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**NCCN Guidelines Panel:** Breast

Dear Panel Members,

On behalf of Natera, I respectfully request the NCCN® Breast Cancer Guideline Panel consider the requested updates and enclosed references, pertaining to the meeting taking place on August 12<sup>th</sup>, 2021.

- a. Operable Disease: Surgical Treatment and Adjuvant Therapy After Preoperative Treatment in BINV-14
- b. Inoperable or Locally Advanced Disease: Surgical Treatment and Adjuvant Therapy After Preoperative Treatment in BINV-15
- c. Adjuvant Systemic Therapy After Preoperative Systemic Therapy in BINV-16
- d. Recurrent/Stage IV (M1) Disease in BINV-18
- e. Principles of monitoring metastatic disease in BINV-S 1 of 3. [BINV-R 1 of 3 is too crowded]
- f. Assessment of response to preoperative systemic treatment in inflammatory breast cancer in IBC-1 and IBC-2
- g. Systemic adjuvant treatment: HR-Negative - HER2-Negative Disease in BINV-10

**Specific Changes:**

- a) *We respectfully request that tumor-informed ctDNA be considered as a test to monitor for preoperative systemic therapy treatment response in BINV-14. Proposed modification of footnote uu in BINV-14: "The accurate assessment of in-breast tumor or regional lymph node response to preoperative systemic therapy is difficult and should include physical examination and performance of imaging studies mammogram and/or breast ultrasound and/or breast MRI) that were abnormal at the time of initial tumor staging. Selection of imaging methods prior to surgery should be determined by the multidisciplinary team. Tumor-informed ctDNA dynamics can complement imaging modalities to monitor disease burden and treatment response."*
- b) *We respectfully request that tumor-informed ctDNA be considered as a test to monitor for preoperative systemic therapy treatment response in BINV-15. Proposed modification of footnote uu in BINV-15: "The accurate assessment of in-breast tumor or regional lymph node response to preoperative systemic therapy is difficult and should include physical examination and performance of imaging studies mammogram and/or breast ultrasound and/or breast MRI) that were abnormal at the time of initial tumor staging. Selection of imaging methods prior to surgery should be determined by the multidisciplinary team. Tumor-informed ctDNA dynamics can complement imaging modalities to monitor disease burden and treatment response."*
- c) *We respectfully request that the presence of post-surgical tumor-informed ctDNA be added as a high-risk factor for recurrence in HR-negative/HER2-negative to consider capecitabine in BINV-16. Proposed modification of footnote yy in BINV-16: "Presence of post-surgical tumor-informed ctDNA is associated with a high risk of recurrence. Recommendations do not apply to residual DCIS (ypTis)."*
- d) *We respectfully request that presence of tumor-informed ctDNA be added as a Workup addition consideration in Recurrent/Stage IV (M1) Disease. Proposed modification of footnote ddd in BINV-18: "In clinical situations where a biopsy cannot safely be obtained but the clinical evidence is strongly supportive of recurrence, treatment may commence based on the ER/PR/HER2 status of the primary tumor. Detection of tumor-informed ctDNA can confirm presence of recurrent disease."*
- e) *We respectfully request that increasing levels of tumor-informed ctDNA be considered as a finding concerning for disease progression and lack of immunotherapy benefit in BINV-S 1 of 3. Proposed*

*modification of “Definition of Disease Progression” Section is addition of bullet point stating: “Increasing levels of tumor-informed ctDNA is associated with progression of disease and lack of immunotherapy treatment benefit.”*

- f) We respectfully request that tumor-informed ctDNA be considered as a test to monitor for preoperative systemic therapy treatment response in IBC-1 and IBC-2. Proposed modification of footnote j in IBC-1: “The accurate assessment of in-breast tumor or regional lymph node response to preoperative systemic therapy is difficult and should include physical examination and performance of imaging studies mammogram and/or breast ultrasound and/or breast MRI) that were abnormal at the time of initial tumor staging. Selection of imaging methods prior to surgery should be determined by the multidisciplinary team. Tumor-informed ctDNA dynamics can complement imaging modalities to monitor disease burden and treatment response.”*
- g) We respectfully request that the presence of post-surgical tumor-informed ctDNA be added as a high-risk factor for recurrence in HR-negative, HER2-negative disease. Proposed modification of footnote cc in BINV-10: “There are limited data to make chemotherapy recommendations for those >70 y of age. Presence of post-surgical tumor-informed ctDNA is associated with a high risk of recurrence.”*

