Appendix C – Summary of the PRISMA Systematic Literature Review

IceCure Medical Ltd. conducted a systematic literature review to develop the performance goal for the rate of ipsilateral breast tumor recurrence (IBTR) through 5 years for the ICE3 clinical study according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidance. The performance goal was based on the standard of care procedure, lumpectomy without adjunctive radiotherapy which was derived from peer-reviewed literature published in scientific journals in a target population comparable to the ICE3 clinical study population.

Methods

The eligibility criteria outlined below were developed according to PICO (Population, Intervention, Outcome, Comparator) guidelines to offer a robust comparison to the ICE3 population. To be included in the review and meta-analysis, the population described by the published article must include patients treated with breast-conserving surgery (i.e., lumpectomy) without adjunctive radiation in the cohort of interest.

Population: The systematic review includes clinical trials of postmenopausal females (age ≥ 50 years) patients who underwent a breast-conserving surgical intervention (i.e., lumpectomy) without adjunctive radiation. The cancer type selected is low risk, early stage (T1), node negative (N0), local (M0), ER/PR positive, and HER2 negative breast cancers.
Intervention: The systematic review considers trials in which patients were treated with breast-conserving surgical intervention (i.e., lumpectomy) without adjunctive radiation. Other adjunctive treatments such as endocrine therapy, chemotherapy, and other non-surgical treatments are admissible. The systematic review excludes trials in which all patients were treated with breast-conserving surgical intervention (i.e., lumpectomy) with radiation or not treated with BSC (i.e., mastectomy).
Outcomes: To be included, the trial must include a defined clinical outcome relating to local tumor recurrence in the breast tissue, specifically ipsilateral breast tumor recurrence (IBTR) and be defined as a recurrent in situ or invasive carcinoma that occurs after breast conserving surgery (BCS) in with the skin or parenchyma of the ipsilateral breast without clinical-radiologic evidence of regional or distant disease. Studies where this variable is not reported and/or not defined are excluded from the review. Disease-free survival and overall survival are variables of interest but do not affect the inclusion or exclusion of the study.
Comparator: Not applicable to this review.

Due to the well-documented impact of adjunctive therapies on local recurrence rates, the population of interest that offers the best comparison to the ICE3 clinical study population is treated with BCS with or without adjunctive endocrine but not radiation therapy. In **Table 1** the screening criteria for the PRISMA literature review are outlined, and low risk of breast cancer recurrence is defined as patients having early-stage tumors that are <2cm in diameter (T1), node negative (N0), ER/PR positive, and HER2 negative.

Table 1: PRISMA Systematic Review and Meta-analysis Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
Articles were **included** if all of the following criteria were met: • Breast-conserving surgery (i.e., lumpectomy) procedure without adjunctive radiation • Cancer type (low risk, lymph node negative, • ER/PR positive, HER2 negative) • Age >50 • Greater than or equal to approximately 100 patients • More than one study site • IBTR evaluable and extractable • English • Full text available	Articles were **excluded** if any of the following criteria were met: • Not breast-conserving surgery (i.e., lumpectomy) procedure **OR** breast-conserving surgery (i.e., lumpectomy) with adjunctive radiation • Cancer type (high risk, lymph node positive, HER2 positive, ER/PR negative) • Age < 50 • Less than approximately 100 patients • Single site study • Book chapters, letters, dissertations, and conference proceedings • IBTR data unavailable/ not extractable • Not English language • Full text unavailable • Duplicate article or population

Relevant published scientific literature was searched in multiple databases including the PubMed, Ovid/Medline, and Embase databases and internet searches of clinical research sites (i.e., ClinicalTrials.gov). Multiple databases were searched to provide comprehensive coverage of the literature related to the target population and intervention. Additionally, these databases encompass a vast collection of US and international medical journals.

- PubMed is a free resource developed and maintained by the National Center for Biotechnology Information at the National Library of Medicine, which is a bibliographic database of citations and abstracts, from approximately 5,400 biomedical journals published in the United States and worldwide. PubMed's coverage extends back to 1948.
- Embase, an online, biomedical and pharmacological search engine that includes peer reviewed publications. It searches scholarly works from more than 8,500 published journals that extends back to 1947.
- Ovid/MEDLINE is the National Library of Medicine's (NLM) premier bibliographic database that contains references to journal articles in life sciences, with a concentration on biomedicine.
- ClinicalTrials.gov is a registry of clinical trials. It is run by the United States National Library of Medicine at the National Institutes of Health, and holds registrations from over 444,000 trials from 221 countries.

