden)
- RCB-II (moderate burden)
- RCB-III (extensive burden)

RCB is estimated from routine pathologic sections of the primary breast tumor site and the regional LNs after the completion of neoadjuvant therapy. A calculation formula includes six variables (d1, d2, %CA, %CIS, LN, dmet) (Figure 2). The evaluation of a primary tumor bed in general terms requires that the pathologist make three judgments about the primary tumor bed:

1) identify the cross-sectional dimensions of the residual tumor bed (d1 and d2)
2) estimate of the proportion of that residual tumor bed area that is involved by cancer (%CA)
3) Estimate the proportion of the cancer that is in situ component (%CIS)

The pathological evaluation of Regional Lymph Nodes includes the number of positive lymph nodes (*LN)* and the measure of the diameter of the largest nodal metastasis (*dmet*).

Figure 3 shows the results of a multicentric pooled analysis carried out by MD Anderson Cancer Center, confirming the strong prognostic value of the RCB index (71).

Breast Cancer Subtype	Pathologic Complete Response	RCB-I	RCB-II	RCB-III
Triple-Negative				
5 yr	91%	80%	66%	28%
10 yr	86%	75%	61%	25%
HR–/HER2+				
5 yr	94%	85%	63%	60%
10 yr	93%	85%	63%	60%
HR+/HER2+				
5 yr	94%	91%	76%	54%
10 yr	91%	83%	64%	45%
HR+/HER2–				
5 yr	88%	91%	80%	71%
10 yr	81%	86%	69%	52%

HR = hormone receptor; RCB = residual cancer burden.

Figure 3 Event-Free Survival at 5 and 10 years (Residual Cancer Burden is Prognostic of Outcomes Across Breast Cancer Subtypes - The ASCO Post, no date)

Secondary Objectives

1) Toxicity

Firstly, acute and late toxicity associated with SBRT boost are evaluated using the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) v.5 scale, which assigns scores ranging from 1 to 5 (72).

What can be expected is some mild acute toxicity after the SBRT boost but an overall decrease in late toxicity compared to standard BC RT treatment due to the surgical removal of the breast tissue that received the higher boost RT dose.

The main expected acute and late toxicities, usually presenting with a severity ≤ grade 2, are summarized in

Table 7 and Table 8, respectively (grade 4 and 5 definitions not reported).

Table 7. Expected acute toxicity.

CTCAE Term	Grade 1	Grade 2	Grade 3
Fatigue	Relieved by rest	Fatigue not relieved by rest; limiting instrumental ADL	Fatigue not relieved by rest; limiting self-care ADL.
Localized oedema	Localized to dependent areas, no disability or functional impairment	Moderate localized edema and intervention indicated; limiting instrumental ADL	Severe localized edema and intervention indicated; limiting self-care ADL
Skin hyperpigmentation	Hyperpigmentation covering <10% BSA; no psychosocial	Hyperpigmentation covering >10% BSA; associated psychosocial impact	
Breast pain	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self-care ADL
Dermatitis radiation	Faint erythema or dry desquamation	Moderate to brisk erythema; patchy moist desquamation, mostly confined to skin folds and creases; moderate edema	Moist desquamation in areas other than skin folds and creases; bleeding induced by minor trauma or abrasion
Radiation recall reaction (dermatologic)	Faint erythema or dry desquamation	Moderate to brisk erythema; patchy moist desquamation, mostly confined to skin folds and creases; moderate edema	Moist desquamation in areas other than skin folds and creases; bleeding induced by minor trauma or abrasion

Table 8. Expected late toxicity.

CTCAE Term	Grade 1	Grade 2	Grade 3
Telangiectasia	Telangiectasias covering <10% BSA	Telangiectasias covering >=10% BSA; associated with psychosocial impact	
Skin induration	Mild induration, able to move skin parallel to plane (sliding) and perpendicular to skin (pinching up)	Moderate induration, able to slide skin, unable to pinch skin, limiting instrumental ADL	Severe induration; unable to slide or pinch skin; limiting joint or orifice movement (e.g., mouth, anus); limiting self-care ADL

2) Cosmesis and Quality of Life (QoL)

Photographs and questionnaires assessing QoL and cosmesis are collected at multiple time points (see Table 13 for a detailed evaluation timetable) to evaluate the effect of the experimental treatment. Breast objective appearance evaluated with photographs can be compared with toxicities registered and patients' reported satisfaction for cosmetic results.

The evaluation of QoL is crucial to determine the feasibility of SBRT boost without significant impact on the patient's overall well-being. Additionally, it explores the possibility of potentially enhancing their well-being by reducing toxicity or improving the pCR.

QoL and cosmesis are assessed with three questionnaires: 2 QoL questionnaires (QLQ) from European Organization for Research and Treatment of Cancer (EORTC) and one developed by Memorial Sloan-Kettering Cancer Center.

- **EORTC QLQ-C30** (73)

The QLQ-C30 comprises both multi-item scales and single-item measures (

Table 9).

These include five functional scales, three symptom scales, a global health status/QoL scale, and six single items. Each multi-item scale consists of a different set of items; no item occurs in more than one scale.

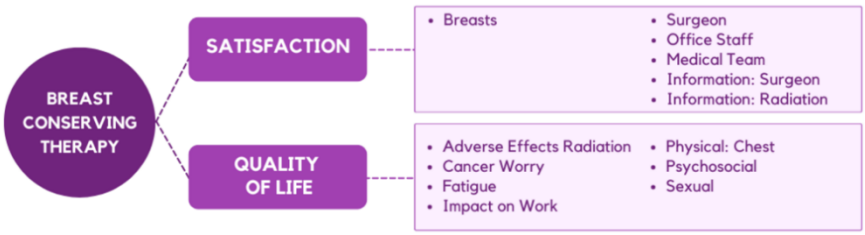
All the scales and single-item measures range in score from 0 to 100. A high scale score represents a higher response level. Thus, a high score for a functional scale represents a high/healthy level of functioning, a high score for the global health status/QoL represents a high QoL, but a high score for a symptom scale/item represents a high level of symptomatology/problems.

- **EORTC QLQ-BR23** (74)

The BC module is a supplementary questionnaire module to be employed with the QLQ-C30. The QLQ-BR23 incorporates five multi-item scales to assess body image, sexual functioning, systemic therapy side effects, breast, and arm symptoms. In addition, single items consider sexual enjoyment, future perspective and being upset by hair loss (Table 10). The scores have the same interpretation used in QLQ-C30.

- **Q-BREAST v.2** (75)

The BREAST-Q is a modular patient-reported outcome measure (PROM) for breast surgery published in 2009, following the standards and guidelines drawn from the literature available at that time. The



content validity of the BREAST-Q was well supported by extensive evidence from qualitative studies. The BREAST-Q conceptual framework covers two domains (Figure 4): quality of life and patient satisfaction. It is composed of multiple independently functioning scales. The variety of scales provides flexibility to choose the subset best suited to measure the outcomes of interest in any study situation.

Figure 4. BREAST-Q framework (76)

Table 9. QLQ-C30 scale components

Domains	Name of the scale	Number of items
Global Health Status/QoL	Global Health Status/QoL	2
Functional scales	Physical functioning	5
	Role functioning	2
	Emotional functioning	4
	Cognitive functioning	2
	Social functioning	2
Symptom scales/items	Fatigue	3
	Nausea and vomiting	2
	Pain	2
	Dyspnoea	1
	Insomnia	1
	Appetite loss	1
	Constipation	1
	Diarrhoea	1
	Financial difficulties	1

Table 10. QLQ-BR23 scale components

Domains	Name of the scale	Number of items
Symptom scales/items	Systemic therapy side effects	7
	Upset by hair loss	1
	Arm symptoms	3
	Breast symptoms	4
Functional scales/items	Body image	4
	Future perspective	1
	Sexual functioning	2
	Sexual enjoyment	1

3) Immune modulation effect

Finally, an exploratory analysis of tumor-associated inflammatory microenvironment modifications following SBRT is conducted by examining biopsies, surgical specimens, and peripheral blood samples. The IHC and molecular qualitative evaluation include markers to define the main inflammatory components in the TME (CD20, CD3, CD8, PD1, PD-L1, CD68, etc.).

These data will be correlated to a Next-Generation Sequencing (NGS) panel used to evaluate pathogenic mutations of target genes in BC in both tumor tissue and plasma from liquid biopsy.

These integrated analyses are conducted on the pre-treatment biopsy, on the biopsy obtained after SBRT and the last administration of taxane, and on the definitive surgical specimen.

Sample Size and Statistical Analysis

The sample size is determined by considering the annual count of patients meeting the inclusion criteria discussed in the multidisciplinary board and considering a pCR rate to standard therapies in this setting of 15% (77) versus an expected result with the addition of the SBRT boost of 35%, consistent with the results of Bondiau et al. (49).

The statistical analysis will include:

- descriptive assessment of sample and treatment characteristics.
- Calculation of the pCR and partial pathological response rates, compared with the literature-reported rate after standard NAC. Although there will not be a direct comparison with the observational cohort, given the non-randomized nature of the study and the difference in sample size, data on pathological response rates will be collected and reported for this cohort as well.
- Acute and late RT-related toxicities will be reported in the SBRT boost group and the observational cohort as incidence rates and compared (with the limitation reported above)
- IHC, molecular, and gene analyses related to the immune microenvironment have an exploratory purpose and, therefore, they will be reported descriptively for both the experimental treatment group and the observational cohort.

Treatments, Visits and Evaluations

Treatments

1) Experimental and preoperative treatments

Patients enrolled in the study are treated with an anticipated SBRT boost associated with standard NAC. The staging procedures and exams are the same as for the standard of care and are reported in Table 13.

Within two weeks from enrollment, patients start NAC. As for clinical practice, the scheme is weekly Paclitaxel at a dose of 80 mg/m^2 for 12 weeks, followed by Epirubicin 90 mg/m^2-Cyclophosphamide 600 mg/m^2 (EC).
EC is administered every three weeks for four cycles or every two weeks for four cycles (dose-dense scheme) associated with granulocyte colony-stimulating factor (G-CSF), depending on the patient's clinical conditions.

After informed consent is released, the pathologist re-evaluates the biopsy sample for study-specific analysis. In all patients, marker clips are placed in the tumor before the therapies start to facilitate tumor bed localization during the surgical procedures after NAC and to perform image-guided SBRT treatment. Patients will be referred to the Radiotherapy Unit for executing the contrast-enhanced planning CT scan, with 2mm slide thickness, and subsequent treatment planning, which will be performed with a DIBH.

Lungs, heart, ipsilateral and contralateral breast, spinal canal, skin (defined as a 5 mm layer from the external body surface) and chest wall (10 mm from the lung, including ribs and intercostal muscles) are contoured as organs at risk (OARs), as per clinical practice (Table 11).
The gross tumor volume (GTV) is outlined on CT, considering all the available imaging exams, defining all included clips, and excluding positive LNs. The clinical target volume (CTV) includes the GTV and a 2-mm margin, limited to the chest wall and skin structures. The planning target volume (PTV) is obtained from CTV plus 5-mm expansion.
The PTV/ipsilateral breast volume ratio should be < 30%. Otherwise, the patient cannot be considered eligible for the experimental treatment.

Table 11. Structures definition and contouring indication.

