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Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: the TONIC trial

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Supplementary Note

Eligibility criteria:

- ◆ Metastatic or incurable locally advanced triple negative breast cancer with confirmation of ER and HER2 negativity (ER <10% and HER2 0,1 or 2 in the absence of amplification as determined by in situ hybridization) on a histological biopsy of a metastatic lesion
- ◆ Metastatic lesion accessible for histological biopsy (Mandatory biopsies: pre-induction treatment, post-induction treatment, 6-weeks. Optional biopsies: 12-weeks, at progression, of irradiated site). The pre-induction treatment biopsy has to contain sufficient tumor content (≥ 100 tumor cells); subjects with samples that have insufficient tumor content will require re-biopsy prior to induction treatment. Interval between last systemic treatment and pre-induction biopsy has to be at least 14 days
- ◆ 18 years or older
- ◆ Maximum of three lines of chemotherapy for metastatic disease and with evidence of progression of disease
- ◆ Measurable or evaluable disease according to RECIST 1.1
- ◆ Metastatic lesion accessible for radiation with 1x20Gy or 3x8Gy
- ◆ No uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris
- ◆ Subjects with brain metastases are eligible if these are not symptomatic. Subjects who received prior treatment for brain metastases should be free of progression on magnetic resonance imaging (MRI) for at least 4 weeks after treatment is completed and prior to first dose of study drug administration. There must also be no requirement for immunosuppressive doses of systemic corticosteroids (> 10 mg/day prednisone equivalents) for at least 2 weeks prior to study drug administration
- ◆ No known history of leptomeningeal disease localization
- ◆ No history of having received other anticancer therapies within 2 weeks of start of the study drug
- ◆ No history of immunodeficiency, autoimmune disease, conditions requiring immunosuppression (>10 mg daily prednisone equivalents) or chronic infections. Subjects with vitiligo, diabetes mellitus type I, psoriasis not requiring systemic treatment or resolved childhood asthma/atopy would be an exception to this rule. Subjects that require intermittent use of bronchodilators, inhaled steroids, or local steroid injections would not be excluded from the study. Subjects with hypothyroidism stable on hormone replacement, Sjögren's syndrome or conditions not expected to recur in the absence of an external trigger will not be excluded from the study. Adrenal replacement doses >10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease
- ◆ No prior treatment with an anti-PD-1, anti-PD-L1, anti-PD-L2, or anti-CTLA-4 antibody
- ◆ No live vaccine within 30 days of planned start of study therapy.

- ◆ No active other cancer.
- ◆ No positive test for hepatitis B surface virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV antibody) indicating acute or chronic infection
- ◆ WHO performance status of 0 or 1 (Appendix B)
- ◆ No history of uncontrolled serious medical or psychiatric illness
- ◆ Absence of any psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule
- ◆ No current pregnancy or breastfeeding. Women of childbearing potential (WOCBP*) must use adequate contraceptive protection. WOCBP should use an adequate method to avoid pregnancy for 23 weeks (30 days plus the time required for nivolumab to undergo five half-lives) after the last dose of investigational drug. WOCBP must have a negative serum or urine pregnancy test
- ◆ $WBC \geq 2.0 \times 10^9/L$, $ANC \geq 1.5 \times 10^9/L$, platelets $\geq 100 \times 10^9/L$, Hemoglobin ≥ 5.0
- ◆ Bilirubin $< 1.5 \times$ upper limit of the normal range (ULN), except subjects with Gilbert Syndrome; alkaline phosphatase $< 2.5 \times$ ULN ($< 5 \times$ ULN in case of liver metastases, and $< 7 \times$ ULN in case of bone metastases); transaminases (ASAT/ALAT) $< 3 \times$ ULN (and $< 5 \times$ ULN in case of liver metastases), LDH $< 2 \times$ ULN
- ◆ Calculated (Cockcroft-Gault) or measured creatinine clearance > 40 mL/min
- ◆ Signed written informed consent

*) Women of childbearing potential” is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or who is not postmenopausal. Menopause is defined clinically as 12 months of amenorrhea in a woman over 45 in the absence of other biological or physiological causes.

Supplementary Table 1. Baseline characteristics of the intention-to-treat population per cohort. Clinical baseline characteristics of all allocated patients separated by induction treatment cohort (n = 70).