The following search terms and limits were utilized in each database:

	Search Terms	Search Limits
1	("Breast-conserving surgery" OR "Lumpectomy") AND ("Irradiation" OR "Radiation")	Clinical Trial, Meta-Analysis, RCT, Controlled Clinical Trial, Systematic Review, Dates 2003-2023, Age (45-80+), Female, English
2	("Early Stage" AND "Breast Cancer") AND ("Lumpectomy" OR "Breast-conserving surgery")	Clinical Trial, Meta-Analysis, RCT, Controlled Clinical Trial, Systematic Review, Dates 2003-2023, Age (45-80+), Female, English

According to item 8 of the 2020 PRISMA checklist, the systematic review was performed by multiple independent reviewers. In an effort to mitigate bias, each article was screened twice by at least two different reviewers. Each article was initially screened by a review of the abstract. If the article appeared to meet the eligibility criteria or if the reviewer could not determine if the article met the inclusion criteria by reviewing the abstract alone, the full text was reviewed. Each reviewer noted in the Screening Log whether the full text or abstract alone was reviewed. If an article did not meet the eligibility criteria, the reviewers documented the reason in the Screening Log. In the case of a disagreement between the two primary reviewers on the eligibility of an article, a third reviewer performed adjudication and provided reason for discordance. The decisions made by the reviewers are documented in the Screening Log.

Within the included studies, multiple treatment arms and cohorts were identified. All data extraction as outlined above was performed on the treatment arm(s) and specific cohort(s) within the treatment arm (if applicable) that was most representative of the ICE3 patient population.

Data extraction and analysis for the local recurrence (IBTR) was performed by two independent reviewers. For extraction of the IBTR rates, "coordinate extraction" of the Kaplan Meier (KM) based cumulative IBTR rates without regard to competing risk was used. When a survival curve was provided for the IBTR recurrence rate, specialized software (DigitizeIt™) was employed to extract the data. The DigitizeIt™ software allows for the extraction of survival estimates based on their x- and y- coordinates as published in the article. This extraction method was selected to reduce bias and ensure consistency across the data extraction process.

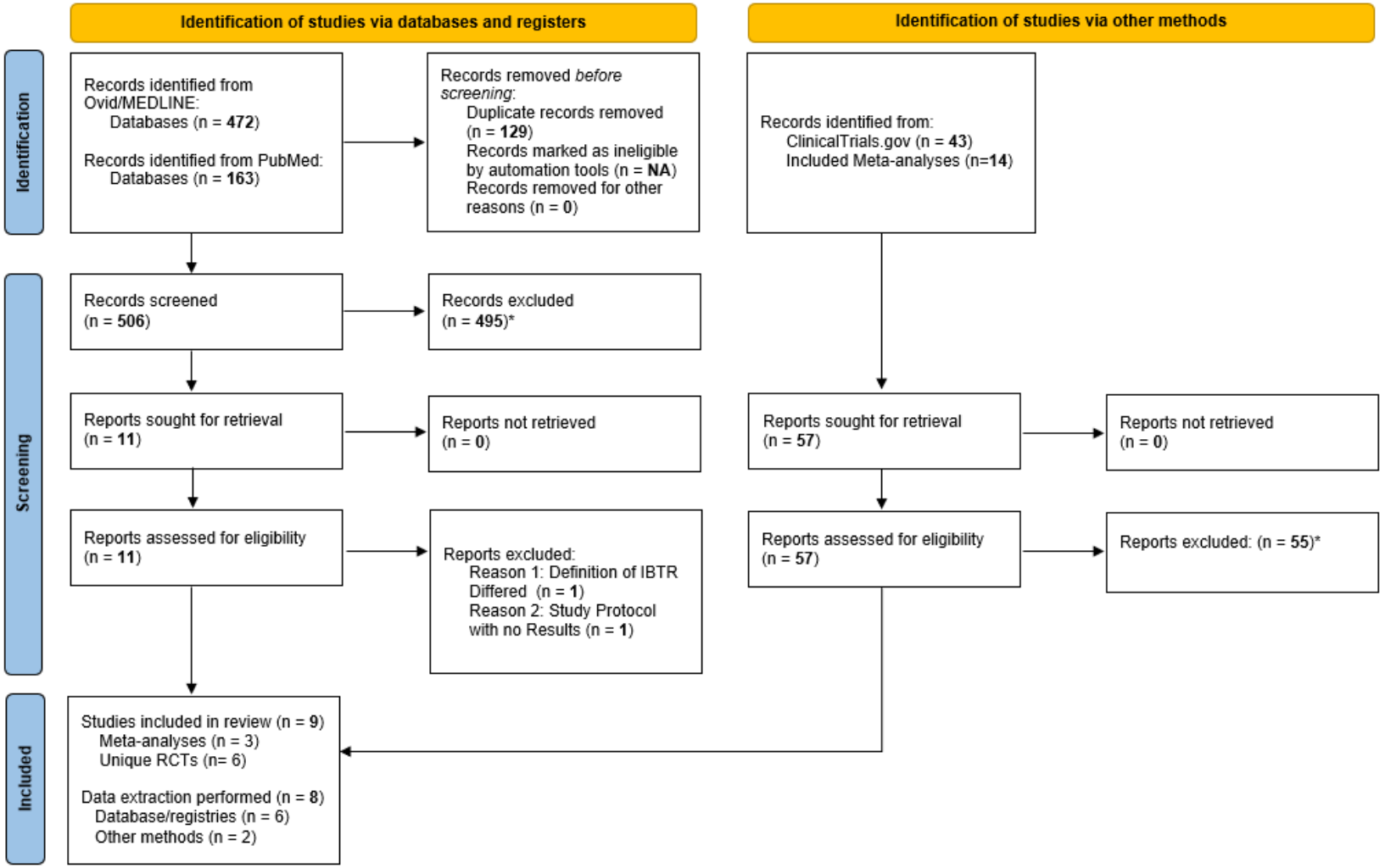
In addition to the measures noted above to address the risk of bias in the subject Systematic Review, risk of bias was also be assessed independently within each included clinical trial according to a validated tool, per PRISMA guidance. Version 2 of the Cochrane risk-of-bias tool for randomized trials (RoB 2) the or the Cochrane risk-of-bias tool for non-randomized trials (ROBINS-I) was used to assess the risk of bias in the included clinical trials. RoB 2 is structured