Structures	Definition and contouring
Breast (ipsilateral and contralateral)	Cranial: Upper border of palpable/visible breast tissue Caudal: Most caudal CT slice with visible breast. Ventral: 5 mm under the skin surface Dorsal: Major pectoral muscle or chest wall Medial: Lateral to the medial perforating mammalian vessels Lateral: Anterior to the lateral thoracic artery
Heart	Contour the heart along with the pericardial sac. Cranial edge: at the bifurcation of the pulmonary trunk and right pulmonary artery Caudal edge: apex of the heart
Lung (right and left)	Limit the contour to the air-inflated lung parenchyma without the inclusion of any fluid visible on CT; exclude the proximal bronchial tree. Do not include the trachea/bronchus
Spinal canal	Consider the volume according to the inner limits of the spinal canal using bone windows
BODY	External body surface
Skin_5mm	Ring structure extending for 5 mm inside the BODY
Skin_3mm	Ring structure extending for 3 mm inside the BODY (dosimetric aim)
Chest wall	10 mm ring around Lung (should include ribs and thoracic wall muscles)
GTV	Consider all the available imaging exams to define tumor extension. Consider visible clips (except lymph nodes clips) and define the included ones
CTV	GTV+2mm
PTV	CTV+5 mm
CTV_eval and PTV_eval	CTV e PTV limited to Skin_5mm (in very selected cases consider Skin_3mm) and Chest wall

Target optimal coverage is set at 95% of the prescribed dose to 95% of PTV_eval volume, eventually accepting as mandatory objective 95% of the prescribed dose to 95% of CTV_eval volume.
The maximum accepted dose within the CTV is 120% (D120%<2cc), while the maximum accepted dose outside the CTV (and within the PTV) is 107% (D107%<2cc)

Table 12 summarizes the SBRT constraints (78,79,80) adapted for this setting, as reported in similar studies (10,13,49).

Table 12. Constraints for Organs at Risk

Organs at risk & avoidance structures	**Mandatory constraints**	**Optimal constraints**
Skin_5mm	V15Gy<10cc V20<1cc	
Skin_3mm	D1cc< 19.2 Gy	D0.1cc<19.2 Gy
Chest wall	V15Gy<10cc	V10Gy<10cc
Heart	V3Gy<5 cc max dose<5Gy mean<3 Gy	 mean<2Gy
Controlateral_lung	V1Gy<1cc	max dose<1 Gy
Ipsilateral_lung	V5Gy<15cc* V2.5Gy<15%	V5Gy<5cc
Total_lungs	mean<5Gy	mean<3Gy
Spinal_cord	3Gy<1cc 10Gy<0.1cc	
Contralateral_breast	ALARA	<1Gy
Ipsilateral_breast (PTV included)	V12Gy<60% V24Gy<30% V10Gy<200 ml	

Priority is given to adhering to the OAR constraints during planning procedures, with particular attention to the skin to prevent surgical complications. Dosimetric data on the total dose administered to the affected breast and OARs are collected for subsequent WBI planning.
Patients are purposed to participate in the observational cohort If their RT plan does not fulfil the constraints and target dosimetric objectives.

No experimental intervention is performed in patients enrolled in the observational cohort; study-specific analyses on specimens and blood samples are carried out and compared with the interventional cohort.

Radiation treatment is administered between the second and fourth cycle of taxane. During the week of the SBRT treatment, taxane is administered the day after the end of RT (not concomitant to it).
The administered dose is 24 Gy in 3 consecutive daily fractions of 8 Gy.
A tumor and a liquid biopsy with a reassessment of the inflammatory microenvironment and NGS evaluation is repeated at the end of the treatment with the taxane, as shown in Figure 5.

At the end of NAC (approximately six months after enrolment), the patients undergo breast surgery associated with a liquid biopsy. The choice between quadrantectomy versus mastectomy and sentinel LN biopsy versus axillary LN dissection depends on the clinical response to NAC, as for clinical practice.

The surgical specimen is analysed to evaluate the pathological response and for study-specific analysis.
The timeline related to the study is shown in Figure 5.

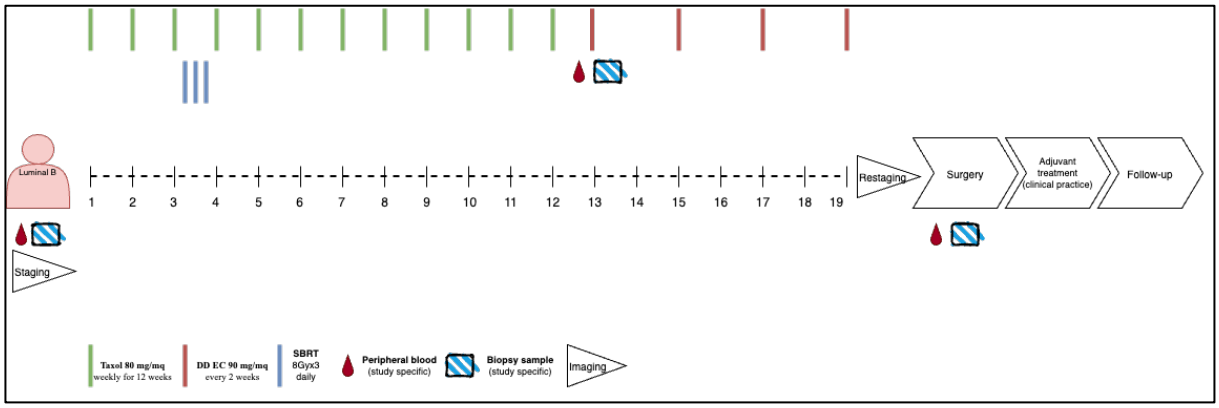


Figure 5. IBISCO trial timeline

2) Post-operative treatments and follow-up

At least one month after surgery, WBI is scheduled, according to clinical practice, at a dose of 40.05 Gy in 15 fractions (or other RT schedules with an equivalent dose in 2 Gy fractions (EQD2)) to the residual breast and the locoregional LN, if clinically indicated. Nonetheless, no boost to the tumor bed is planned, as preoperative SBRT is already considered an anticipated boost. The planning and dosimetric evaluation of WBI considers the previous preoperative treatment characteristics. According to clinical practice, adjuvant endocrine treatment is also prescribed (Table 13).

Adhering to the standard of care, the follow-up regimen includes a physical examination every six months and annual mammograms. During the follow-up period, standard and experimental treatment toxicities are assessed.

Table 13. Diagnostic and therapeutic procedures

	Diagnostic phase	**Pre-treatment evaluation**	**Baseline evaluation**	**Pre-op boost***	**End of taxane**	**End of EC**	**Surgery**	**WBI**	**6-month FU**	**12-month FU**
Mammograms and Ultrasound	X				X	X				X
Diagnostic biopsy	X									
Informed consent			X							
Breast Magnetic resonance		X				X				
Staging imaging (mostly CT-PET)		X								
Blood test			X		X	X	X		X	X
Check for inclusion criteria		X	X							
Pre-operative RT BOOST				X						
Biopsy/surgical specimen evaluation (study-related analysis)			X		X		X			
Liquid biopsy evaluation (study-related analysis)					X		X		X	
QoL, cosmesis and adverse effects assessment			X	X	X	X	X	X	X	

Legend: the blue background is for study-related activities; the white background is for clinical practice

*Between 2° and 4° taxane dose

3) Concomitant treatments

Given the non-pharmacological nature of the study, patients are allowed to use drugs for chronic and pre-existing comorbidities and drugs for intercurrent diseases. Of course, supportive care needed during chemotherapy is allowed.

Visits and Evaluations

Patients' discussion is carried on during the multidisciplinary meeting when data on mammograms, breast ultrasound and diagnostic biopsy are available. After discussing the cases, patients eligible for the trial have the first consultation with the medical and radiation oncologists. During that visit, enrollment in the trial is proposed, and the patients sign the informed consent. All the routine staging exams are planned (breast MR, CT-PET, and other evaluations still missing), and the chemotherapy usually starts within a couple of weeks.
The planning CT is planned shortly after the beginning of chemotherapy, and on the same day, patients can have an additional consultation with the radiation oncologist if needed. The experimental treatment is carried on between the 2° and the 4° taxane administration.

During the SBRT boost administration, patients are evaluated every day by the radiation oncologist to check for any acute toxicity. After the treatment, other visits are scheduled after 1 and 3 weeks to check toxicity and complete QoL and cosmesis questionnaires.

At the end of taxane administration (usually 14 weeks after enrollment), patients are re-evaluated for toxicity, QoL and cosmesis questionnaires, mammograms and breast ultrasounds are performed, and the intermediate biopsy is planned.

Patients are re-assessed at the end of NAC (5 to 6 months after enrollment), with repetition of breast MR, mammograms, breast ultrasound and toxicity, QoL and cosmesis evaluation.
After re-evaluation, the case is discussed again in the multidisciplinary meeting to define surgical procedures and adjuvant treatments.

In the adjuvant phase, another consultation with a radiation oncologist is scheduled for WBI planning.
Toxicity, QoL and cosmesis are evaluated again before WBI and 1 and 6 months after.
The follow-up (FU) visits with physical examination are planned twice a year, and mammograms are repeated annually.

Haematological and urine tests are performed per routine clinical practice during the different stages of treatment and follow-up. An additional blood test tube is collected for liquid biopsy evaluation at the baseline, at the end of taxane and concomitant to definitive surgery.

Results

Patients Characteristics

Patient screening and enrollment started in February 2023. Ten patients (age 35-73) with invasive ductal BC were enrolled in the first year, and their characteristics are summarized in Table 14.

Eight patients had cT2 tumors; the fourth had a cT1c multiple tumors composed of two adjacent lesions and the tenth had a cT1c tumor. Five patients had right breast tumors, of which four were in the upper-outer quadrant. Five patients had left-sided tumors, three of them in the outer quadrant.

Globally, 50% of the lesions were in the upper-outer part of the breast, consistently with the distribution in the general population.

Three out of ten lesions were in unfavorable parts of the breast: one right lower-inner quadrant, one subareolar left region, one upper-inner left quadrant.

All patients had Luminal B-like BC, characterized by Estrogen Receptor (ER) positivity and high proliferative index. Four patients had ER-positive associated with low Progesterone Receptor (PR). Ki67 varied between 30 and 60, and TILs ranged from 5 to 15%. HER2 status was negative in six patients (score 0-1+), while four had a HER2 score of 2+, not amplified at the evaluation with the in-situ hybridization (ISH) technique.

Five patients had a fine needle aspiration resulting in cytologic-proven positive LN (maximum diameter between 10 and 16 mm). All patients were M0 confirmed at staging exams.

Table 14. Patient characteristics.