	Cohort 1, Control/no induction (n = 12)	Cohort 2, Irradiation (n = 14)	Cohort 3, Cyclophosphamide (n = 13)	Cohort 4, Cisplatin (n = 14)	Cohort 5, Doxorubicin (n = 17)
Median age, years (range)	53 (33-69)	47 (33-68)	51 (35-68)	53 (41-70)	46 (29-68)
Female, n (%)	12 (100)	14 (100)	13 (100)	14 (100)	17 (100)
WHO performance status, n (%)					
0	7 (58)	6 (43)	6 (46)	8 (57)	14 (82)
1	5 (42)	8 (57)	7 (54)	6 (43)	3 (18)
gBRCA1/2, n (%)					
Mutation	3 (25)	0 (0)	0 (0)	1 (7)	2 (12)
Wildtype	7 (58)	12 (86)	11 (85)	8 (57)	12 (71)
Unknown	2 (17)	2 (14)	2 (15)	5 (36)	3 (18)
Location of metastasis, n (%)					
Lymph node only	1 (8)	1 (7)	0 (0)	0 (0)	4 (24)
Visceral metastasis	7 (58)	12 (86)	11 (85)	10 (71)	10 (59)
Other metastasis	4 (33)	1 (7)	2 (15)	4 (29)	3 (18)
No. of prior therapies for metastatic disease, n (%)					
0	4 (33)	4 (29)	2 (15)	1 (7)	6 (35)
1	6 (50)	7 (50)	6 (46)	7 (50)	8 (47)
2-3	2 (17)	3 (21)	5 (38)	6 (43)	3 (18)
Previous neoadjuvant or adjuvant therapy, n (%)	11 (92)	13 (93)	11 (85)	10 (71)	14 (82)
Previous chemotherapy exposure, n (%)					
Taxane	11 (92)	13 (92)	12 (93)	12 (86)	16 (94)
Anthracycline	11 (92)	14 (100)	11 (85)	10 (71)	14 (82)
Platinum	12 (100)	7 (50)	5 (38)	8 (57)	10 (59)
Capecitabine	6 (50)	6 (43)	8 (62)	8 (57)	6 (35)
Disease free interval, n (%)					
De novo metastatic disease	1 (8)	1 (7)	2 (15)	3 (21)	2 (12)
Disease free interval ≤ 12 months	4 (33)	8 (57)	5 (38)	2 (14)	4 (24)
Disease free interval > 12 months	7 (58)	5 (36)	6 (46)	9 (64)	11 (65)
LDH level, n (%)					
≤ ULN	7 (58)	7 (50)	8 (62)	6 (43)	11 (65)
> 2x ULN	5 (42)	7 (50)	5 (38)	8 (57)	6 (35)
Stromal tumor infiltrating lymphocytes, n (%)					
Not available	0 (0)	2 (14)	0 (0)	1 (7)	2 (12)
< 5%	4 (33)	7 (50)	4 (31)	4 (29)	6 (35)
≥ 5%	8 (67)	5 (36)	9 (69)	9 (64)	9 (53)
PD-L1 expression on tumor cells, n (%)					
Not available	0 (0)	2 (14)	1 (8)	1 (7)	1 (6)
≥ 1% on tumor cells	8 (67)	10 (71)	9 (69)	8 (57)	9 (53)
≥ 5% on tumor cells	3 (25)	7 (50)	5 (38)	4 (29)	4 (24)
PD-L1 expression on immune cells, n (%)					
Not available	0 (0)	2 (14)	1 (8)	1 (7)	1 (6)
≥ 1% on immune cells	11 (92)	10 (71)	12 (92)	12 (86)	15 (88)
≥ 5% on immune cells	9 (75)	8 (57)	8 (62)	11 (79)	11 (65)
No. of nivolumab cycles, median (range)	5 (2-20)	2 (0-24)	5 (1-16)	6 (0-42)	6 (1-37)

Supplementary Table 2. Baseline characteristics of the efficacy analysis population.
Clinical baseline characteristics of the patients included in the efficacy analysis (n = 66).