into a fixed set of domains of bias, focusing on different aspects of trial design, conduct, and reporting. The ROBINS-I tool is similar to the RoB2 but is tailored to assessing risk of bias in non-randomized studies of interventions, rather than risk of bias in randomized clinical trials.

Results (Overall)

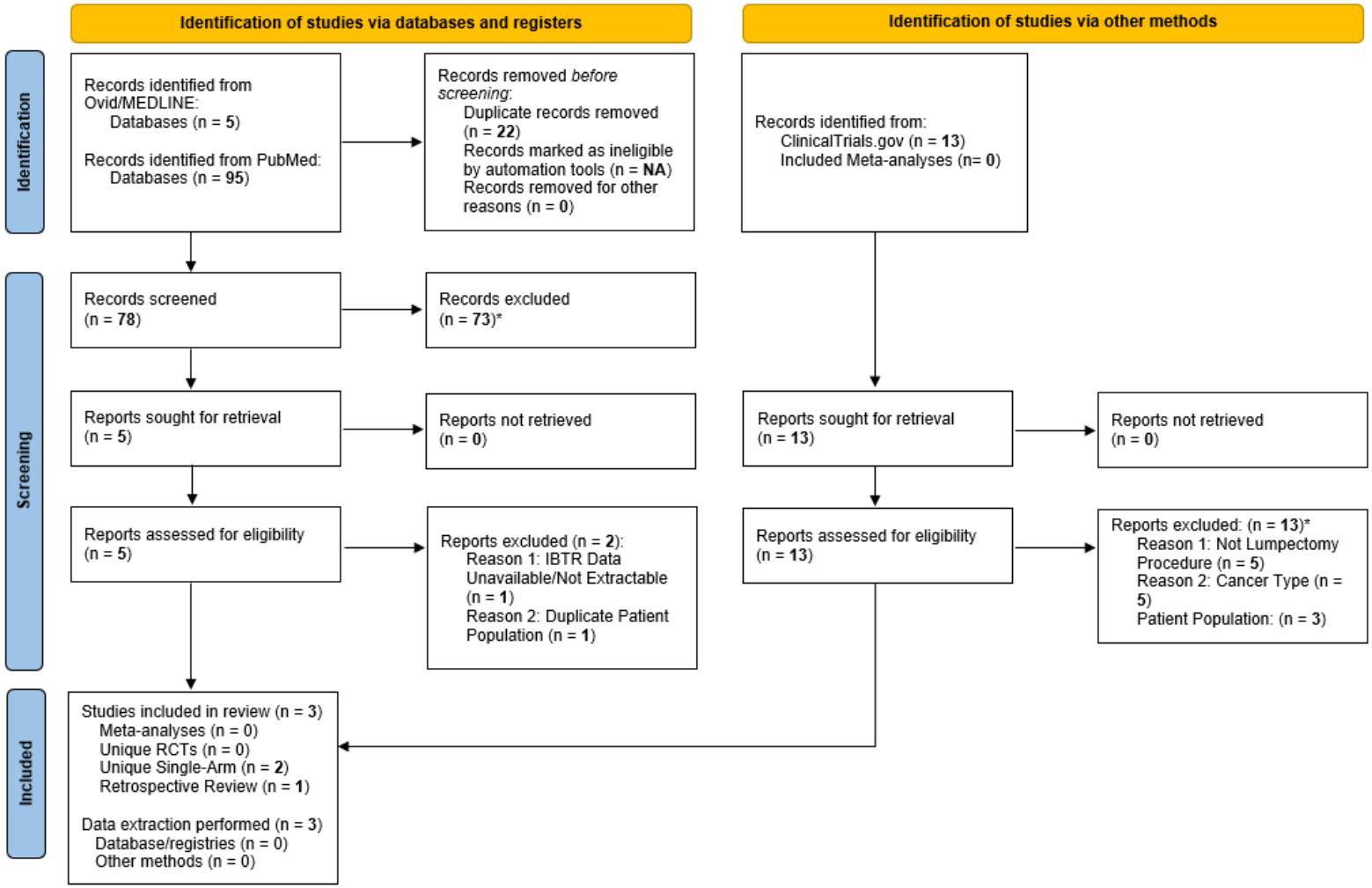
This literature search was originally conducted in April 2023 and returned 635 citations. The original search criteria resulted in a total of eight (8) unique articles for data extraction. An Addendum to the original search was performed in February 2024 that included articles published between April 2023 and February 2024, to bring the search current. The Addendum returned 100 additional citations, which were screened per the original search criteria, and resulted in the inclusion of three (3) additional unique articles for data extraction. ***In total, eleven (11) unique articles reporting clinical studies containing data from 3,718 patients were included and analyzed in this review and meta-analysis.***

PRISMA Flow diagrams for the original (**Figure 1**) and addendum (**Figure 2**) reports are provided below.