Patient	**Age**	**Comorbidities and chronic medications**	**Site and breast quadrant**	**Clinical stage**	**Size T (mm)**	**N (number, dimension)**	**Histology and grade**		**Molecular characteristics**						
									ER (%)	PR (%)	Ki67 (%)	p53	HER2	Bcl2	TILs (%)
1	65	Synchronous primitive lung cancer	R, upper-outer quadrant	cT2N0	37x30 (d max); 33 (CC)	0	CDI	G2	100	40	55	wt	2+, ISH not amplified	+	15
2	49	None	R, lower-inner quadrant	cT(m)1N0	27x22, 12x12	0	CDI	G2-3	90	70	60	wt	0	int	10
3	56	None	R, upper-outer quadrant	cT2N1	30x22	1 (16 mm)	CDI	G2	90	2	50	null	1+	+	<10
4	67	None	L, upper-inner quadrant	cT2N0	45x30	0	CDI	G2-3	90	5	30	NR	1+	NR	5
5	50	None	R, upper-outer quadrant	cT2N1	22x12	1 (15mm)	CDI	G1-2	10	5	33%-5%	wt	2+, ISH not amplified	-	10
6	73	Hypertensive heart disease	R, upper-outer quadrant	cT2N1	42x34x32	1 (14mm)	CDI	G2	98	30	50	abn	0	int	5
7	55	None	L, subareolar	cT2N0	29x34x28	0	CDI	G2-3	100	0	40	wt	2+, ISH not amplified	+	10

8	35	None	L, upper-outer quadrant	cT2N0	24x27x26	0	CDI	G2-3	100	70	32	NR	1+	NR	10
9	70	None	L, outer-equatorial quadrant	cT2N1	25x18; 27 (CC)	1 (10mm)	CDI	G2-3	100	90	37	wt	2+, ISH not amplified	int	5
10	73	None	L, outer-equatorial quadrant	cT1cN1	14x10	1 (12mm)	CDI	G3	95	80	32	null	1+	int	NR

Abbreviations: abn: abnormal, CC: craniocaudal extension; CDI: ductal invasive carcinoma; d max: maximum diameter; ISH: in-situ hybridization; int: intermediate; L: left; m: multiple; NR: not reported; R: right; TILs: Tumor-infiltrating lymphocyte; wt: wild type

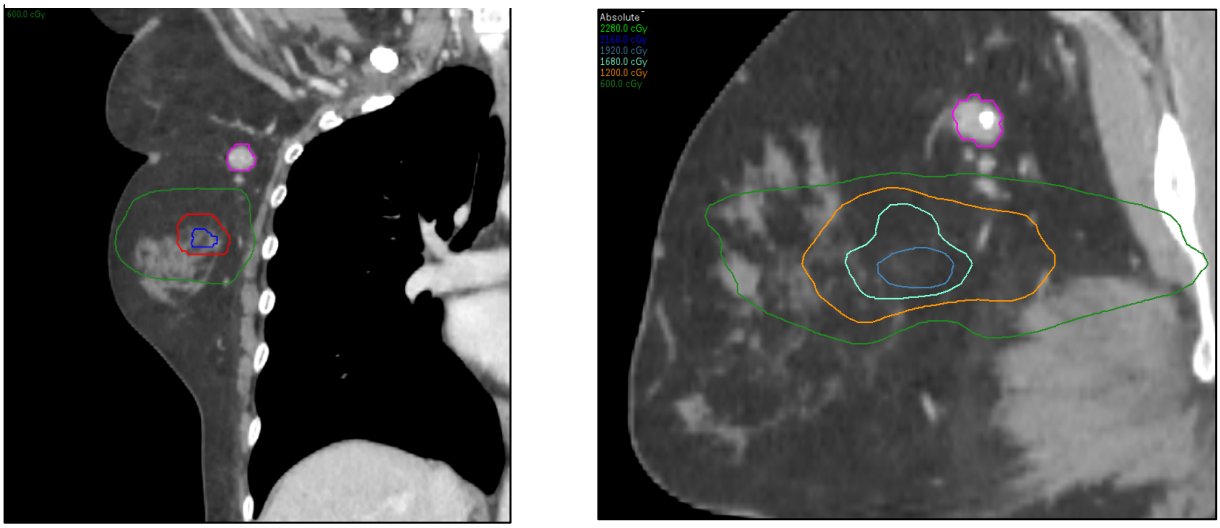
Treatment Characteristics

For all patients, the RT preoperative boost was planned between the third and fourth administration of paclitaxel, approximately one month after the enrollment. The planned and delivered dose was 24 Gy in 3 daily 8 Gy-fractions for all patients, as for protocol. For all patients, a Volumetric Modulated Arc Therapy (VMAT) technique was used.

The GTV volumes ranged between 1.2 and 15.1 cm^3, while the final PTV were between 12.2 and 50.9 cm^3. The PTV/breast ratio varied, of course, with PTV and breast dimensions. However, it was always below 15%. Table 15 shows the details of RT treatments.

In patients with a positive LN, it was delineated on the planning CT to check the received dose and special attention was kept in excluding it from the high dose volumes (Figure 6). This choice was made to ensure that no significant RT effect could be found on it at the definitive LN staging procedure to avoid any possible undertreatment. In all but one case, given the cranial position of the LN compared to the target, no coverage compromises were necessary to achieve this objective.

Figure 6. Coronal (left) and Sagittal (right) view of the LN (in pink) of patient 3. CTV in blue, PTV in red.



In patient 6 it was necessary to conform the treatment further to achieve low doses to the positive LN since it was at a lower level compared to the other four patients (Figure 7).

Figure 7. Treatment conformation and dose distribution in patient 6 to avoid high dose to positive LN (in pink). PTV in red.

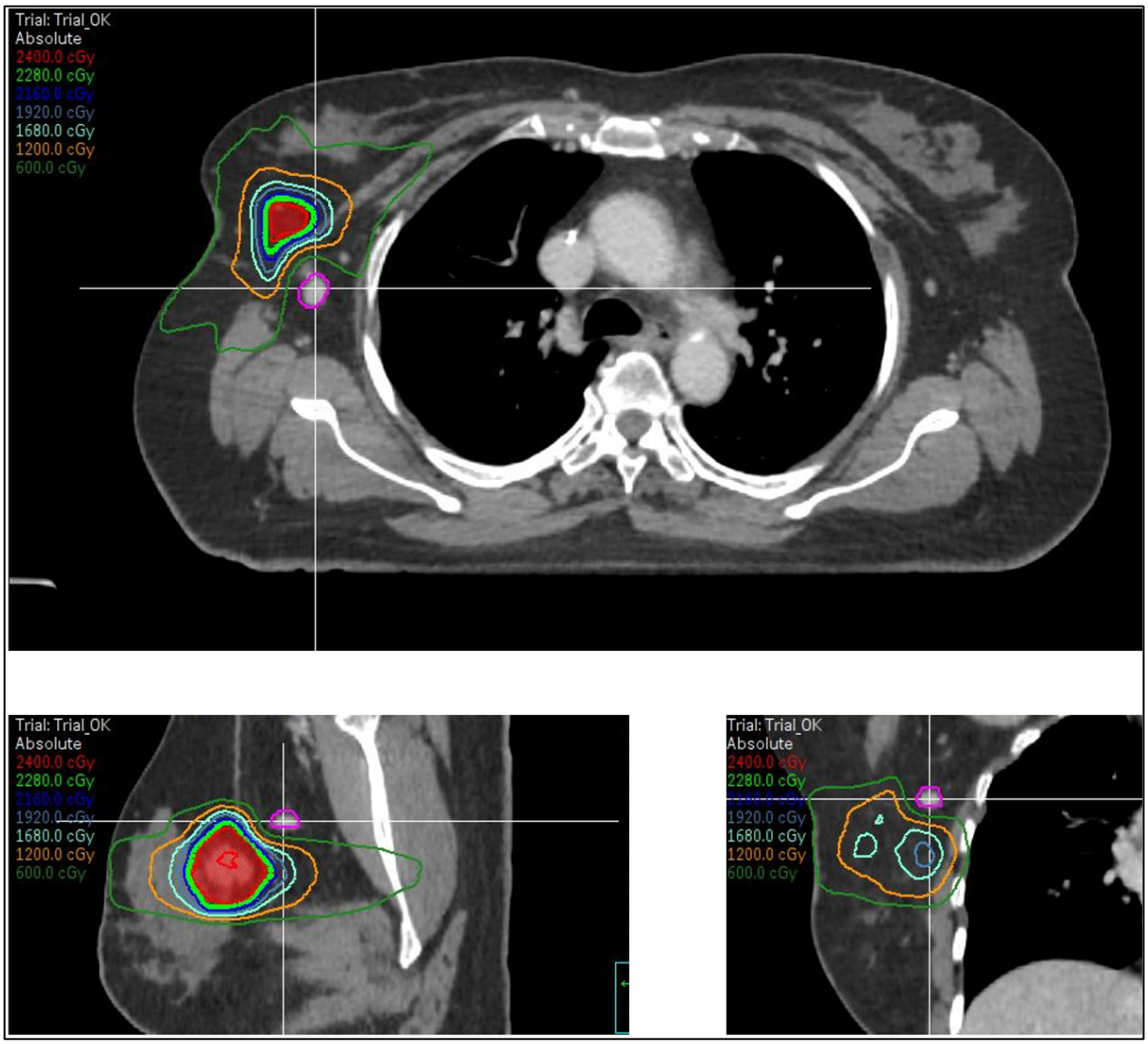


Table 15 summarizes the maximum punctual doses administered to positive LN in patients who had cytologically proven LN metastases.

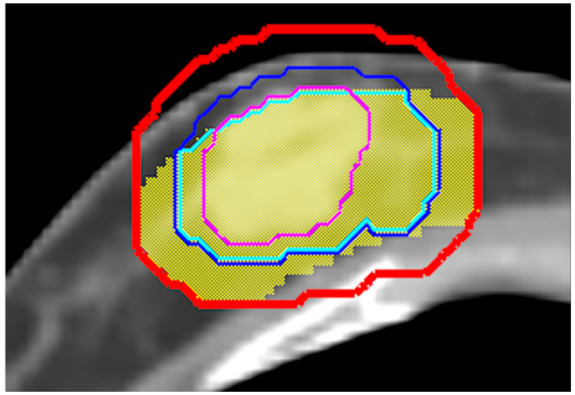
Table 15. Treatment characteristics

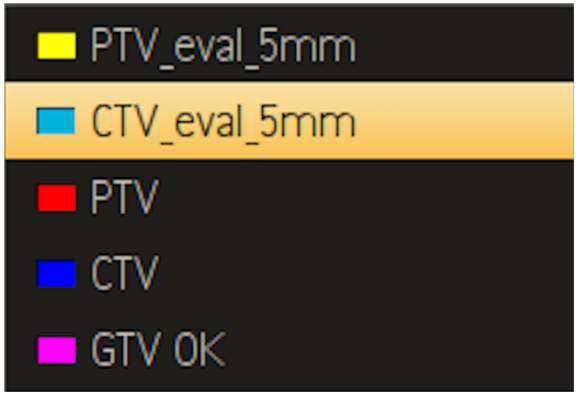
Patient	GTV (cm^3)	CTV (cm^3)	PTV (cm^3)	Ipsilateral breast (cm^3)	Volume Ratio PTV/ipsilateral breast (%)	Max puntctual dose to LN+ (Gy)
1	10.0	18.0	37.4	822.2	4.5	
2	6.8	11.9	21.4	232.5	9.2	
3	9.5	16.7	35.7	1291.6	2.8	0.77
4	15.1	25.6	50.9	934.0	5.4	
5	2.6	6.2	16.2	727.2	2.2	0.14
6	12.8	20.8	40.6	604.7	6.7	2.66
7	9.3	15.2	32.9	814.2	4.0	
8	7.5	13	31.0	212.1	14.6	
9	4.6	9.9	29.8	1353.6	2.2	0.19
10	1.2	3.1	12.2	664.3	1.8	0.05

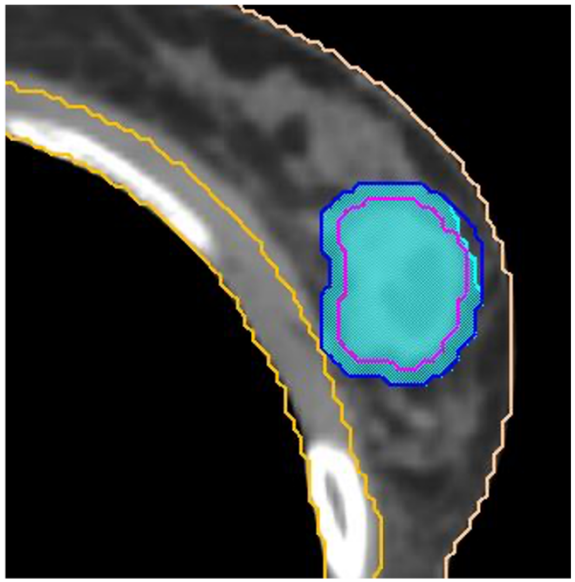
Dosimetric Planning Constraints

The CTV was cropped to avoid skin and chest wall in 4 patients (1, 2, 8, 9), and the new volume was identified as CTV_eval. The most relevant difference was seen in patient 2 (Figure 8-upper image), while in the other three cases, the differences between the two structures were minimal (Figure 8-lower image).