**FDA Clearance:** A personalized and tumor-informed 16-plex PCR, next generation sequencing assay measuring circulating tumor DNA assay is a laboratory developed test (LDT) performed in the central laboratory of Natera, Inc., which is ISO 13485-certified and is regulated under the Clinical Laboratory Improvement Amendments (CLIA) and the College of American Pathologists (CAP).

On May 3, 2019, FDA granted a breakthrough device designation for this personalized and tumor-informed 16-plex PCR, next generation sequencing assay in patients with localized and advanced colorectal cancer.

On August 22, 2019, Medicare published a proposed Local Coverage determination for this personalized and tumor-informed 16-plex PCR, next generation sequencing assay for patients with stage II/III CRC.

**Rationale:** Circulating tumor DNA (ctDNA) provides a direct measurement of residual molecular or micro-metastatic disease that originated directly from the tumor. Below we provide supporting evidence from literature that highlights the feasibility of ctDNA testing for breast cancer in the neoadjuvant and adjuvant setting, demonstrating **(a)** the presence of post-surgical ctDNA (MRD detection) to be associated with high-risk of recurrence, **(b)** the association of ctDNA with tumor burden and aggressive tumor biology, **(c)** the clinical implications of ctDNA clearance, which can predict pCR to treatment, and **(d)** the association of ctDNA-positivity after NAT with patient outcomes.

**Adjuvant Setting:** In a study published in clinical cancer research (Coombes et al. 2019), the authors demonstrated ctDNA analysis through Signatera to be a robust biomarker for disease surveillance in breast cancer patients. The study showed presence of post-surgical ctDNA at first time point and in follow-up plasma samples to be significantly associated with poorer prognosis (HR, 11.8; 95% CI: 4.3-32.5 and HR, 35.8; 95% CI: 8.0-161.3), respectively. All ctDNA-positive patients relapsed within 50 months after surgery and molecular relapse through ctDNA analysis was detected up to 2 years prior to clinical relapse with a median of 266 days (range: 14-721 days) or 8.9 months (range: 0.5–24.0 months). On monitoring ctDNA with other monitoring tests, such as CA 15-3, interestingly for patients with CA 15-3 positive status, ctDNA was detected on average over 200 days ahead of significant CA 15-3 levels. The test showed a sensitivity and specificity of 89% and 100%, respectively.

**Neoadjuvant Setting:** Based on the results obtained from the I-SPY 2 TRIAL, (Magbanua et al, 2020), published in Annals of Oncology, the significance of serial ctDNA testing for predicting pCR and risk of recurrence is clearly demonstrated. The study showed that the lack of ctDNA clearance after NAC was significantly associated with metastatic recurrence (HR: 22.4, 95% CI 2.5-201, p<0.001). All the patients who achieved pCR did not have a positive Signatera test before the surgery, while ctDNA-positive patients who did not achieve pCR had worse distant-recurrence free survival (DRFS) (HR: 10.4, 95%CI 2.3-46.6). More importantly, the study showed that the prognostic impact of ctDNA detection was the strongest after NAC (before surgery) for risk stratification of patients. This highlights the potential utility of monitoring

ctDNA dynamics to guide treatment in the adjuvant setting. This association remained significant for the sub-cohort of TNBC patients (HR=11.4, 95% CI: 3.6-35.4, p=0.0001).