*Refer to the Screening Log for reason for exclusion

Figure 1: Flow Diagram for Systematic Review Methodology in with PRISMA Guidelines (Original Report 2023)



*Refer to the Screening Log for reason for exclusion

Figure 2: Flow Diagram for Systematic Review Methodology in with PRISMA Guidelines (Addendum Report 2024)

The PRISMA Systematic Review and Meta-analysis was conducted to support the performance goal IBTR rate and is the most scientifically robust estimation of the rate of recurrence following lumpectomy. All literature screening and data extraction was performed by two independent reviews, as recommended in the PRISMA guidelines. Data extraction was performed using specialized software (DigitizeIt™) to analyze the KM curves and extract IBTR data at each follow-up timepoint. Within the included studies, multiple treatment arms and subpopulations were identified by the authors. Data extraction as outlined above was limited to the treatment arm and specific subpopulation(s) within the treatment arm (if applicable) that were most closely met the inclusion criteria. The treatment arm and subpopulation (if applicable) are noted in **Table 2** below.

As recommended in PRISMA guidance, a certainty assessment was performed systematically to evaluate the confidence in the body of evidence for the outcome measures sought. Generally, most studies met the most rigorous inclusion criterion. Some studies had patient groups that were slightly different when compared to the inclusion criteria. The studies were appraised as follows:

- P1 Applicable: >50% alignment with the ICE3 tumor characteristics*
    - P1(a) ideal: >90% alignment with the ICE3 tumor characteristics*
    - P1(b) sufficient: <90% and >75% alignment with the ICE3 tumor characteristics*
    - P1(c) limited: <75% and >50% alignment with the ICE3 tumor characteristics*
- P2 Insufficient: ≤50% alignment with the ICE3 tumor characteristics*
- P3 Different Population

**T1N0M0, ER/PR+, HER2-, Grade I and II*

The study appraisal exercise was performed by two independent reviewers as pre-specified in the PRISMA Systematic Review protocol. The certainty assessment resulted in the downweighing of articles in the meta-analysis that did not offer an "ideal" (P1(a)) match with the ICE3 population. For each study reported as having "sufficient" (P1(b)) alignment with the ICE3 study instead of "ideal", the at-risk sample size was reduced by 25% to decrease its contribution to the meta-analysis.

The goal of this weighting methodology was to promote further alignment of the studies by down-weighting the contribution of studies with less-than-ideal patient groups. This is effectively accomplished by decreasing the at-risk sample size of the studies with P1(b) patient groups in the meta-analysis model. Four articles were appraised as having "sufficient" (P1(b)) alignment and down weighted accordingly: *Fastner (2020), Hughes (2013), Kunkler (2023),* and *Fisher (2002).*

Table 2: Selected Article Summary

Citation	Study Details and Treatment Arm/Cohort	5-Year IBTR
Fastner (2020)[1]	This citation published long-term follow-up data from the randomized, controlled Austrian Breast and Colorectal Cancer Study Group (8A Trial) (N=430). The treatment arm of interest was treated with lumpectomy with endocrine therapy. The average age of the study was slightly lower than that that of the ICE3 cohort (66.1 years of age). Patients' baseline tumor characteristics matched sufficiently with that of the ICE3 population, but less restrictive compared to the ICE3 cohort. *This study was appraised as P1(b) and its contribution downweighed due in the meta-analysis due to sufficient (<90% and >75%) alignment with the ICE3 population tumor characteristics.*	3.6%
Fryles (2004)[2]	This citation published results from a randomized controlled trial conducted at multiple study sites in Canada (N=305). The treatment arm of interest was treated with lumpectomy with endocrine therapy. A subgroup analysis was performed in the study on patients with a "good prognosis" defined as exclusively T1 tumors that were either positive for endocrine receptors or had an unknown endocrine receptor status. This subgroup was used in meta-analysis. Considering this subgroup, patients' baseline characteristics were an excellent match with that of the ICE3 patients . Average age was slightly lower than that of the ICE3 cohort (68 years of age).	5.8%
Hughes (2013)[3]	This citation published long term follow-up data from the Cancer and Leukemia Group B CALGB 9343 randomized, controlled trial (N=319). The treatment arm of interest was treated with lumpectomy with endocrine therapy. The average age of the study was consistent with that of the ICE3 cohort (>75 years of age). Patients ' baseline tumor characteristics matched sufficiently with that of the ICE3 population, but less restrictive compared to the ICE3 cohort. *Therefore, this study was appraised as P1(b) and its contribution downweighed in the meta-analysis due to sufficient (<90% and >75%) alignment with the ICE3 population tumor characteristics.*	4.8%

[1] Fastner, G., et al. (2020). "Endocrine therapy with or without whole breast irradiation in low-risk breast cancer patients after breast-conserving surgery: 10-year results of the Austrian Breast and Colorectal Cancer Study Group 8A trial." Eur J Cancer 127: 12-20.