Figure 8. cropped CTV in patients 2 (upper) and 8 (lower)





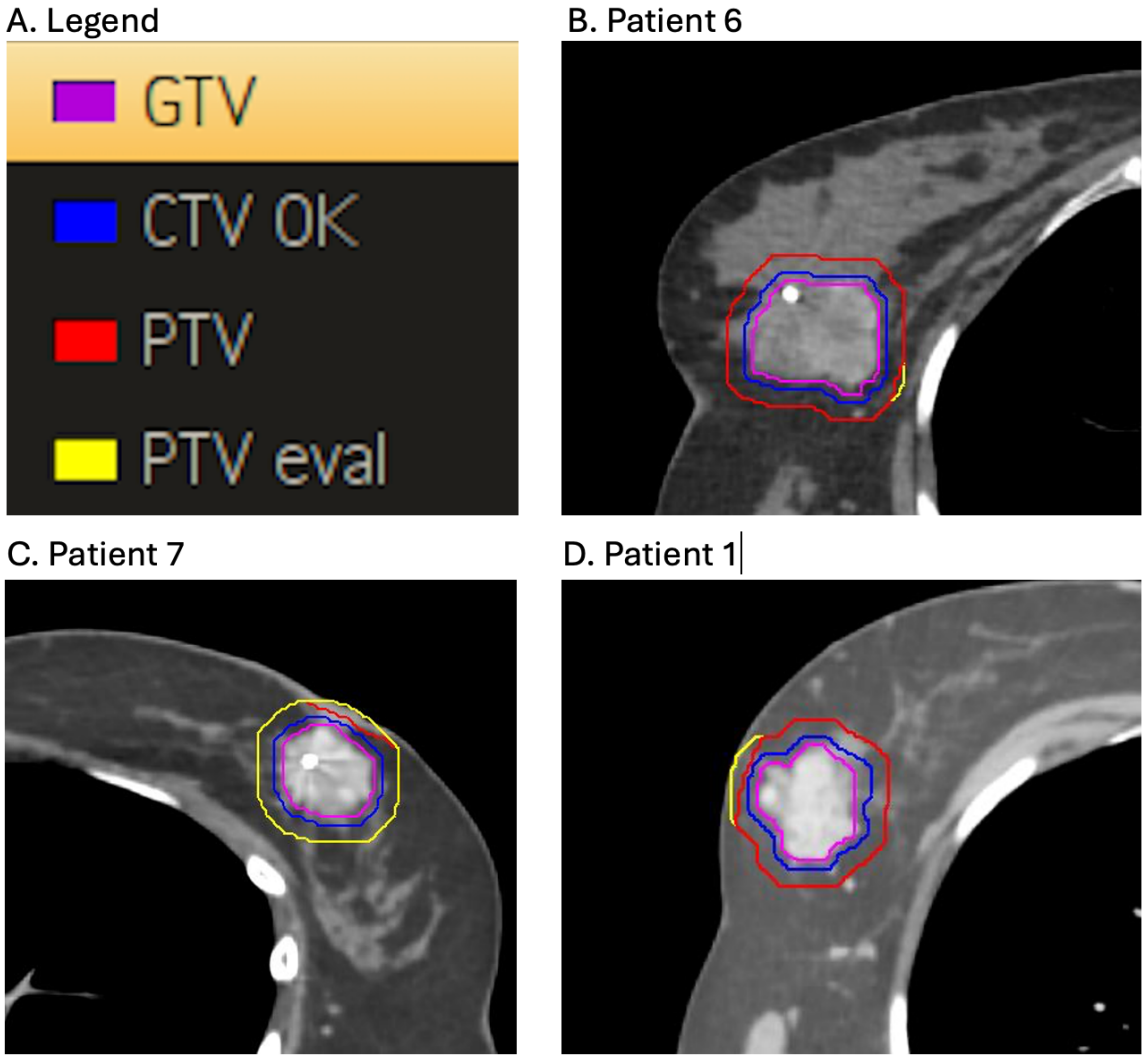


In both cases, CTV is cropped to avoid the skin, but without compromising GTV. In patient 2 (upper figure) the difference between CTV and CTV_eval is more relevant since it follows the whole superficial profile of the structure, while in patient 8 (lower figure) there are only some CTV's more superficial parts that need to be cropped, resulting in a minimal volume difference between the two structures.

Interestingly, patient 2 exhibited a mean value of GTV and ipsilateral breast volume (I.e., not an “extreme” case). However, the lesion was positioned medially in an area with limited breast parenchymal thickness, where the skin and chest wall were relatively close, and the lesion was situated in between.

In the remaining 6 patients, some of whom are shown in Figure 9, the lesion was located within the densest breast parenchyma, requiring no reduction of CTV margins.

Figure 9. Examples of cases without CTV cropping



As expected, in the RT plan of patients 2 and 7 and 8, the constraints on the skin were more difficult to respect: the volume of skin receiving 15Gy was below the limit of 10 cm^3, and the volume of skin receiving 20Gy was below the limit of 1 cm^3 (6 and 0.8 cm^3, 5.1 and 0.9 cm^3, 7.5 and 0.7 cm^3 for the two constraints in patient 2, 7 and 8 respectively), but higher if compared with the volumes receiving these doses in the other plans. Considering the constraints adopted for the avoidance structure Skin_3mm, which represents the punctual maximum dose that can be accepted, we only had a minor deviation from 19.2 Gy to 19.4 and 19.8 Gy for patients 2 and 7, respectively, which we considered acceptable. However, the mandatory constraint ($D1cm^3$<19.2Gy) was respected for all patients. Considering the chest wall, the optimal constraint was not respected for two patients where the PTV was located next to it, with a volume receiving 10 Gy of 10.2 and 12.1 cm^3 in patients 2 and 4, respectively (instead of <10 cm^3). However, the mandatory constraint was largely respected for both. Moreover, it should be kept in mind that the most relevant structures that benefit from a strict constraint on the chestwall are the ribs. Hence, a personalized check was conducted in these patients to be sure that the higher dose volumes were not massively involving bone structures (Figure 10).

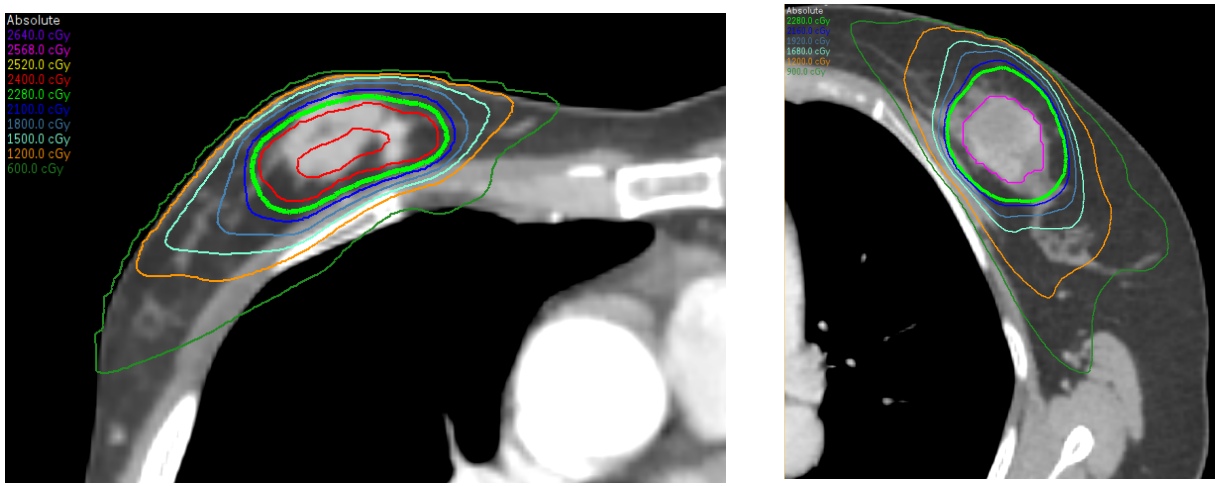


Figure 10 Visualization of the target coverage for patient 2 (left) and 4 (right), showing that ribs are spared from isodoses>15Gy.

The analysis of the constraints for OARs demonstrated that the doses to the heart were maintained at exceptionally low levels in terms of maximum dose. The mean dose was also far below the optimal and mandatory constraints, ranging from 0.04 to 0.37 Gy.

Contralateral lung was almost completely spared in all the treatment plans, with a maximum dose ranging from 0.45 and 1.06 Gy and a mean dose extremely low, considering that the mean dose of the Total_lungs was for all patients below 0.70 Gy (range 0.20-0.70 Gy).
Dose constraints were also restrictive for the ipsilateral lung, and in all ten treatments, the registered dose was far below them. The volume receiving 2.5 Gy ranged between 1.0 and 9.7%, while the volume receiving 5 Gy dose was neglectable (significantly below 1% of the lung volume). Only patient 2, as expected, had a slightly higher ipsilateral lung volume receiving 5 Gy (8.80 cm^3, below 1% of the ipsilateral lung volume), still in between the optimal and mandatory constraint for this OaR.

The spinal cord and contralateral breast were almost unreached from the RT dose, with a maximum (punctual) value of 1.26 Gy and 0.97 Gy, respectively.

Finally, the conformation of the RT plan resulted in a significant sparing of the ipsilateral breast: the volume receiving half the prescribed dose ranged from 7 to 32.5%, indicating a rapid dose reduction just beyond the PTV. The less sparing results were in patients 2 and 8, who notably had the smaller breast volume (232.5 cm^3 and 212.1 cm^3, respectively) and consequently higher PTV/breast ratio (9.2% and 14.6%, respectively.

As described in Materials and Methods, for RT planning, priority was given to OARs sparing. As a result, when needed, target coverage was sacrificed. The most relevant case was again patient 2 (**Errore. L'origine riferimento non è stata trovata.**), where, as anticipated previously, the lesion was close to the skin and chest wall. For her plan, we agreed to reach a good coverage of GTV (97.4% of the volume receiving 95% of the prescribed dose) and CTV_eval (96.6% and of the volume receiving 95% of the prescribed dose).

Patient 8 had a similar situation (Figure 12) with the lesion located between the chestwall and skin in a medium-sized breast, but a good coverage of GTV, CTV and PTV_eval while sparing OaRs was easier to obtain given the favourable position in the upper-outer quadrant.