**Neoadjuvant Setting:** Further exploratory work from the I-SPY2 TRIAL (Magbanua et al, 2021), published in NPJ Breast Cancer demonstrated that combining serial ctDNA monitoring with magnetic resonance imaging (MRI)-based functional tumor volume (FTV) analysis in high-risk patients significantly improved prediction of pCR and DRFS (p<0.05). The study showed that ctDNA-positivity was significantly associated with higher FTV (p<0.05), also ctDNA dynamics during the course of treatment correlated with changes in FTV (p<0.05). Importantly, the study demonstrated that at the post-NAC timepoint, both ctDNA (HR: 11.50, 95% CI 2.87-46.14, p=0.0006) and FTV (HR: 1.03, 95% CI 1.01-1.04, p=0.0005) were significantly prognostic of DRFS. Furthermore, adding ctDNA-positivity to FTV at this time point showed a strong positive relationship for prediction of pCR (p=0.002). Thus, highlighting that imaging modalities like MRI could potentially be complemented with ctDNA analysis to monitor disease burden and treatment response for guiding therapeutic decision-making in the adjuvant setting.

**Table** summarizing key studies in neoadjuvant and adjuvant settings in both early-and advanced-stage breast cancers, which show the presence of ctDNA, as determined by different methodologies, to be associated with poor outcomes. In most of the studies listed below, ctDNA status was independently associated with the outcome after adjusting for known clinicopathologic risk factors.

**Table: Association of ctDNA with clinical outcomes in breast cancer**

Reference	Treatment Setting	Cancer type and stage	No. of patients enrolled	ctDNA detection technique	Clinical outcome: Hazard Ratio, p-value	Percentage of ctDNA-positive patients at MRD who eventually relapsed
Magbanua MJM, <i>et al</i> , 2021, NPJ Breast	NAT	BC, Stages II-III	84	Signatera™	<b>DRFS:</b> 11.5, p=0.0006	-
Magbanua MJM, <i>et al</i> , 2020, Ann Oncol	NAT	BC, Stages II-III	84	Signatera™	<b>DRFS:</b> 11.5, p=0.001	90% (9/10)
Coombes RC, <i>et al</i> , 2019, Clin Canc Res	NAT and Adjuvant	BC, Stages I-III	49	Signatera™	<b>RFS:</b> <i>Post-surgical:</i> 11.8, P<0.001 <i>Longitudinal:</i> 35.80, P<0.001	89% (16/18)
Martinez-Saez O, <i>et al</i> , 2021, NPJ Br Canc	NAT	HR+/HE R2-, MBC	45	74-gene panel Guardant360 V2.11	<b>PFS:</b> 0.31, P=0.049	25% (5/20)
Zhou Y, <i>et al</i> , 2021, Br Canc Res Treat	NAT	BC, Stages IIb-IV	32	NGS panel (1021 genes)	<b>DFS:</b> 23.53, P<0.0001	100% (5/5)

Darrigues L, <i>et al</i> , 2021, Br Canc Res	NAT	MBC	25	ddPCR	<b>PFS:</b> 5.1, P=0.02 Longitudinal: 7.2, P=0.004	100% (5/5)
Muendlein A, <i>et al</i> , 2021, Sci Reports	NAT	ER+/HER2-, MBC	59	PCR	<b>PFS:</b> 2.0, P<0.001 (ctDNA load) <b>OS:</b> 1.93, P=0.001 (ctDNA load)	84% (32/38)
O’Leary B, <i>et al</i> , 2021, J Nat Canc Inst	NAT	ER+, Stages II-III	521	dPCR	<b>PFS:</b> 1.2, P <0.001	42% (131/310)
Cavallone L, <i>et al</i> . 2020, Sci Reports	NAT	TNBC, Stages I-III	26	ddPCR	<b>RFS:</b> 0.29, P=0.046 <b>OS:</b> 0.27, P=0.043	50% (5/10)
Li S, <i>et al</i> , 2020, JCO-PO	NAT	BC, Stages I-III	52	NGS panel (1021 genes)	<b>DFS:</b> 5.72, P= 0.011 <b>OS:</b> 11.27, P=0.004	50% (7/14)
Radovich BJ, <i>et al</i> . 2020, JAMA Oncol	NAT	TNBC, Stages I-III	196	Safe-SeqS	<b>DDFS:</b> 2.99, P=0.006 <b>DFS:</b> 2.67, P=0.009 <b>OS:</b> 4.16, P=0.002	79% (23/29)
Yoshinami T, <i>et al</i> . 2020, Transl Oncol	NAT	BC, Stages I-II	62	Molecular barcode (MB) NGS	<b>DDFS:</b> 9.34, P=0.004	57% (4/7)
Cullinane C, <i>et al</i> . 2020, JAMA Open	NAT	BC, Stages I-III	739 (meta-analysis from 8 studies)	PCR, NGS	<b>DFS:</b> 4.44, P<0.001	-
Butler TM, <i>et al</i> , 2019, Cold Spr Harb Mol Case Stud	NAT	BC, Stages I-III	10	Safe-SeqS, Dual-Indexed Degenerate Adapter (DIDA)	<b>pCR:</b> P<0.02	100% (2/2)
Hrebian S, <i>et al</i> , 2019, Annals Oncol	NAT	ER+, MBC	42	ddPCR	<b>PFS:</b> 0.20, P<0.0001	74% (26/35)
McDonald BR, <i>et al</i> . 2019, Sci Transl Med	NAT	BC, Stages I-III	33	Targeted digital sequencing (TARDIS)	<b>pCR:</b> P=0.0057	77% (17/22)