[2] Fyles, A. W., et al. (2004). "Tamoxifen with or without breast irradiation in women 50 years of age or older with early breast cancer." N Engl J Med 351(10): 963-970.

[3] Hughes, K. S., et al. (2013). "Lumpectomy plus tamoxifen with or without irradiation in women age 70 years or older with early breast cancer: long-term follow-up of CALGB 9343." J Clin Oncol 31(19): 2382-2387.

Citation	Study Details and Treatment Arm/Cohort	5-Year IBTR
Kunkler (2023)[4]	This citation published results of the randomized, controlled PRIME II trial (N=593). The treatment arm of interest was treated with lumpectomy with endocrine therapy. A subgroup analysis was performed in the study of patients with ER-high status. Data from this subgroup was used in meta-analysis. The average age of this study was slightly lower than that of the ICE3 (70.8 years of age). Patients ' baseline tumor characteristics matched sufficiently with that of the ICE3 population, but less restrictive compared to the ICE3 cohort. *Therefore, this study was appraised as P1(b) and its contribution downweighed in the meta-analysis due to sufficient (<90% and >75%) alignment with the ICE3 population tumor characteristics.*	3.9%
Stenmark Tulberg (2021)[5]	This citation published results from the randomized, controlled SweBCG91RT clinical trial. The treatment arm of interest was treated with lumpectomy with or without endocrine therapy. A subgroup analysis was performed in the study on patients >65 years of age with T1N0, ER-positive breast cancer (N=84). Data from this subgroup was used in meta-analysis and offered an excellent comparison to the tumor characteristics of the ICE3 population. Results from this cohort as also detailed in Killander (2016).[6] A low rate of adjunctive endocrine therapy (8% of the study population) was reported in this study. Adjunctive treatment with endocrine therapy has an established effect on local recurrence in endocrine receptor positive tumors. Therefore, the rate of local recurrence is expected to be higher than that of the ICE3 population.	15.4%
Wickberg (2018)[7]	This citation published results of a RCT conducted at multiple study sites in Sweden. The treatment arm of interest was treated with lumpectomy without endocrine therapy. A subgroup analysis was performed in the study on patients with Luminal A, non-lobular cancer and >55 years of age (N=49). No patients received adjunctive endocrine therapy. Adjunctive treatment with endocrine therapy has an established effect on local recurrence in endocrine receptor positive tumors. Therefore, the rate of local recurrence is expected to be higher than that of the ICE3 population.	10.8%

[4] Kunkler, I. H., et al. (2023). "Breast-Conserving Surgery with or without Irradiation in Early Breast Cancer." N Engl J Med 388(7): 585-594.

[5] Stenmark Tullberg, A., et al. (2021). "Immune Infiltrate in the Primary Tumor Predicts Effect of Adjuvant Radiotherapy in Breast Cancer; Results from the Randomized SweBCG91RT Trial." Clin Cancer Res 27(3): 749-758.

[6] Killander, F., et al. (2016). "No breast cancer subgroup can be spared postoperative radiotherapy after breast-conserving surgery. Fifteen-year results from the Swedish Breast Cancer Group randomised trial, SweBCG 91 RT." Eur J Cancer 67: 57-65.

[7] Wickberg, Å., et al. (2018). "Influence of the subtype on local recurrence risk of breast cancer with or without radiation therapy." Breast 42: 54-60.

Citation	Study Details and Treatment Arm/Cohort	5-Year IBTR
Fisher (2002)[8]	This citation published results from a randomized, controlled trial conducted in the US and Canada (N=334). The treatment arm of interest received lumpectomy with adjunctive endocrine therapy. The average age of the treatment cohort was >50 years of age. Patients ' baseline tumor characteristics matched sufficiently with that of the ICE3 population, but less restrictive compared to the ICE3 cohort. *Therefore, this study was appraised as P1(b) and its contribution downweighed in the meta-analysis due to sufficient (<90% and >75%) alignment with the ICE3 population tumor characteristics.*	10.5%
Blamey (2013)[9]	This citation published results of the British Association of Surgical Oncology (BASO) II Trial. Two treatment arms of interest were identified in this 2x2 clinical trial design: **1.** Lumpectomy without radiotherapy and with endocrine therapy (N=95) **2.** Lumpectomy without radiotherapy and without endocrine therapy (N=106) The average age of the two treatment cohorts was 57 years of age. No patients in the second treatment arm received adjunctive endocrine therapy. Adjunctive treatment with endocrine therapy has an established effect on local recurrence in endocrine receptor positive tumors. Therefore, the rate of local recurrence is expected to be higher than that of the ICE3 population in Treatment Arm 2 and lower than ICE3 in Treatment Arm 1.	Arm 1: 2.0% Arm 2: 13.1%
Jagsi (2024)[10]	This citation published results from a single-arm, multicenter, prospective cohort trial (NCT2400190) (N=200). The treatment of interest was treated with lumpectomy with adjunctive endocrine therapy. Results were presented separately for patients 50-59 years of age and patients 60-69 years of age. All patients received adjunctive endocrine therapy. Adjunctive treatment with endocrine therapy has an established effect on local	(50-59): 3.4% (60-69): 0.0%