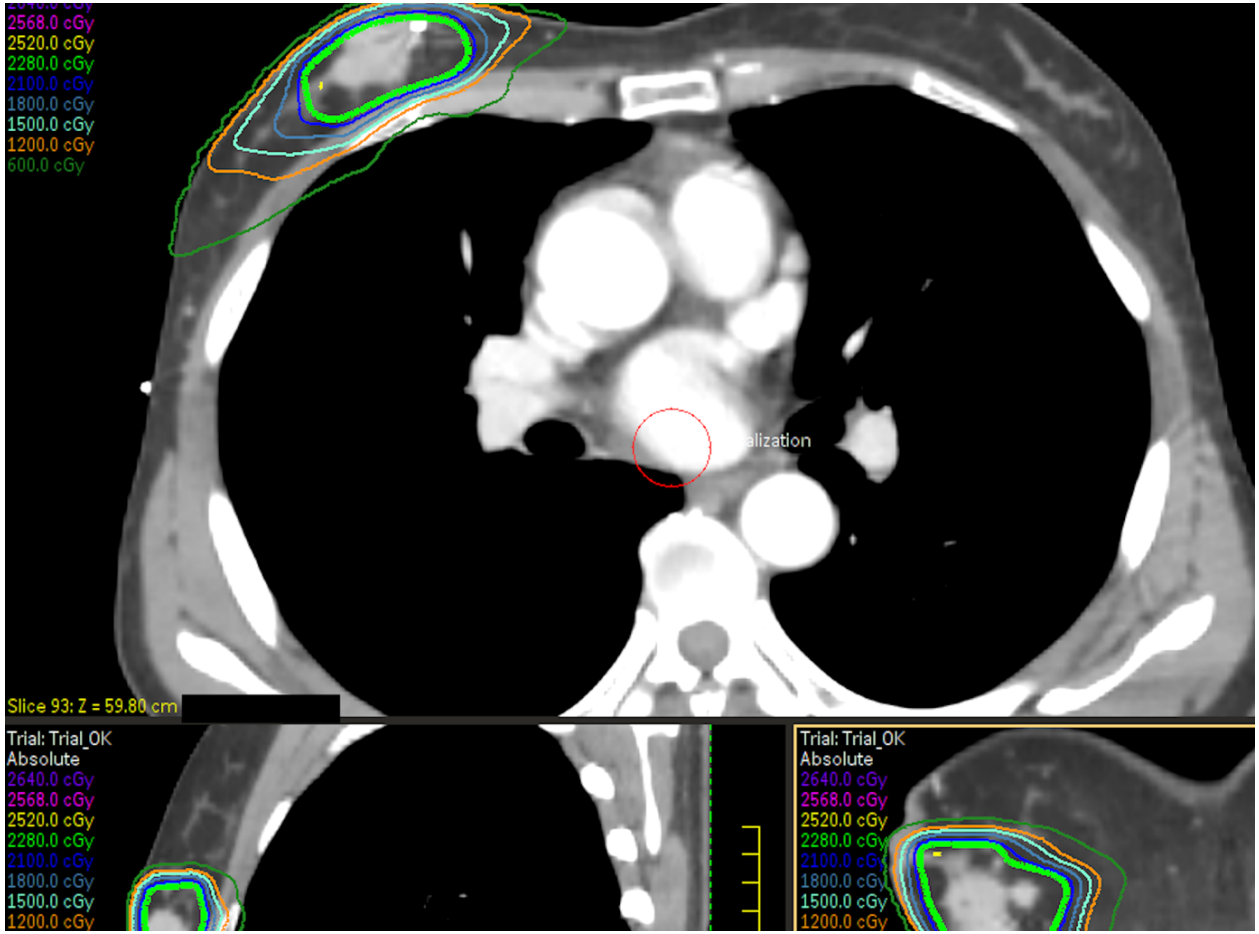
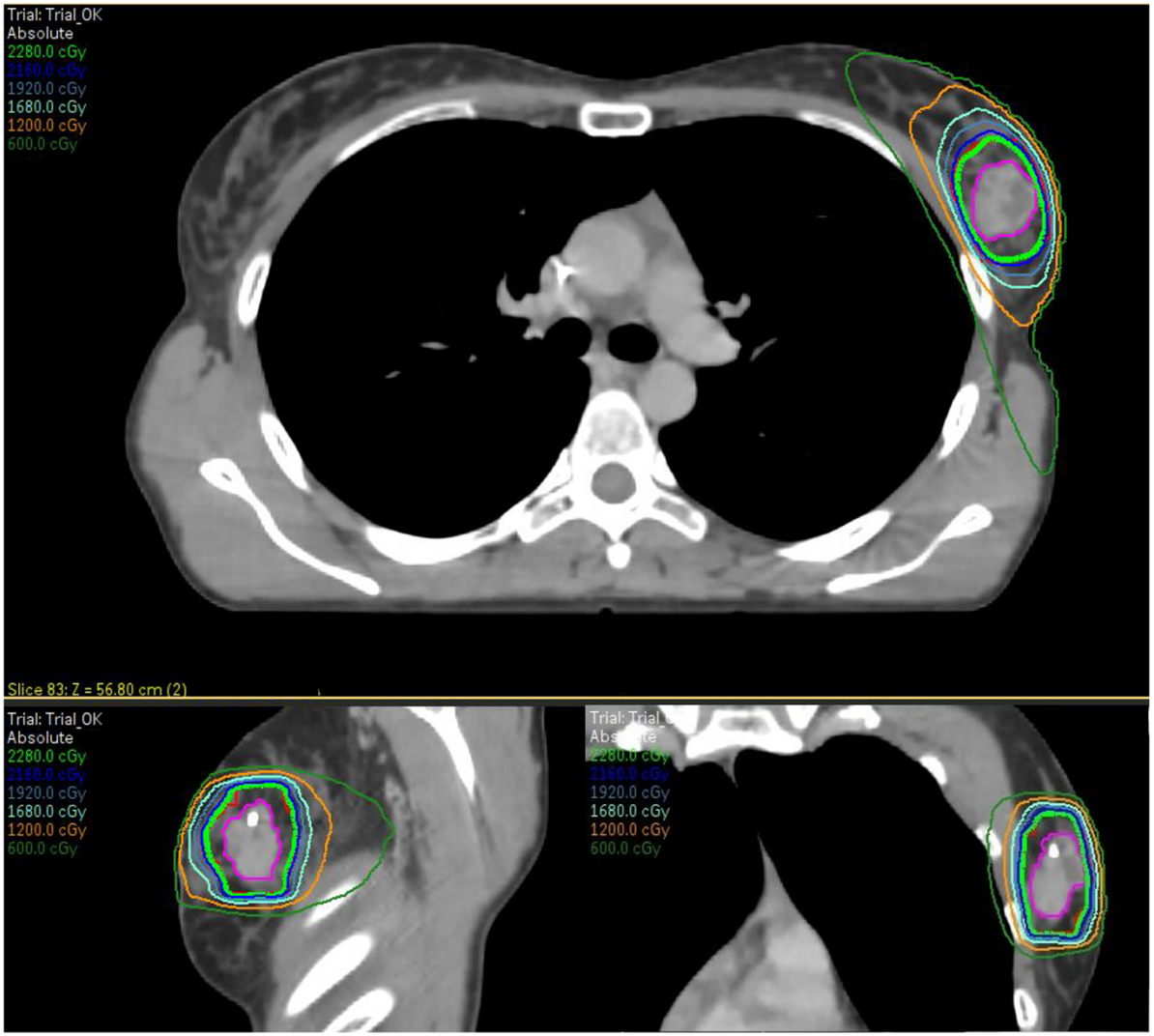


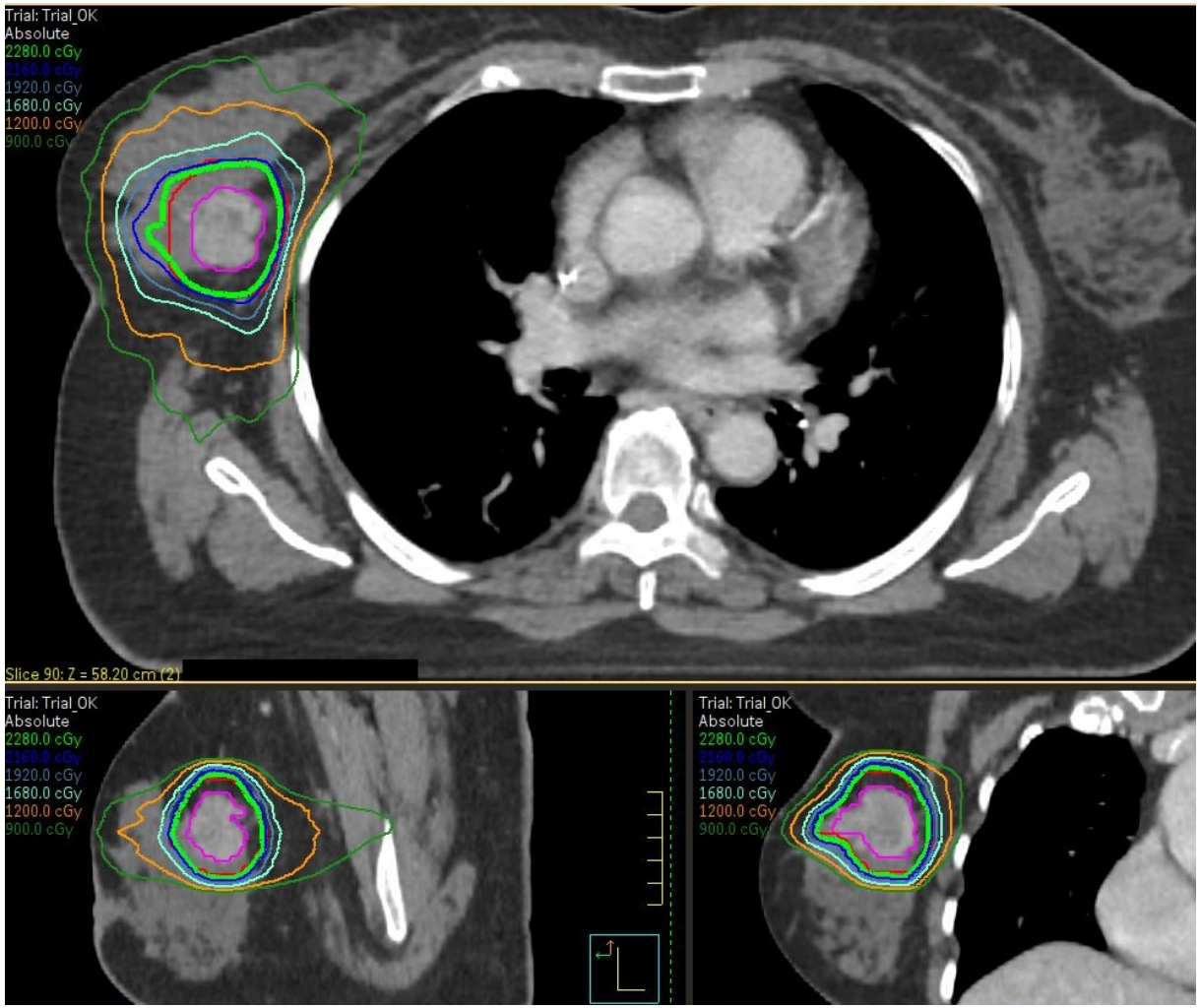
Figure 11. Target coverage of patient 2. Legend: GTV (in pink), PTV (in red). The light green and blue isodoses represent 95% and 90% of the prescribed dose to the PTV, respectively.

Figure 12. Target coverage of patient 8. Legend: GTV (in pink), PTV (in red). The light green and blue isodoses represent 95% and 90% of the prescribed dose to the PTV, respectively.


Trial: Trial_OK
Absolute
2280.0 cGy
2160.0 cGy
1920.0 cGy
1680.0 cGy
1200.0 cGy
600.0 cGy
Slice 83: Z = 56.80 cm (2)
Trial: Trial_OK
Absolute
2280.0 cGy
2160.0 cGy
1920.0 cGy
1680.0 cGy
1200.0 cGy
600.0 cGy
1920.0 cGy
1680.0 cGy
1200.0 cGy
600.0 cGy

Patient 6 had a coverage of PTV_eval just below the optimal objective when considering the 95% of the dose, while the isodose 90% reached 98.3% of the volume. This can be explained both by the large volume of the lesion and its location relatively close to the chestwall. Even though in this case it was not necessary to significantly crop the target volumes to prevent overlaps with OaRs, the final dose distribution was conformed to respect OaRs constraints.

Figure 13. Target coverage of patient 6. Legend: GTV (in pink), PTV (in red). The light green and blue isodoses represent 95% and 90% of the prescribed dose to the PTV, respectively.



Similarly, for patients 1 (Figure 14) and 4 (Figure 15), the coverage of GTV and CTV was optimal (100% and 99.9-100%, respectively). The PTV_eval was well covered when considering 90% of the prescribed dose (covering 99.5% and 99.2% of the volume, respectively). In comparison, the 95% isodose covered almost 94% of the PTV and 95.4 and 94.5% of the PTV_eval, respectively, to respect skin and chest wall constraints.

