

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☐ ☒ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☐ ☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Data was collected in Microsoft Excel v.16.16.8 and in .csv files. Clinical data was collected in electronic Case Report Forms.

Data analysis

Clinical statistics: SAS v9.4 for windows; IBM SPSS Statistics 23
Whole exome sequencing: Burrows-Wheeler Aligner, Picard MarkDuplicates, MuTect, SnpEff 4.3t (build 2017-11-24 10:18), Sequenza (version 2.1.2)
Neoantigen predictions: NetChop (version 3.1); netMHCpan (version 4)
RNA sequencing: Salmon (version 0.11.0), R Bioconductor package tximport (version 1.4.0), Bioconductor package (version 3.18.1), flexgsea-R package,
TCR sequencing: ImmunoSeq Analyzer by Adaptive Biotechnologies
Bayesian modelling: rstan (version 2.17.3)
All other: GraphPad Prism v7.0, R v3.3.2

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

DNA and RNA sequencing data have been deposited into the European Genome-phenome Archive (EGA) under accession number EGAS0001003535. and will be made available upon reasonable request. Data requests will be reviewed by the institutional review board of the NKI and applying researchers will need to sign a

data access agreement with the NKI after approval. The TCR sequencing data are available from Adaptive Biotechnologies but restrictions apply to their availability. Data are however available from the authors upon reasonable request and with permission of Adaptive Biotechnologies.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For metastatic TNBC patients, no first or second line 'standard' therapies have been defined. The median PFS in first-line typically lies between 4-6 months. Based on four phase II trials in TNBC allowing 1 or 2 previous lines of chemotherapy a median PFS between 2 and 4 months was anticipated. Therefore, the investigators considered a proportion of >30% of the patients having a PFS of at least 12 weeks as potentially interesting. The null hypothesis that the true PFS rate as binary endpoint at 12 weeks is 30% was tested against an alternative of 50%. A Simon two-stage minimax design with a one-sided alpha of 15% and 85% power, was also optimal with respect to the expected sample size. A sample size of 10 evaluable patients in the first stage required early discontinuation of a particular treatment cohort if less than 4 out of 10 patients were free of progression and alive at 12 weeks. A patient was considered evaluable when at least one cycle of nivolumab was administered and both the pre-induction treatment biopsy and the post-induction treatment biopsy were available for immunohistochemistry. Because the number of patients in each cohort is larger than 10 (due to collection of 10 paired biopsies), the decision about discontinuation of a cohort was based on the first 10 patients.
Data exclusions	Eligible patients were 18 years or older with histologically confirmed triple negative metastatic breast cancer, had a World Health Organization (WHO) performance status of 0 or 1, lactate dehydrogenase (LDH) levels below 2x upper limit normal and received a maximum of three lines of palliative chemotherapy. In total 70 patients were randomized in the TONIC-trial. 2 subjects dropped out during the induction treatment, due to progressive disease and were not evaluable for safety analysis. 1 subject was excluded from the analysis since she was not eligible in retrospect (ER-positive metastasis upon revision). Furthermore, 1 subject had no evaluable disease according to RECIST and was excluded from objective response analysis. Exclusion criteria were prespecified.
Replication	The current study was not attempted to be replicated. However, the most important findings will be tested in the second stage of the trial.
Randomization	Patients were randomized into one of five cohorts by the independent trial office of the NKI. The first 17 patients were stratified based on PD-L1 expression on tumor cells. Due to logistical problems (unnecessary prolongation of the time between screening and randomization) this was stopped. The remaining 53 patients were randomly allocated to the five different cohorts.
Blinding	Since the TONIC-trial is the first trial testing induction by (low-dose) chemotherapy or radiation, followed by anti-PD-1, physicians needed to know what patients received in order to ensure safety. Pathologists were blinded for clinical outcome and treatment group upon scoring of the biopsies.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Immunohistochemistry:
 CD8: clone C8/144B (Agilent, DAKO). Lot no: 20011401. Dilution 1:200
 CD4: clone SP35 (CellMarque). Lot no.: 1625107B. Dilution 1:50
 FOXP3: clone 236A/E7 (AbCam). Lot no.: GR3220121-1. Dilution 1:200
 PD-L1: clone 22C3 (Agilent, DAKO). Lot no. 10141044. Dilution 1:40

Validation

All antibodies are commercially available. CD8, CD4 and PD-L1 are diagnostic markers and were validated on human diagnostic tissue by the local pathology department. FOXP3 has been validated for research purposes and validated on human tonsil and appendix tissue by the local pathology department.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

70 patients with metastatic triple negative breast cancer were included in the study. All participants were female and median age was 51 years. Visceral metastasis were present in 71% of patients. All patients received prior chemotherapy, either (neo) adjuvant or palliative. 24% of patients received the study treatment as first line of palliative systemic treatment. Detailed patient characteristics are described in table 1 and supplementary table 2, patient characteristics per cohort are described in supplementary table 1.

Recruitment

All eligible patients with metastatic TNBC were offered to participate in the trial.

Ethics oversight

Local medical-ethical committee of the NKI

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration

NCT02499367

Study protocol

All eligibility criteria are listed in the Supplementary Note. The full protocol will be made available upon publication of the results of the second stage of the TONIC-trial.

Data collection

Between September 2015 and November 2017, 70 patients were randomized. All patients were treated in the NKI. Data was collected in electronic case report forms and extracted directly from the patient records.

Outcomes

The primary endpoint of the study was progression free survival (PFS), assessed from randomization (PFS1) to tumor progression or death from any cause as defined by RECIST1.1. Secondary endpoints of the study were objective response rate (ORR), defined as the percentage of patients with a best overall response of complete response (CR) or partial response (PR) according to RECIST1.1 and iRECIST; clinical benefit rate (CBR), defined as the percentage of patients with best overall response of CR, PR and stable disease (SD) for 24 weeks, according to RECIST1.1 and iRECIST; PFS1 as defined by iRECIST; PFS, assessed from nivolumab treatment initiation (PFS2) to tumor progression or death from any cause as defined by RECIST1.1 and iRECIST; overall survival, defined as time from nivolumab initiation to death from any cause and percentage of patients with toxicity according to CTCAE v4.03 and immune-related toxicity. Translational objectives included: effects of the induction treatments on the anti-cancer immune response evaluated using immune-related gene expression signatures; T-cell influx determined using H&E and immunohistochemistry (IHC) and T-cell receptor (TCR) sequencing; the exploration of putative predictive biomarkers.