[8] Fisher, B., et al. (2002) "Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer." N Engl J Med. 2002 Oct 17;347(16):1233-41. doi: 10.1056/NEJMoa022152.

[9] Blamey RW., et al. (2013) "Radiotherapy or tamoxifen after conserving surgery for breast cancers of excellent prognosis: British Association of Surgical Oncology (BASO) II trial." Eur J Cancer. 2013 Jul;49(10):2294-302. doi: 10.1016/j.ejca.2013.02.031.

[10] Jagsi R, Griffith KA, Harris EE, Wright JL, Recht A, Taghian AG, Lee L, Moran MS, Small W Jr, Johnstone C, Rahimi A, Freedman G, Muzaffar M, Haffty B, Horst K, Powell SN, Sharp J, Sabel M, Schott A, El-Tamer M. Omission of Radiotherapy After Breast-Conserving Surgery for Women With Breast Cancer With Low Clinical and Genomic Risk: 5-Year Outcomes of IDEA. J Clin Oncol. 2024 Feb 1;42(4):390-398. doi: 10.1200/JCO.23.02270.

Citation	Study Details and Treatment Arm/Cohort	5-Year IBTR
	recurrence in endocrine receptor positive tumors. Therefore, the rate of local recurrence is expected to be lower than that of the ICE3 population.	
Whelan (2023)[11]	This citation published results from a single-arm, multicenter, prospective cohort trial (N=500). The treatment arm of interest received lumpectomy with adjunctive endocrine therapy and without radiotherapy. The average age of the population was 67.1 years of age. This study included only patients with luminal A tumor characteristics with positive margins, representing a population at lower risk of recurrence compared to the ICE3 population. Additionally, all patients in this cohort were treated with endocrine therapy. Adjunctive treatment with endocrine therapy has an established effect on local recurrence in endocrine receptor positive tumors. Therefore, the rate of local recurrence is expected to be lower than that of the ICE3 population.	2.3%
Rodin (2023)[12]	This citation published a population-based retrospective analysis of administrative database held ICES (formerly known as the institute for Clinical Evaluative Sciences and Cancer Car Ontario (CCO)) (N=703). The treatment arm of interest received lumpectomy with endocrine therapy. Additionally, all patients in this cohort were treated with endocrine therapy. Adjunctive treatment with endocrine therapy has an established effect on local recurrence in endocrine receptor positive tumors. Therefore, the rate of local recurrence is expected to be lower than that of the ICE3 population.	2.8%

[11] Whelan TJ, Smith S, Parpia S, Fyles AW, Bane A, Liu FF, Rakovitch E, Chang L, Stevens C, Bowen J, Provencher S, Théberge V, Mulligan AM, Kos Z, Akra MA, Voduc KD, Hijal T, Dayes IS, Pond G, Wright JR, Nielsen TO, Levine MN; LUMINA Study Investigators. Omitting Radiotherapy after Breast-Conserving Surgery in Luminal A Breast Cancer. N Engl J Med. 2023 Aug 17;389(7):612-619. doi: 10.1056/NEJMoa2302344.

[12] Rodin D, Sutradhar R, Jerzak KJ, Hahn E, Nguyen L, Castelo M, Fatiregun O, Fong C, Mata DGMM, Trebinjac S, Paszat L, Rakovitch E. Impact of non-adherence to endocrine therapy on recurrence risk in older women with stage I breast cancer after breast-conserving surgery. Breast Cancer Res Treat. 2023 Aug;201(1):77-87.