	Efficacy analysis population (n = 66)	
Median age, years (range)	51 (29-70)	
WHO performance status, n (%)		
0	39	59%
1	27	41%
gBRCA1/2, n (%)		
Mutation	6	9%
Wildtype	47	71%
Unknown	13	20%
Location of metastasis, n (%)		
Lymph node only	6	9%
Visceral metastasis	47	71%
Other metastasis	13	20%
No. of prior therapies for metastatic disease, n (%)		
0	15	23%
1	33	50%
2-3	18	27%
Previous neoadjuvant or adjuvant therapy, n (%)	56	85%
Previous chemotherapy exposure, n (%)		
Taxane	60	91%
Anthracycline	56	85%
Platinum	38	58%
Capecitabine	33	50%
Disease Free Interval, n (%)		
De novo metastatic disease	8	12%
Disease Free Interval ≤ 12 months	21	32%
Disease Free Interval > 12 months	37	56%
LDH level, n (%)		
≤ ULN	38	58%
≤ 2x ULN	28	42%
PD-L1 expression on tumor cells, n (%)		
Not available	2	3%
≥ 1% on tumor cells	41	62%
≥ 5% on tumor cells	23	35%
PD-L1 expression on immune cells, n (%)		
Not available	2	3%
≥ 1% on immune cells	59	89%
≥ 5% on immune cells	46	70%

Supplementary Table 3. Maximum grade of nivolumab or induction treatment-related adverse events. Maximum grade of treatment-related adverse event, separated by induction treatment cohort. Denoted are the maximum grade adverse events during nivolumab treatment and during induction treatment.

¹Two patients with clear evidence of progression died on study. One patient died from bacterial peritonitis probably due to progressive intestinal metastasis. One patient with progressive pleural fluid and lymphangitic carcinomatosis died from acute respiratory insufficiency not otherwise specified while being hospitalized.

	Any grade, n (%)	Grade 3, n (%)	Grade 4, n (%)	Grade 5, n (%)
Induction related (n = 68)	19 (28)	2 (3)	0	0
Control/no induction (n = 12)	1 (8)	0	0	0
Irradiation (n = 13)	3 (23)	0	0	0
Cyclophosphamide (n = 13)	3 (23)	1 (8)	0	0
Cisplatin (n = 13)	8 (62)	1 (8)	0	0
Doxorubicin (n =17)	4 (24)	0	0	0
Nivolumab related (n = 68)	55 (81)	9 (13)	2 (3)	2 (3) ¹
Control/no induction (n = 12)	9 (75)	2 (17)	0	1 (8)
Irradiation (n = 13)	10 (77)	3 (23)	0	1 (8)
Cyclophosphamide (n = 13)	13 (100)	2 (15)	2 (15)	0
Cisplatin (n = 13)	11 (85)	2 (15)	0	0
Doxorubicin (n =17)	12 (71)	0	0	0

Supplementary Table 4. Nivolumab related adverse events. Nivolumab related adverse events of any grade, occurring in at least 5% of patients, and all grade 3-5 adverse events, all immune-related adverse events and immune-related events of special interest.

¹ One patient had lymphangitic carcinomatosis in which an immune-related component could not be excluded.

N = 68	Any grade, no. (%)	Grade 3, no. (%)	Grade 4, no. (%)	Grade 5, no. (%)
Fatigue	16 (24)	0	0	0
Alanine aminotransferase increased	13 (19)	0	0	0
Aspartate aminotransferase increased	13 (19)	1 (1)	0	0
Hypothyroidism	12 (18)	0	0	0
Diarrhea	9 (13)	0	0	0
Dyspnea ¹	8 (12)	2 (3)	0	0
Gamma-glutamyltransferase increased	8 (12)	2 (3)	1 (1)	0
Alkaline phosphatase increased	7 (10)	1 (1)	0	0
Infusion related reaction	7 (10)	0	0	0
Fever	6 (9)	0	0	0
Flu like symptoms	5 (7)	0	0	0
Pain	5 (7)	0	0	0
Serum amylase increased	4 (6)	0	1 (1)	0
Hyperthyroidism	4 (6)	0	0	0
Sarcoidosis	4 (6)	1 (1)	0	0
Lipase increased	3 (4)	1 (1)	0	0
Anemia	2 (3)	1 (1)	0	0
Abdominal infection	1 (1)	0	0	1 (1)
Anorexia	1 (1)	1 (1)	0	0
Febrile neutropenia	1 (1)	1 (1)	0	0
Hypertension	1 (1)	1 (1)	0	0
Hyponatremia	1 (1)	1 (1)	0	0
Respiratory insufficiency	1 (1)	0	0	1 (1)
Syncope	1 (1)	1 (1)	0	0
Immune-related colitis	1 (1)	0	0	0
Immune-related gastritis	1 (1)	0	0	0
Immune-related pneumonitis	1 (1)	0	0	0

Supplementary Table 5. Efficacy of nivolumab in the efficacy analysis population.