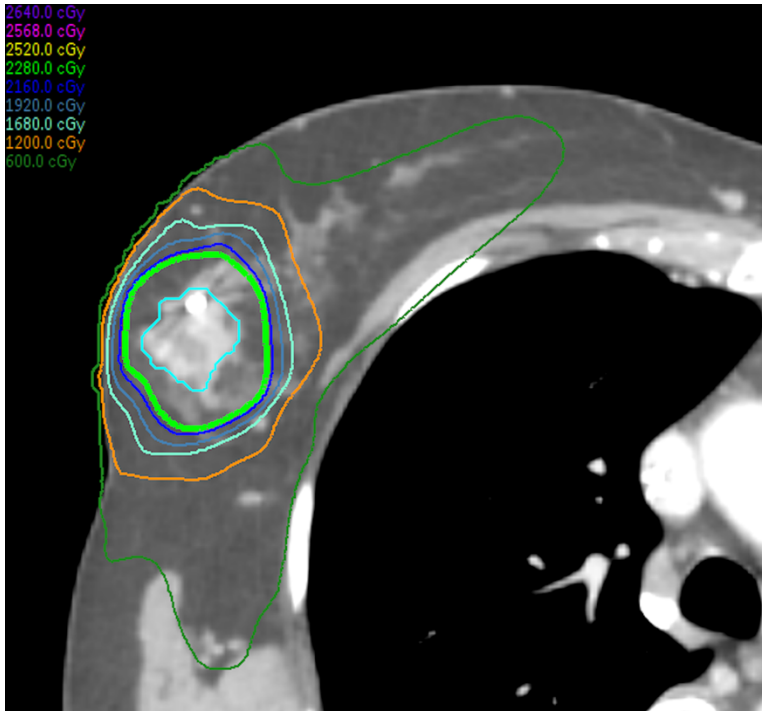


Figure 14 Target coverage of patient 1. Legend: GTV (in light blue). The light green and blue isodoses represent 95% and 90% of the prescribed dose to the PTV, respectively.

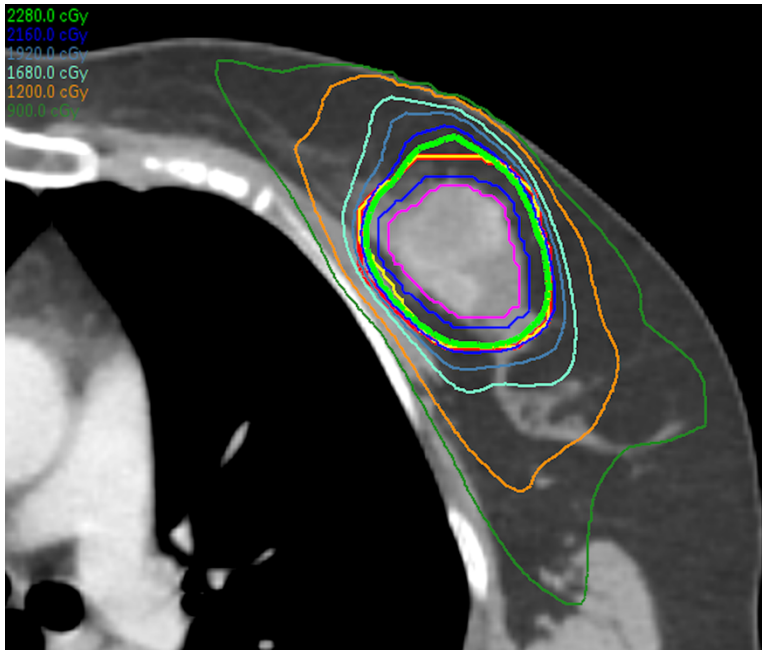


Figure 15 Target coverage of patient 4. Legend: GTV (in pink), CTV (in blue), PTV (in red), PTV_eval (in yellow). The light green and blue isodoses represent 95% and 90% of the prescribed dose to the PTV_eval, respectively.

In patient 3 and 5 (Figure 16 and Figure 17, respectively), no volume contraction was necessary, both due to the size of the lesions and their favourable positions. Target coverage met all dose constraints, notably achieving 97-98% coverage of the PTV with 95% of the prescribed dose.

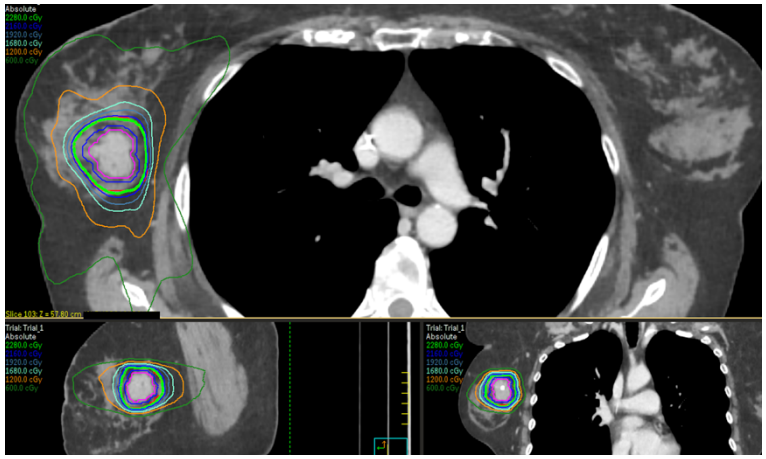


Figure 16 Target coverage of patient 3. Legend: GTV (in pink), CTV (in blue), PTV (in red). The light green and blue isodoses represent 95% and 90% of the prescribed dose to the PTV, respectively.

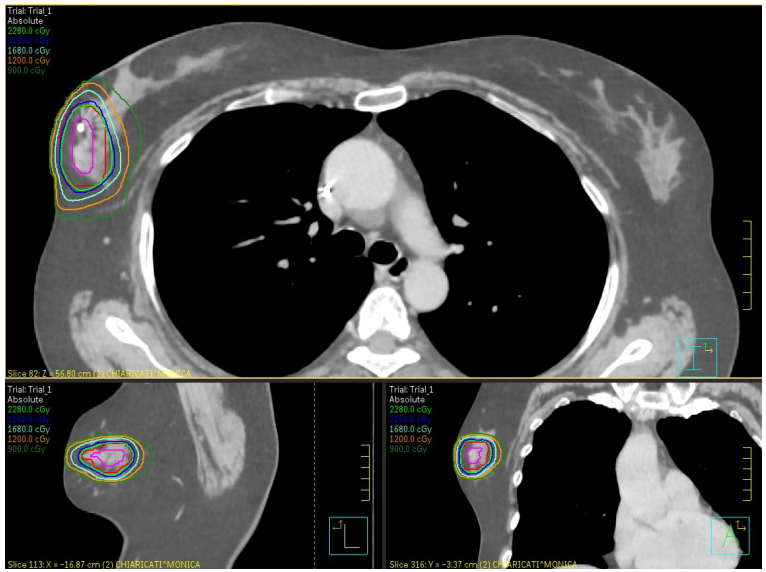


Figure 17. Target coverage of patient 5. Legend: GTV (in pink), PTV (in red). The light green and blue isodoses represent 95% and 90% of the prescribed dose to the PTV, respectively.

Organs At Risk	Constraints	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Skin_5mm	V15Gy<10 cm3 (mandatory)	3 cm3	6 cm3	0 cm3	1.7 cm3	2.5 cm3	0 cm3	5.1 cm3	7.5 cm3	3.6 cm3	2.7 cm3
	V20Gy<1 cm3 (mandatory)	0.3 cm3	0.8 cm3	0 cm3	0 cm3	0.1 cm3	0 cm3	0.9 cm3	0.7 cm3	0.3 cm3	0.3 cm3
	$DMAX_{(0.1cm3)}$<19.2Gy	18.7Gy	19.4Gy	8.5 Gy	17.7Gy	16.7 Gy	0 Gy	19.8 Gy	19.1 Gy	19.1 Gy	17.9 Gy

	(optimal)										
Skin_3mm	D1cm3<19.2Gy (mandatory)	15.3Gy	16.8Gy	6.9Gy	13.5Gy	14.2 Gy	0 Gy	17.2 Gy	16.9 Gy	16.0 Gy	14.6 Gy
Chest wall	V10Gy<10 cm3 (optimal)	0.9 cm3	10.2 cm3	0.04 cm3	12.1 cm3	0 cm3	7.0 cm3	4.2 cm3	9.2 cm3	0 cm3	0 cm3
	V15Gy<10 cm3 (mandatory)	0	3.5 cm3	0 cm3	3.1 cm3	0 cm3	0.8 cm3	0.1 cm3	2.6 cm3	0 cm3	0 cm3
Heart	V3Gy<5 cm3 (mandatory)	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3
	DMAX<5Gy (mandatory)	0.18 Gy	2.54 Gy	0.65 Gy	0.14 Gy	0.3 Gy	1.7 Gy	0.9 Gy	1.0 Gy	1.3 Gy	1.2 Gy
	DMEAN <2Gy (optimal) DMEAN <3Gy (mandatory)	0.04 Gy	0.36 Gy	0.07 Gy	0.16 Gy	0.04 Gy	0.3 Gy	0.08 Gy	0.09 Gy	0.37 Gy	0.28 Gy
Controlateral_lung	V1Gy<1 cm3	0 cm3	0 cm3	0 cm3	0.01 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3
	DMAX<1Gy	0.45Gy	0.96 Gy	0.59Gy	1.06Gy	0.65 Gy	0.94 Gy	0.70 Gy	0.70 Gy	0.72 Gy	0.77 Gy
Ipsilateral_ lung	V5Gy<15 cm3 (mandatory) V5Gy<5 cm3 (optimal)	0.58 cm3	8.80 cm3	1.23 cm3	4 cm3	0 cm3	3.6 cm3	3.4 cm3	3.6 cm3	0 cm3	0 cm3
	V2.5Gy<15%	2.0%	4.0%	1.0%	5.4%	1.0%	9.7%	0.8%	2.4%	0.2%	1.0%

Total_lungs	DMEAN<3Gy (optimal) DMEAN<5Gy (mandatory)	0.29 Gy	0.33 Gy	0.27 Gy	0.46Gy	0.20 Gy	0.70 Gy	0.20 Gy	0.30 Gy	0.30 Gy	0.24 Gy
Spinal_cord	V3Gy<1 cm3 (mandatory)	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3
	V10Gy<0.1 cm3 (mandatory)	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3	0 cm3
	$DMAX_{(0.1cm3)}$ <10Gy	1.26 Gy	0.86 Gy	0.77 Gy	1.16 Gy	0.67 Gy	0.95 Gy	0.46 Gy	0.68 Gy	0.88 Gy	1.15 Gy
	D1cm3<3Gy	1.08 Gy	0.71 Gy	0.78 Gy	1.06 Gy	0.59 Gy	0.83 Gy	0.42 Gy	0.60 Gy	0.84 Gy	1.10 Gy
Controlateral_breast	ALARA DMAX<1Gy	0.27 Gy	0.97 Gy	0.51 Gy	0.76 Gy	0.38 Gy	0.52 Gy	0.71 Gy	0.73 Gy	0.56 Gy	0.65 Gy
Ipsilateral_breast including target	V12Gy<60%	14.5%	29.3%	10.5%	16.7%	8.0 %	19.1%	12.7%	32.5%	7%	7%
	V24Gy<30%	2.7%	6%	1.6%	3.6%	1%	5%	2.5%	7.3%	2%	2.0%
	V10Gy<200 cm3	145.9 cm3	78.5 cm3	189.0 cm3	196.7 cm3	74 cm3	143.1 cm3	129.9 cm3	77.7 cm3	124 cm3	59.6 cm3

reports the dosimetric planning constraints for OARs, and

Table 17 the target coverages.

Table 16. Dosimetric planning constraints for OARs. Highlighted in yellow the dose constraints out of the optimal range.