Table 3. Detailed Demographic and Tumor Characteristics

Study Citation	Year	Study Design	Number of Patients Treated[a]	Avg. Age (Range) [Years]	Tumor Characteristics							
					Avg. Tumor Size [cm]	Nottingham Tumor Score / Histological Grade	Size (T-Stage)	Metastasis (M-Stage)	Lymph Nodes (N-Stage)	ER+	PR+	Her2-
ICE3 Clinical Study												
ICE3	-	Single Arm Prospective	194	75.7 (55-94)	≤1.5	Low I – 51% Intermediate II – 49% High – 0%	T1: 100%	M0: 100%	N0: 100%	100%	92.9%	100%
Systematic Review												
Fastner (2020)	2020	RCT	430	66.1 (46-80)	<2[f]	Grade x: 8.4% Grade I: 31.4% Grade II: 60.1%	T1: 89.4%	M0: 100%	N0: 100%	97.4%	79.3%	90.8%
Fyles (2004)	2004	RCT	305	68[b]	<2[f]	NR	T1: 100%	M0: 100%	N0: 100%	100%	100%	NR
Hughes (2013)	2013	RCT	319	>75	<2[f]	NR	T1: 100%	M0: 100%	N0: 100%	97%	77%	NR
Kunkler (2023)	2023	RCT	593	70.8 (67-73)	<2[f]	Grade I: 40.6% Grade II: 55.1% Grade III: 3.4%	T1: 87.4%	M0: 100%	N0: 100%	NR[d]	NR[d]	NR
Stenmark Tullberg (2021)	2021	RCT	84	>65	1.2	NR[e]	T1:100%	M0: 100%	N0: 100%	100%	100%	NR
Wickberg (2018)	2018	RCT	49	61	<2[f]	NR	T1: 100%	M0: 100%	N0: 100%	100%	100%	100%
Fisher (2002)	2002	RCT	334	>50[c]	<1[f]	NR	T1: 100%	M0: 100%	N0: 100%	82%	NR	NR
Blamey (2013)	2013	RCT	Treatment Arm (a): 95 Treatment Arm (b): 106	57 (33-69)	<2[f]	Grade I: 100%	T1: 100%	M0: 100%	N0: 100%	NR	NR	NR

Study Citation	Year	Study Design	Number of Patients Treated[(a)]	Avg. Age (Range) [Years]	Tumor Characteristics							
					Avg. Tumor Size [cm]	Nottingham Tumor Score / Histological Grade	Size (T-Stage)	Metastasis (M-Stage)	Lymph Nodes (N-Stage)	ER+	PR+	Her2-
Jagsi (2024)	2024	Prospective Cohort	200	62 (50-69)	1.0	Grade 1: 42.5% Grade 2: 54.5% Grade 3: 3%	T1: 100%	M0: 100%	N0: 100%	100%	100%	100%
Whelan (2023)	2023	Prospective Cohort	500	67.1 (62.9-71.6)	1.0	Grade 1: 66% Grade 2: 34%	T1: 100%	M0: 100%	N0: 100%	100%	100%	100%
Rodin (2023)	2023	Retrospective Analysis	703	74 (69-74)	<2.0	Low I: 34.5% Intermediate II: 54.3% High III 8.4%	T1: 100%	M0: 100%	N0: 100%	98.1%	89.7%	100%

(a) Number of patients treated within treatment arm and subgroup of interest (if applicable)
(b) Fyles 2004 reported median age
(c) Fisher 2002 did not provide an average age for the treatment cohorts of interest; however, based on the inclusion/exclusion criteria of the study, the average age was over >50 years
NR = not reported

All eleven (11) studies were assessed by two independent reviewers via the Cochrane risk-of-bias tool for randomized trials (RoB 2) or Cochrane risk-of-bias tool for non-randomized trials (ROBINS-I) tool and were concluded to be at low risk for bias.

The results from the PRISMA Systematic Review and Meta-analysis represent the established and accepted outcomes of the existing standard of care treatment lumpectomy without radiotherapy and constitute a valid reference rate for comparison to investigational treatments. The PRISMA literature review derived comparator rate resulted in an **estimated 5-year IBTR rate of 3.52% with a 95% CI from 2.08% to 5.77%.**

The meta-analysis interval provided a two-sided 95% CI of 2.08% to 5.77%. The true overall survival rate can be as small as 2.08% or as large as 5.77%. The CI was calculated using a Monte-Carlo (MC) approach to generate the 95% interval defined as the 2.5% and 97.5% percentiles from 10,000 sample.

The IBTR rates of from the PRISMA selected literature and the ICE3 clinical study are summarized in **Figure 3.** All articles were published in the last 25 years (oldest published in 2002), with 58.3% (7/12) published in the 5 years.