Garcia-Murillas I, <i>et al.</i> 2019, JAMA Oncol	NAT and Adjuvant	BC, Stages I-III	101	dPCR	<b>RFS:</b> <i>Baseline:</i> 5.8, P=0.01 <i>Longitudinal:</i> 25.2, P<0.001	79.3% (23/29)
Rothé F, <i>et al.</i> 2019, Clin Canc Res	NAT	HER2-amplified, Stages I-III	69	ddPCR	<b>pCR:</b> 0.15, P=0.0089	15% (6/40)
Li H, <i>et al.</i> 2018, Am J Canc Res	NAT	BC, Stages I-III	65	NGS identifying <i>PIK3CA</i> mutations	<b>PFS:</b> P=0.006 <b>OS:</b> P=0.043	5/11
Chen Y-H, <i>et al.</i> 2017, NPJ Br Canc	NAT	TNBC, Stages I-III	38	Oncomine Research Panel	<b>DFS:</b> 12.6, P<0.0001	31% (4/13)
Riva F, <i>et al.</i> 2017, Clin Chem	NAT	TNBC, Stages I-III	36	ddPCR	<b>DFS:</b> P<0.001 <b>OS:</b> P=0.0006	100% (3/3)
Garcia-Murillas I, <i>et al.</i> 2015, Sci Transl Med	NAT	BC, Stages I-III	55	dPCR	<b>RFS:</b> <i>Post-surgical:</i> 25.1, p<0.0001 <i>Longitudinal:</i> 12.0, p<0.0001	80% (12/15) 50% (6/12)
Olsson <i>et al.</i> 2015, EMBO Mol Med	Adjuvant	BC, Stages I-III	20	ddPCR	<b>PFS (odds ratio):</b> 2.1, P=0.02 <b>OS (odds ratio):</b> 1.3, P=0.04	92.8% (13/13)
Dawson <i>et al.</i> 2013, NEJM	Adjuvant	MBC	30	Tagged-amplicon deep sequencing	OS (p<0.001)	17/19 (89%)
Fernandez-Garcia <i>et al.</i> 2019, Breast Cancer Research	Adjuvant	MBC	194	qPCR	PFS (HR: 1.193, p=0.042)	-
Ye <i>et al.</i> 2019, European Journal of Cancer	Adjuvant	MBC	117	qPCR, Qubit fluorometer	PFS (HR: 2.37)	-
Guan <i>et al.</i> 2020, The Breast	NAT, Adjuvant, Palliative	BC, Stages II-III, and MBC (or Stage IV)	105	NGS	PFS (p<0.001)	17/18 (94.4%)

**Abbreviations:** BC, Breast cancer; MBC, metastatic breast cancer; RFS, Recurrence free survival; OS: overall survival; DFS, disease-free survival; DDFS, distant disease-free survival; NAT, neoadjuvant, HER2, human epidermal growth factor receptor 2; ER, estrogen receptor

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