Appendix C – Key ICE3 Study Protocol Modifications

The first version of the clinical protocol used in the clinical study was Version 13, dated May 11, 2014. During the course of the study, the protocol was amended to include changes to the eligibility criteria. The key changes and their associated date are listed below.

Changes from Protocol 13 to Protocol 15

	Protocol 13 (11-May-2014)	Protocol 15 (23-May-2015)	Rationale for changes
Inclusion Criteria	Nottingham grade 1-2	Nottingham grade 1-2. Specifically, nuclear and mitotic scores must be less than or equal to 2.	The investigators and DSMB recommended that the Nottingham score and its components are what define the level of risk and are sufficient to replace the Ki-67 <14% inclusion criterion.
	Estrogen receptor positive, progesterone receptor positive, HER2 negative, Ki-67<14%	Estrogen receptor positive, and or progesterone receptor positive, HER2 negative	Per the company, it was determined that low risk is characterized by the presence of ER and/or PR and the absence of HER, and that Ki-67 is not a test routinely or consistently obtained throughout the USA. Its reliability as an indicator of low risk is inconclusive in the literature,[1] whereas histologic grade is evaluated routinely with every histopathologic exam, do not add to testing costs, and provide similar proliferation rate information.[2]
		History of previously treated ipsilateral or contralateral breast carcinoma is not an exclusion criteria if the investigator is certain newly diagnosed carcinoma is new unifocal primary tumor.	Per clinicaltirals.gov, the inclusion criteria appeared first on record on 2015-07-23. Protocol 15 (23-May-2015) was not on the record at clinicaltrials.gov.
Exclusion Criteria		Nottingham score of 3 (specifically nuclear and or mitotic score >2)	Same as above for Nottingham grade inclusion criteria
	ER/PR negative, Her2 positive, or Ki-67>14% noted on pre-cryo biopsy	ER AND PR negative, or Her2 positive noted on pre-cryo biopsy	Same as above for receptor status inclusion criteria

[1] Louis DM, Nair LM, Vallonthaiel AG, Narmadha MP, Vijaykumar DK. Ki 67: a Promising Prognostic Marker in Early Breast Cancer-a Review Article. Indian J Surg Oncol. 2023 Mar;14(1):122-127.

[2] Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, Fox SB, Ichihara S, Jacquemier J, Lakhani SR, Palacios J, Richardson AL, Schnitt SJ, Schmitt FC, Tan PH, Tse GM, Badve S, Ellis IO. Breast cancer prognostic classification in the molecular era: the role of histological grade. Breast Cancer Res. 2010;12(4):207. doi: 10.1186/bcr2607. Epub 2010 Jul 30.

Changes from Protocol 15 to Protocol 16

	Protocol 15 (23-May-2015)	Protocol 16 (01-May-2017)	Rationale for Changes
Inclusion Criteria	Age ≥65	Age ≥60	The age was reduced to increase enrollment rate and still include patients with a low risk profile.

Changes from Protocol 16 to Protocol 17

	Protocol 16 (01-May-2017)	Protocol 17 (28-Jun-2017)	Comments
Inclusion Criteria	Age ≥65	Age ≥50	Local IRB only