Efficacy analysis was performed using the efficacy analysis population (n = 66) as determined by iRECIST. Complete and partial responses had to be confirmed on at least one subsequent CT scan. 1 patient was non-evaluable according to RECIST1.1, but had clinical benefit for more than 24 weeks. Progression-free survival was calculated in the per protocol population (n = 67) from randomization to date of progression according to RECIST1.1 or iRECIST. Eleven patients had unequivocal clinical progression before the first response evaluation. Duration of response was assessed using Kaplan Meier curves, with censoring of ongoing responses, and calculated from time of first PR or CR until progression according to iRECIST.

Objective response rate iRECIST, n, % (95% CI)	13	20% (11-31)
Clinical benefit rate iRECIST, n, % (95% CI)	14	21% (12-33)
Best overall response iRECIST, n (%)		
Complete response	2	3%
Partial response	11	17%
Stable disease > 24 weeks	1	2%
Non-evaluable, n (%)	1	2%
Median progression-free survival RECIST1.1, months (95% CI)	1.9 (1.8-2.0)	
Median progression-free survival iRECIST, months (95% CI)	1.9 (1.8-3.2)	
Duration of response, months (95% CI)	9.0 (4.7 – NR)	

Supplementary Table 6. Characteristics of irradiation cohort. The irradiated lesions per patient, volume and dose of the irradiation, radiation technique and response of both the irradiated lesions and the other lesions (abscopal effect) after irradiation and the best response on nivolumab are depicted. In the rightmost column, the absolute change in stromal tumor-infiltrating lymphocytes (TIL) after irradiation is depicted for a biopsy from a non-irradiated lesion. Gy: Grey; CTV: clinical target volume; PTV: planning target volume; VMAT: volumetric modulated arc therapy; 3D-CRT: conventional radiotherapy with anterior-posterior fields or tangential fields; 3D-IMRT: intensity modulated radiotherapy.

Patient ID	Irradiated lesion	Dose	Volume (mL)	Volume type	Radiation technique	Response irradiated lesion after irradiation	Response non-irradiated lesions after irradiation	Best response irradiated lesion after nivolumab	Best overall response non-irradiated lesions after nivolumab	Absolute change in TIL after irradiation in biopsied lesion (non-irradiated)
Pat_5	Lymph nodes left mediastinum	3 x 8 Gy	32.643	PTV	VMAT	Non-CR/non-PD	SD	Non-CR/non-PD	PR	-12.5%
Pat_7	Breast right	3 x 8 Gy	401.136	PTV	3D-CRT	Non-CR/non-PD	SD	Non-CR/non-PD	PD	+2%
Pat_9	Lymph nodes neck left	3 x 8 Gy	43.317	PTV	3D-IMRT	Non-CR/non-PD	Non-CR/non-PD	NA	PD (clinical)	-4.5%
Pat_17	Lymph nodes axilla left	3 x 8 Gy	45.2	PTV	VMAT	Non-CR/non-PD	SD	Non-CR/non-PD	PD	NA
Pat_28	Hip right	3 x 8 Gy	235.6	CTV	3D-CRT	Non-CR/non-PD	PD	NA	PD (clinical)	+4%
Pat_37	Lymph nodes supraclavicular right	3 x 8 Gy	12.2	CTV	3D-CRT	Non-CR/non-PD	SD	Non-CR/non-PD	SD > 24 weeks	+7%
Pat_38	Right breast	3 x 8 Gy	635.86	CTV	3D-CRT	PD	PD	PR	PD	-2%
Pat_47	Axilla right	3 x 8 Gy	56.997	PTV	3D-IMRT	Non-CR/non-PD	SD	NA	PD (clinical)	-32.5%
Pat_53	Lymph nodes neck right	3 x 8 Gy	35.64	PTV	VMAT	Non-CR/non-PD	PD	NA (not imaged)	PD	-2%
Pat_60	Liver segment 6/7	3 x 8 Gy	129.64	PTV	VMAT	PD	PD	PD	PD	+2.5%
Pat_67	Lung middle lobe	3 x 8 Gy	38.628	PTV	VMAT	PD	PD	NA	PD (clinical)	NA
Pat_68	Sternum	3 x 8 Gy	64.38	PTV	VMAT	Non-CR/non-PD	SD	Non-CR/non-PD	PD	+4%