# nature research

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# **Reporting Summary**

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For	all statistical ar	nalyses, 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 stateme	ent 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.					
$\boxtimes$	A description of all covariates tested					
$\boxtimes$	A descript	tion of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons				
$\boxtimes$	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)					
$\boxtimes$	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted Give <i>P</i> values as exact values whenever suitable.					
$\boxtimes$	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings					
$\boxtimes$	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes					
$\boxtimes$	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), indicating how they were calculated					
		Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.				
So	ftware an	d code				
Poli	cy information	about <u>availability of computer code</u>				
Da	ata collection	ta collection Mantra Quantitative Pathology Workstation				
Da	ata analysis	QuPath: 0.2.3, Python: 3.7.3, R 4.0.2				
		g custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and 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 <u>availability of data</u>

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

The datasets generated during and/or analyzed during the current study are available from the corresponding authors on reasonable request.

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Please select the or	ne 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 t	the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>			
Life scier	nces study design			
All studies must dis	close on these points even when the disclosure is negative.			
Sample size	10 paired triple-negative breast cancer biopsies (obtained before and 2 weeks after the injection of bevacizumab) were included in this study. No sample size calculation was performed. In a previous study we showed that 7 paired human triple-negative breast cancer samples was sufficient to find significant differences in vascular parameters between pre- and post-bevacizumab samples (Tolaney et al., PNAS 2015).			
Data exclusions	For the analysis of stromal tumor-infiltrating lymphocytes 2/10 paired-biopsy samples were excluded because there was insufficient stroma in those samples.			
Replication	No specific study has been performed yet to reproduce the specific effects of bevacizumab on the recruitment of immune cells in triplenegative breast cancer. However, in other human tumor types bevacizumab also increased the recruitment of T cells and the expression of MHC-I.			
Randomization	This study was not randomized.			
Blinding	The scientists who performed the imaging and quantitative analysis of immune cells and blood vessels were blinded.			
<del> </del>	g for specific materials, systems and methods on from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material			
	ed 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 & exp	perimental systems Methods			
n/a Involved in th	n/a Involved in the study			
Antibodies	ChIP-seq			
Eukaryotic	cell lines			
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Animals an	d other organisms			
	earch participants			
	Clinical data			
Dual use re	esearch of concern			
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Antibodies				
Antibodies used	Antibody Clone Company Catalogue # CD68 PGM1 Agilent Dako M0876			
	CD163 10D6 Leica NCL-L-CD163			
	CD11c 5D11 Leica CD11C-563-L-CE CD8 C8/144B Agilent Dako M710301			
	PD-1 EH-33 Cell Signaling Tech. 43248S			
	CD31 Polyclonal Abcam AB28364			
	Ang2 F-1 Santa Cruz SC-74403 CD4 4B12 Dako M731029			
	FOXP3 206D BioLegend 320102			
	CD45RA 4KB5 Thermofisher MA5-12490 CD45RO UCHL1 Dako M0742			
	MHC-I EMR8-5 Abcam AB70328			
	Granzy- GrB7 Dako M7235 me B			
Validation	CD68 Agilent Daki website: monoclonal mouse anti-human CD68, immunohistochemistry			

Validation

Leica website: monoclonal mouse anti-human CD163. immunohistochemistry CD163 CD11c Leica website: monoclonal mouse anti-human CD11c, immunohistochemistry CD8 Agilent Dako website: monoclonal mouse anti-human CD8, immunohistochemistry PD-1 Cell Signaling Technology website: monoclonal mouse anti-human PD-1, immunohistochemistry CD31 Abcam website: polyclonal rabbit anti-human CD31, immunohistochemistry  $Santa\ Cruz\ website: monoclonal\ mouse\ anti-human\ Ang 2, immunohistochemistry$ Ang2 FOXP3 BioLegend website: monoclonal mouse anti-human FOXP3, immunohistochemistry CD45RA Thermofisher website: monoclonal mouse anti-human CD45RA, immunohistochemistry CD45RO Dako website: monoclonal mouse anti-human CD45RO, immunohistochemistry MHC-I Abcam website: monoclonal mouse anti-human MHC-I, immunohistochemistry Granzyme B Dako website: monoclonal mouse anti-human granzyme B, immunohistochemistry

### Human research participants

Policy information about studies involving human research participants

Population characteristics

Enrollment required a pathological diagnosis of adenocarcinoma of the breast. Eligible TNBC patients were negative for ER, PR, and HER2, had a breast lesion  $\geq 1.5$  cm, and no evidence of distal metastasis. Patients with bilateral cancers were eligible as long as one cancer was eligible. Patients also required sufficient hematopoietic, hepatic, and renal function, along with a left ventricular ejection fraction  $\geq 50\%$ . Patients with any HER2-positive disease (amplified by FISH or IHC), a history of prior myocardial infarction, uncontrolled hypertension,  $\geq$  grade 2 neuropathy, significant bleeding within 6 months of study entry, or urine protein: creatinine ratio > 1 were excluded.

Recruitment

Enrollment in this phase II trial required a pathological diagnosis of adenocarcinoma of the breast. Eligible TNBC patients were negative for ER, PR, and HER2, had a breast lesion ≥ 1.5 cm, and no evidence of distal metastasis.

Ethics oversight

This study was approved by the Dana–Farber/Harvard Cancer Center Institutional Review Board.

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

NCT00546156

Study protocol

https://clinicaltrials.gov/ct2/show/record/NCT00546156

Data collection

The core biopsies used in the current study were obtained in the Dept of Radiation Oncology at Dana-Farber Cancer Institute and Massachusetts General Hospital between 2007 and 2011. The multiplex immunofluorescence and quantitative analysis were performed between March 2017 and January 2021.

Outcomes

The primary and secondary outcomes of the study were not correlated with the immune cell and blood vessel data of the current article.