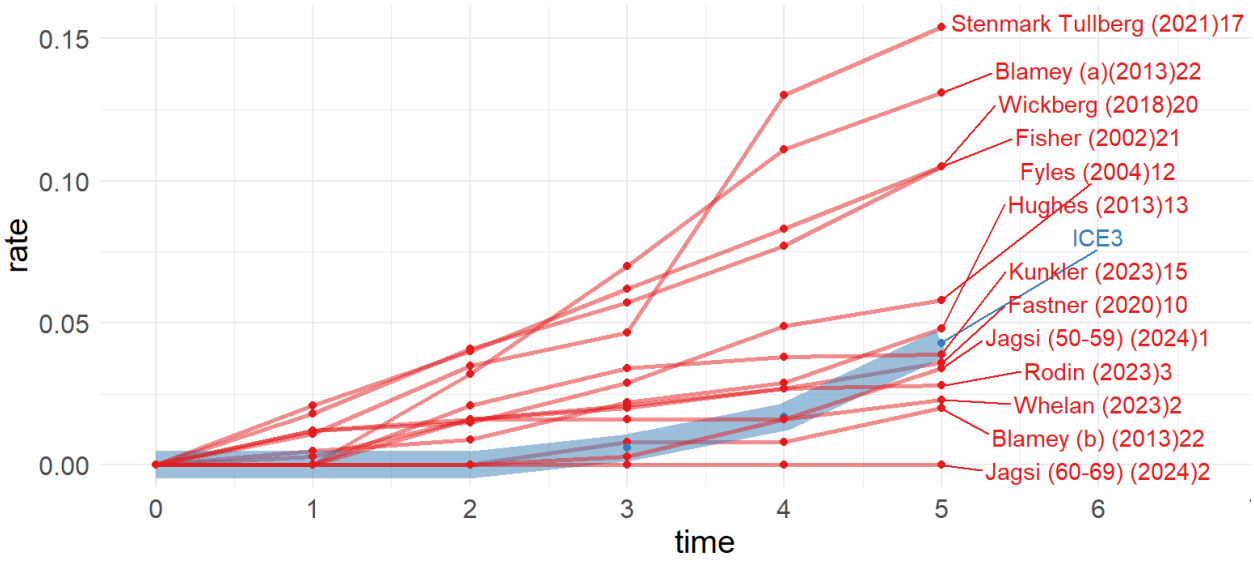


Figure 3. Ipsilateral Breast Tumor Recurrence (IBTR) Rate Up to 5-years for the ICE3 Clinical Study and Each Study Included in the PRISMA Systematic Literature Review and Meta-analysis

Table 4. Pooled Rate for the Probability of Recurrence up to 5-years with Confidence Intervals Based on Reported Recurrence Rates Included in the Meta-Analysis Using a Random Effects Meta-analysis Model Adjusted (Weighted) for Sample Size Based on Appraisal of the Patient Group for Alignment with the ICE3 Study.

Time	Pooled Rate (Recurrence Probability)	95% LB [2]	95% UB [2]	90% LB [3]	90% UB [3]
Year 0 [1]	0.0000	--	--	--	--
Year 1	0.0038	0.0008	0.0100	0.011	0.089
Year 2	0.0106	0.0043	0.0214	0.0053	0.0193
Year 3	0.0184	0.0094	0.0330	0.0107	0.0304
Year 4	0.0282	0.0157	0.0471	0.0176	0.0443
Year 5	**0.0352**	**0.0208**	**0.0577**	**0.0228**	**0.0536**

Notes:
[1] Modeled as 0.001. This has no impact on the results.
[2] 2.5% to 97.5% distribution-free CI.
[3] 5% to 95% distribution-free CI.

The 5-year IBTR in the ICE3 Primary Analysis Set is 4.3% (95% CI UB 8.7%). Therefore, the ICE3 clinical study primary outcome is acceptable when compared to the PRISMA derived comparator 5-year recurrence rate of 3.52% with a 95% CI from 2.08% to 5.77%.

Results (Sensitivity Analysis)

IceCure performed a sensitivity analysis of the PRISMA Systematic Review and Meta-analysis results to evaluate the 5-year IBTR rate excluding studies without the use of adjuvant endocrine therapy. The purpose of this sensitivity analysis is to determine the ipsilateral breast tumor recurrence (IBTR) rate in low-risk, early-stage, node-negative, ER/PR positive, and HER2-negative breast cancers treated with BCS **without** adjunctive radiation **and with adjunctive hormone therapy.** Studies in which <50% of subjects received adjective hormone therapy were excluded from the sensitivity analysis. Accordingly, Stenmark Tullberg (2021), Wickberg (2018), and Blamey (2013) (a) were excluded in the results presented below.

Table 5. Pooled Rate for the Probability of Recurrence up to 5-years with Confidence Intervals Based on Reported Recurrence Rates Included in the Meta-Analysis Using a Random Effects Meta-analysis Model Adjusted (Weighted) for Sample Size Based on Appraisal of the Patient Group for Alignment with the ICE3 Study – Endocrine Therapy-Only Subgroup Sensitivity Analysis

Time	Pooled Rate (Recurrence Probability)	95% LB [2]	95% UB [2]	90% LB [3]	90% UB [3]
Year 0 [1]	0.0000	--	--	--	--
Year 1	0.0033	0.0005	0.0100	0.0008	0.0088
Year 2	0.0087	0.0032	0.0192	0.0039	0.0177
Year 3	0.0152	0.0072	0.0292	0.0085	0.0271
Year 4	0.0226	0.0123	0.0397	0.0139	0.0371
Year 5	**0.0282**	**0.0162**	**0.0483**	**0.0183**	**0.0451**

Notes:

[1] Modeled as 0.001. This has no impact on the results.

[2] 2.5% to 97.5% distribution-free CI.

[3] 5% to 95% distribution-free CI.

The statistical methods employed demonstrate that the applicable IBTR rate in low-risk, early-stage, node-negative, ER/PR positive, and HER2-negative breast cancers treated with BCS without adjunctive radiation and with hormone therapy at 5-years follow-up is observed to be 2.82% with a 95% CI from 1.62% to 4.83%.