Organs At Risk	Constraints	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Skin_5mm	V_{15Gy}<10 cm^3 (mandatory)	3 cm^3	6 cm^3	0 cm^3	1.7 cm^3	2.5 cm^3	0 cm^3	5.1 cm^3	7.5 cm^3	3.6 cm^3	2.7 cm^3
	V_{20Gy}<1 cm^3 (mandatory)	0.3 cm3	0.8 cm3	0 cm3	0 cm3	0.1 cm^3	0 cm^3	0.9 cm^3	0.7 cm^3	0.3 cm^3	0.3 cm^3
Skin_3mm	$D_{MAX(0.1cm^3)}$<19.2Gy (optimal)	18.7Gy	19.4Gy	8.5 Gy	17.7Gy	16.7 Gy	0 Gy	19.8 Gy	19.1 Gy	19.1 Gy	17.9 Gy
	D_{1cm^3}<19.2Gy (mandatory)	15.3Gy	16.8Gy	6.9Gy	13.5Gy	14.2 Gy	0 Gy	17.2 Gy	16.9 Gy	16.0 Gy	14.6 Gy
Chest wall	V_{10Gy}<10 cm^3 (optimal)	0.9 cm^3	10.2 cm^3	0.04 cm^3	12.1 cm^3	0 cm^3	7.0 cm^3	4.2 cm^3	9.2 cm^3	0 cm^3	0 cm^3
	V_{15Gy}<10 cm^3 (mandatory)	0	3.5 cm^3	0 cm^3	3.1 cm^3	0 cm^3	0.8 cm^3	0.1 cm^3	2.6 cm^3	0 cm^3	0 cm^3
Heart	V_{3Gy}<5 cm^3 (mandatory)	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3
	D_{MAX}<5Gy (mandatory)	0.18 Gy	2.54 Gy	0.65 Gy	0.14 Gy	0.3 Gy	1.7 Gy	0.9 Gy	1.0 Gy	1.3 Gy	1.2 Gy
	D_{MEAN} <2Gy (optimal)										
	D_{MEAN} <3Gy (mandatory)	0.04 Gy	0.36 Gy	0.07 Gy	0.16 Gy	0.04 Gy	0.3 Gy	0.08 Gy	0.09 Gy	0.37 Gy	0.28 Gy

Controlateral_lung	V_{1Gy}<1 cm^3	0 cm^3	0 cm^3	0 cm^3	0.01 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3
	D_{MAX}<1Gy	0.45Gy	0.96 Gy	0.59Gy	1.06Gy	0.65 Gy	0.94 Gy	0.70 Gy	0.70 Gy	0.72 Gy	0.77 Gy
Ipsilateral_ lung	V_{5Gy}<15 cm^3 (mandatory) V_{5Gy}<5 cm^3 (optimal)	0.58 cm^3	8.80 cm^3	1.23 cm^3	4 cm^3	0 cm^3	3.6 cm^3	3.4 cm^3	3.6 cm^3	0 cm^3	0 cm^3
	$V_{2.5Gy}$<15%	2.0%	4.0%	1.0%	5.4%	1.0%	9.7%	0.8%	2.4%	0.2%	1.0%
Total_lungs	D_{MEAN}<3Gy (optimal) D_{MEAN}<5Gy (mandatory)	0.29 Gy	0.33 Gy	0.27 Gy	0.46Gy	0.20 Gy	0.70 Gy	0.20 Gy	0.30 Gy	0.30 Gy	0.24 Gy
Spinal_cord	V_{3Gy}<1 cm^3 (mandatory)	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3
	V_{10Gy}<0.1 cm^3 (mandatory)	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3	0 cm^3
	$D_{MAX(0.1cm^3)}$ <10Gy	1.26 Gy	0.86 Gy	0.77 Gy	1.16 Gy	0.67 Gy	0.95 Gy	0.46 Gy	0.68 Gy	0.88 Gy	1.15 Gy
	D_{1cm^3}<3Gy	1.08 Gy	0.71 Gy	0.78 Gy	1.06 Gy	0.59 Gy	0.83 Gy	0.42 Gy	0.60 Gy	0.84 Gy	1.10 Gy
Controlateral_breast	ALARA D_{MAX}<1Gy	0.27 Gy	0.97 Gy	0.51 Gy	0.76 Gy	0.38 Gy	0.52 Gy	0.71 Gy	0.73 Gy	0.56 Gy	0.65 Gy
	V_{12Gy}<60%	14.5%	29.3%	10.5%	16.7%	8.0 %	19.1%	12.7%	32.5%	7%	7%

Ipsilateral_breast including target	V_{24Gy}<30%	2.7%	6%	1.6%	3.6%	1%	5%	2.5%	7.3%	2%	2.0%
	V_{10Gy}<200 cm^3	145.9 cm^3	78.5 cm^3	189.0 cm^3	196.7 cm^3	74 cm^3	143.1 cm^3	129.9 cm^3	77.7 cm^3	124 cm^3	59.6 cm^3

Table 17. Target coverages. In red, the suboptimal coverages of the various targets.

Target	Coverage	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
PTV_eval	V95>95 %	95.4 %	92.5 %	-	94.5 %	97.8 %	93.4 %	95.2 %	95.4 %	97.3 %	96.6 %
	V90>95 %	99.5 %	99.5 %	-	99.2 %	99.6 %	98.3 %	99.3 %	99.4 %	99.7 %	99.6 %
(PTV)	V95>95 %	93.8 %	63.3 %	98%	93.6 %	97.0 %	93.1 %	93.4 %	80.8 %	93.3 %	94.1 %
CTV_eval	V95>95 %	99.9 %	96.6 %	-	-	-	-	-	99.1 %	99.0 %	-
(CTV)	V95>95 %	99.9 %	86.7 %	100%	100%	100%	100%	99.9 %	98.1 %	99.0 %	99.7 %
GTV	V95>95 %	100%	97.4 %	100%	100%	100%	100%	100%	99.9 %	99.9 %	100%

Evaluation Of Physical Parameters During Radiation Treatment

All the treatments were planned with a VMAT technique using a 6 MV photon beam with a flattering filter free (FFF). In SBRT treatments, FFF can be used to obtain higher doses in the target and a steep dose falling outside it. Moreover, since the use of FFF is associated with a higher dose rate, the delivery of the treatment is fast, allowing the delivery of more than 1500 Monitor Unit (MU) at the same time as a "conventional" breast treatment (of almost 700 MU).

As shown in Table 18, all but one patient were treated with two arcs; patient 2 was treated with three arcs (MU range: 431.1-1118.7 per arc), and the total MU per treatment ranged between 1242.6 and 2063.6 MU.

The treatments were all planned with DIBH technique (except patient 6, clinically not suitable for it) to obtain the highest OAR-sparing situation. Despite the high number of MU and the complexity of VMAT treatment, the delivery was so fast that most sessions were completed with no more than 1-2 apneas per arc (Table 19).
Among the 27 RT sessions delivered with DIBH, 13 were conducted with 2-3 apneas, 12 with four apneas, and only two involving multiple apneas, probably related to a technical issue in the surface image-guided system.
The treatments were never stopped during a DIBH, meaning that intrafraction positioning was optimal considering our threshold of 5mm vector for 3-dimension shifts.

Patient positioning was performed using skin tattoos.

Table 20 shows the difference between the shifts suggested (and not applied) by the surface image-guided system after patients' positioning and the shifts applied after Cone Beam CT (CBCT). The results are encouraging since they show that the tattoo-based positioning was accurate (only one measure out of 36 performed was just above the optimal acceptance threshold of 5 mm), and the surface image-guided system did not reflect the "real" shifts present.
Of course, this is not surprising considering that the two systems (surface image guided and CBCT) scan different volumes; however, the CBCT shifts are much more relevant in SBRT treatments because they reflect the target shifts instead of the body surface shifts and are evaluated during DIBH, so in the same condition of the treatment delivery.

Interestingly, the number of apneas needed for the CBCT was similar to those required for the whole treatment in all the patients, especially in the first session. It means that the positioning-verifying procedures are as stressful as the treatment itself. Of course, CBCT is the method of choice for positioning verification for SBRT treatments, but this observation is relevant to define a protocol for CBCT that can be appropriate for the target but as fast as possible to further reduce patients' discomfort.

Table 18. Physical parameters of RT

Patient	Fields	Start-stop angle °	Collimator angle°	Monitor Unit	Total Monitor Unit
1	A1	20-220	10	893.7	1511.4
	A2	220-20	350	617.7	
2	A1	60-230	10	508.3	1615.3
	A2	230-60	350	675.9	
	A3	60-230	0	431.1	
3	A1	20-180.1	10	906.4	1784.1
	A2	180.1-20	350	877.7	
4	A1	135-310	10	745.3	1740.9
	A2	310-135	350	995.6	
5	A1	30-200	350	715.3	1596.9
	A2	200-30	10	881.6	
6	A1	40-200	10	1118.7	2063.6
	A2	200-40	350	944.9	
7	A1	160-310	10	720.2	1477.9
	A2	310-160	350	757.7	
8	A1	150-320	10	750.6	1435.7
	A2	320-150	350	685.1	
9	A1	180-340	10	705.5	1388.7
	A2	340-180	350	683.2	
10	A1	180-340	10	567.1	1242.6
	A2	340-180	350	675.5	

Table 19. Number of DIBH during RT

Patient	Session	Beam	DIBH
1	1	CBCT	2
		A1	1
		A2	1
	2	CBCT	4
		A1	2
		A2	2
	3	CBCT	2
		A1	1
		A2	1
2	1	CBCT	2
		A1	1
		A2	1
		A3	1
	2	CBCT	2
		A1	1
		A2	1
		A3	1
	3	CBCT	3
		A1	5
		A2	3
		A3	2
3	1	CBCT	3
		A1	2
		A2	2
	2	CBCT	4
		A1	2
		A2	2
	3	CBCT	2
		A1	2
		A2	2
4	1	CBCT	2
		A1	1
		A2	2
	2	CBCT	2
		A1	2
		A2	1
	3	CBCT	1
		A1	1
		A2	2
5	1	CBCT	4
		A1	2
		A2	2
	2	CBCT	3
		A1	2
		A2	2

	3	CBCT	3
		A1	2
		A2	2
6	1	CBCT	Free
		A1	Free
		A2	Free
	2	CBCT	Free
		A1	Free
		A2	Free
	3	CBCT	Free
		A1	Free
		A2	Free
7	1	CBCT	4
		A1	2
		A2	2
	2	CBCT	3
		A1	2
		A2	2
	3	CBCT	2
		A1	1
		A2	2
8	1	CBCT	5
		A1	1
		A2	2
	2	CBCT	4
		A1	2
		A2	2
	3	CBCT	2
		A1	1
		A2	1
9	1	CBCT	3
		A1	2
		A2	3
	2	CBCT	2
		A1	2
		A2	1
	3	CBCT	2
		A1	2
		A2	1
10	1	CBCT	2
		A1	1
		A2	2
	2	CBCT	2
		A1	2
		A2	2
	3	CBCT	2
		A1	2
		A2	2

Table 20. Displacements identified by Catalyst for patient positioning (cPosition) and by Cone Beam CT (CBCT) during verification.

		LATERAL (mm)		LONG (mm)		VERTICAL (mm)	
Patient	Session	cPosition Results	CBCT	cPosition Results	CBCT	cPosition Results	CBCT
1	1	-0.9	0.0	+1.7	-2.0	-2.3	-2.0
	2	-0.9	-1.0	3.3	-3.0	-0.3	-2.0
	3	-1.9	-3.0	+2.8	0.0	-2.5	-3.0
2	1	2.4	4.0	-0.2	2.0	-0.7	2.0
	2	2.1	3.0	-0.2	3.0	-1.5	2.0
	3	2.0	1.0	-1.4	5.0	-2.7	2.0
3	1	0.4	-2.0	5.9	6.0	-3.1	-3.0
	2	-2.9	-5.0	3.4	5.0	-1.2	-1.0
	3	-2.2	-4.0	1.7	5.0	1.2	3.0
4	1	2.2	-3.0	-2.0	2.0	-4.5	1.0
	2	6.5	5.0	2.3	1.0	4.5	5.0
	3	3.0	-4.0	3.8	5.0	-4.8	0.0
5	1	0.1	0.4	3.8	2.4	-2.3	-0.6
	2	-2.6	-2.6	4	5.2	-2.4	0.2
	3	0.3	0.4	4.9	2.4	-1.3	-0.6
6	1	4.0	2.0	1.6	-3.0	-2.3	-2.0
	2	2.4	-2.9	-3.7	-6.6	1.3	4.6
	3	-2.5	-4.5	-2.9	-2.5	-2.2	-1.2
7	1	-2.4	5.1	1.9	0.9	0.8	0.6
	2	-2.3	-0.8	-2.8	3.6	-0.4	0.2
	3	-2.6	2.5	-1.3	6.7	-2.3	-0.6
8	1	0.2	0.4	3.1	-1.0	-1.9	-4.8
	2	-3.3	-1.9	-0.2	-3.6	-8.4	-7.0
	3	-2.9	-3.4	1.7	-0.9	-4.0	-4.8
9	1	3.2	5.1	4.5	0.5	-0.9	1.9
	2	-1.2	0.8	4.7	0.5	-2.3	-7.7
	3	0.9	5.1	5.0	0.5	-0.6	1.9
10	1	-1.5	-6.9	4.3	5.5	0.7	4.9
	2	1.6	1.5	0.2	2.4	-3.7	2.2
	3	3.1	6.5	2.5	3.5	1.4	6.3

Pathological response, adjuvant RT and toxicity

Table 21 illustrates the toxicities registered after pre-operative RT: none of the patients showed any acute skin toxicity, nor other organ adverse events at a minimal FU of 2 months (range 2-6 months).

Four patients completed the primary phase of the treatment with BCS. No surgical complications were reported.
Surgical procedures and pathological findings are shown in Table 22.
Of note, a partial or complete response to neoadjuvant treatments was seen in all patients as a reduction of the tumor volume of more than 50%. Moreover, it's relevant to point out that the values of Ki67 in the final specimen were much lower than the initial value, consistently with the evidence that chemotherapy is more effective versus highly aggressive cell subclones, while the most indolent ones are less affected by this treatment.
However, two patients initially staged as N0 were found node positive during sentinel LN evaluation, thus undergoing axillary LN dissection.

Patient 1 underwent surgery sooner than initially scheduled because she was found to have primitive left lung cancer (Figure 18), so combined surgery for breast and lung was planned after primary systemic therapy with anthracyclines.

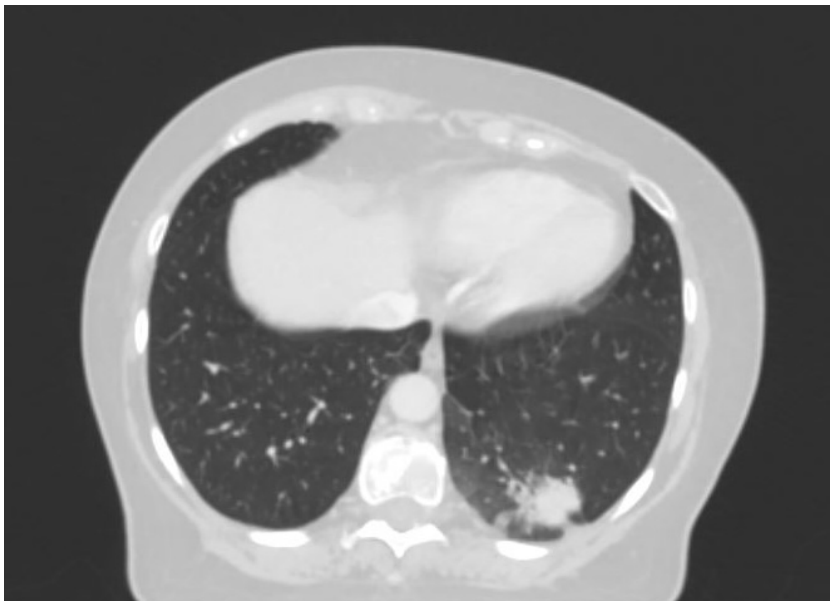


Figure 18. Patient 1: left lung primary tumor

Then she completed the systemic treatment for BC with adjuvant taxanes, and approximately one month after the 12th administration underwent a 5-day WBI scheme to allow for initiation of adjuvant systemic therapy with Pembrolizumab for lung cancer.

Except for patient 1, the other three patients underwent WBI 4-8 weeks after surgery. For patient 4, regional node irradiation (level III-IV) was also delivered.

The treatment was overall well tolerated, with three patients who showed no acute toxicity, and one presented grade 2 skin toxicity (erythema and breast oedema) that required an RT replanning during treatment but healed soon after WBI conclusion. No interruptions of RT treatment were necessary.

WBI plans were prepared considering the dose already received, as shown in Figure 19. A deformation tool was needed to adapt the two different anatomies of the patients (before and after surgery) for estimating the total dose received in the tissues around the surgical bed.

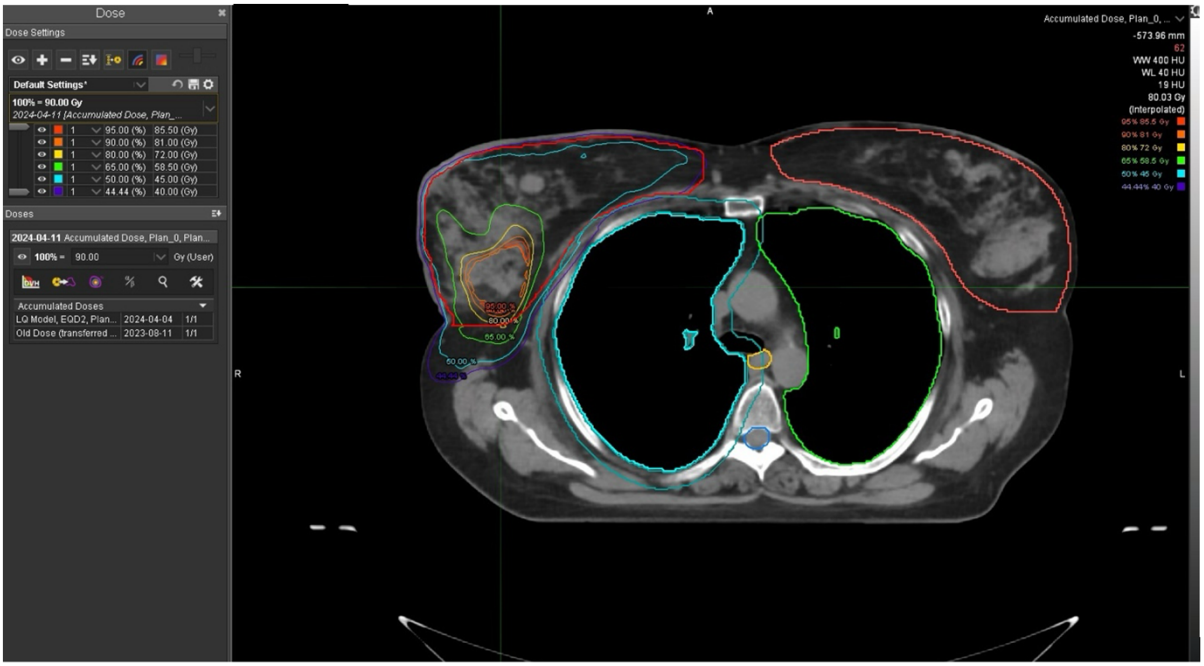


Figure 19. Sum plan for WBI evaluation

Table 21. Acute toxicity (CTCAE v.5)

Patient	Acute toxicity after pre-operative boost	Months of FU	Adjuvant WBI (dose/fraction, volumes)	Max acute toxicity after WBI (CTCAE grade)	Weeks of FU after WBI
1	0	3 (until surgery)	26 Gy/5 fr Right breast	0	20
2	0	5 (until surgery)	40.05 Gy/15 fr Right breast	0	12
3	0	6 (until surgery)	40.05 Gy/15 fr Right breast	2	1 week
4	0	6 (until surgery)	40.05 Gy/15 fr Left breast+level III-IV	0	1 week
5	0	6	-	-	-
6	0	6	-	-	-
7	0	4	-	-	-
8	0	4	-	-	-
9	0	2	-	-	-
10	0	2	-	-	-

Legend: FU: follow-up; WBI: whole breast irradiation

Table 22. Surgery characteristics and pathological tumor response

Patient	Initial clinical stage	Initial T dimension (mm)	Histology and grade	ypTNM	LVI	ECIS	RCB Class	Molecular characteristics						
								ER (%)	PR (%)	Ki67 (%)	p53	HER2	Bcl2	TILs (%)
1	cT2N0	37x30x33	CDI G3	ypT1c (11mm) ypN0	NO	NO	II	98	85	1	wt	1+	int	20%
2	cT(m)1N0	27x22, 12x12	CDI G2	ypT(m)1b (6mm) ypN1mic (1mic/32)	NO	NO	II	95	0	8	-	neg	-	5
3	cT2N1	30x22	-	ypT0 ypN0 (0/4)	NO	NO	0	-	-	-	-	-	-	-
4	cT2N0	45x30	CDI G3	ypT(m)1c (13mm) ypN1a (3/16)	NO	NO	II	100	10	1	-	1+	-	5

Conclusions

The preliminary results of IBISCO trial showcase the feasibility and safety of integrating a preoperative SBRT boost in conjunction with NAC in Luminal B BC treatment. Specifically, acute skin toxicities were not observed in all the ten patients treated, highlighting the efficacy of modern RT fractionation and techniques (VMAT) that likely will overpass the limits from the past in applying preoperative RT.
These modalities allow for a high dose rate and precise dose distribution, ensuring excellent target coverage even in challenging anatomical positions while minimizing toxicity to OARs.
Furthermore, in our initial experience, the diagnostic and treatment workflow proceeded without any delays, emphasizing exceptional collaboration among the multidisciplinary team involved.
These two elements lead to a potentially very tolerable treatment that could be easily implemented in clinical practice, and lower late toxicity compared to standard RT in the same setting could also be expected, given the surgical removal of the breast tissue that received the higher boost RT dose.

Another interesting observation, rising from our initial experience, is that even in a relatively homogenous group of patients there is a huge variability in tumor boost contouring and characteristics. However, a personalized approach is feasible only when the tumor is still on-site, as in this trial. So, this modality could represent in the future another potential advantage of boosting the tumor preoperatively.

However, it's also important to acknowledge that our results are not mature enough to assess the impact of the RT boost on the pathological and immunological response of the neoplasm.
Despite the reassuring results (1/4 patients undergoing BCS with pCR), it should be underlined that two patients had an LN status upstage at final surgery. This could be related to an initial under-staging (one patient had suspicious LN but negative fine needle biopsy and the other was staged in another hospital with contrast-enhanced instead of PET-TC, as usually performed in our centre). However, it cannot be excluded that the disease progressed during NAC as well, so the attention should be kept high on intermediate clinical and instrumental evaluations.

As already mentioned, the setting of Luminal B BC is of special interest concerning these topics because traditionally, it has a worse response to PST compared to TN and HER2-positive disease but pCR is a positive prognostic factor for it as well.

In the future, the availability of a prognostic and predictive test to fine-tune preoperatively the selection of patients for whom chemotherapy is indicated will probably increase the number of patients enrolled in trials that include it. Conversely, for doubtful situations in clinical practice nowadays it is usually preferred to proceed with primary surgery and decide on adjuvant systemic treatments based on the pathological result. This approach limits the cohort of patients suitable for the IBISCO trial enrolment.

IBISCO trial aims to increase pCR in Luminal B cancers, both through the direct cytotoxic effect of SBRT and the potential enhancement of host immune response, and the final results of the trial will hopefully be useful to develop targeted strategies to optimize the association between RT and PST.

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