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Evidence of β-blockers drug repurposing for the treatment of triple negative breast cancer: A systematic review

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Triple negative breast cancer (TNBC) is a particularly aggressive subtype of breast cancer (BC) for which limited therapeutic options are available. Recently, β-blockers (BBs) have been suggested to have favorable effects in the treatment of BC. The aim of this systematic review was to collect evidence from preclinical and clinical studies concerning the scientific evidence for the repurposing of BBs in TNBC treatment. PubMed database was searched to retrieve studies of interest published up to 30/01/2018. All preclinical studies using TNBC *in vitro* and *in vivo* models and assessing the effect of any molecule with sympatholytic or sympathomimetic activity on adrenergic receptors were included. Clinical studies concerning BBs were considered eligible. The Newcastle-Ottawa scale was used for the quality assessment of clinical studies. A total of 614 study references were retrieved. Forty-six preclinical studies were included. In *in vitro* studies, propranolol, a non-selective BB, significantly decreased proliferation, migration and invasion of TNBC cells. Consistently, in *in vivo* studies, propranolol inhibited metastasis, angiogenesis and tumor growth. Clinical studies, reporting evidence from a total of four distinct retrospective observational cohort studies, showed a beneficial effect of BBs in TNBC treatment. The overall quality of the clinical evidence collected was low. Preclinical evidence collected in this systematic review are in line with the results reported in the clinical studies retrieved, pointing towards a beneficial effect of BB in the treatment of TNBC. However, given the overall low quality of available evidence, no definite conclusion may be drawn.

Key words: triple negative breast cancer, β-blockers, systematic review, drug repurposing, propranolol

Breast cancer (BC) is the second leading cause of cancer death among women in Western countries [1, 2]. BC is a heterogeneous disease composed of several biological subtypes having different prognosis and outcome. Based on the immunohistochemical classification, approximately 16% of all the BCs do not express estrogen receptors, progesterone receptors, erb-b2 receptor tyrosine kinase 2/Neu (HER2) and lack HER2 amplification. This molecular profile defines the triple-negative BC (TNBC).

Population-based studies have shown a higher incidence of TNBC in women under the age of forty [3]. High aggressiveness and lack of response to hormone and targeted therapies, limits the number of therapeutic opportunities and makes the prognosis for TNBC patients poor [4, 5]. Compared to other BC subtypes, TNBC has a lower 5-year survival rate and, in the first three years of diagnosis, a higher risk of relapse [6]. Nowadays, novel treatment approaches, such as immunotherapy, are under investigation for the treatment of TNBC [7].

On the other hand, the repurposing, in term of efficacy, of known drugs that are currently used for indication other than TNBC is also under investigation. In particular, preclinical evidence on the effect of norepinephrine on tumor progression together with the observed activity of β-adrenergic receptor (β-AR)-mediated pathways on tumor development, angiogenesis and apoptosis [8–11], led the attention of the scientific community on the potential antineoplastic properties of β-AR blocker agents, a class of drugs that is widely used in clinical practice for cardiovascular indications. A comprehensive list of β-blocker drugs (BBs) on the basis of selective or not selective binding to β-ARs (Table 1) and on the basis of its documented anticancer-properties was presented (Table 2).

A number of *in vitro* studies have demonstrated the antiproliferative, anti-migratory and cytotoxic properties of BBs [12, 13]. Propranolol, whose activity seems to be due to the non-selective antagonism of the β1 and β2-ARs, was also found to have potent anti-angiogenic effects through direct

mechanisms on vascular endothelial cells and by decreasing pro-angiogenic signaling in cancer cells [14]. Some of these promising anti-cancer properties have been also confirmed *in vivo,* in animal models [15]. Therefore, given the anti-neoplastic potential of BB and the lack of effective treatment for TNBC, BB repurposing for TNBC treatment was suggested [16].

The aim of this systematic review was to collect the available evidence from preclinical and clinical studies concerning the scientific foundation for the repurposing of BBs in TNBC treatment and to provide recommendation for future clinical studies on this topic.

Materials and methods

Study eligibility criteria. Any type of preclinical study assessing the effect of any molecule with adrenergic activity on TNBC models were included. Clinical studies concerning the exposure to any BBs in TNBC patients were considered eligible for inclusion. Studies written in any language other than English or for which full-text was not available were excluded.

Search strategy. We searched PubMed database for retrieving all the studies of interest published up to January 30, 2018. The search string is available in Supplemental Table S1. A snowball search (such as pursuing references of references from full text included) was conducted in order to retrieve and assess for inclusion also references cited in systematic reviews and meta-analyses on the same/similar topic.

Study selection. Two authors (AS and GR) reviewed and screened independently titles and abstracts of retrieved references. Potentially relevant studies were assessed for inclusion through examination of full texts. Disagreement of the judgment of the two reviewers was solved through discussion.

Data extraction. One author (AS) extracted the following information from preclinical studies: i) *in vivo/in vitro* methods, AR agonist or BB exposure and study objective; ii) *in vitro* AR expression; iii) AR agonist or BB activity on cancer; iv) pathways associated to BBs on cancer activity. A second author (LB) validated the extracted data.

GR extracted the following information from clinical studies: i) data source name, data source type, country; ii) study population; iii) study design, exclusion criteria and covariates; iv) efficacy outcome and effect size measures reported in the included studies. RG reviewed the extracted information.

Quality assessment of clinical studies. GR and RG evaluated, blinded to each other, the quality of the included studies. Disagreement of the judgment of the two reviewers was solved through discussion among study authors. For the purposes of this review, the Newcastle-Ottawa scale was applied [17].

Protocol registration. The study protocol was published in advance with respect to the initiation of the present review on the International Prospective Register of Systematic Reviews (https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=89131)

Table 1. BB drugs subdivided by selective or not selective binding.

Non selective BBs	Beta 1 selective BBs	Beta 2 selective BBs	Non selective BBs with additional alpha blocking action
Alprenolol	Acebutolol	ICI 118,551	Carvedilol
Carteolol	Atenolol		Bucindolol
Nadolol	Betaxolol		Labetalol
Oxprenolol	Bevantolol		
Penbutol	Bisoprolol		
Pindolol	Celiprolol		
Propranolol	CGP-20712A		
Sotalol	Esmolol		
Timolol	Metoprolol		
Tertalol	Nebivolol		
	Practolol		

The table shows all BBs on the market and involved in experimentation.

Results

Study selection. A total of 614 study references were retrieved from PubMed (Figure 1). Six records were retrieved through snowball search [18–23]. Titles and abstracts of 614 individual study records were screened. Potentially eligible studies (n=150) were further evaluated for inclusion through full-text examination (Table S2, the entire list of full-text assessed and reason of exclusion).

A total of 46 preclinical studies [12–15, 18–59] and 3 clinical studies [16, 60, 61] fulfilled the eligibility criteria and were finally included into the review.

Preclinical studies. Forty-six studies assessed TNBC models as cell cultures and/or animal studies. In total we retrieved 20 in *in vitro*, 9 *in vivo* and 17 in *in vivo/in vitro* studies (Table S3).

Evidences about β-adrenergic stimulation in TNBC

***In vitro* studies.** Several studies showed that isoproterenol, a nonselective β agonist, increased migration [25, 40] and invasion of MDA-MB-231 breast cancer cells [25, 27, 31, 40], while reduced their proliferation [26, 35, 49]. One of the selected studies [27], showed enhanced matrix metalloproteinase expression in MDA-MB-231 cancer cells after the treatment with this drug. Similarly, 10 μM terbutaline, a selective β2 agonist treatment, has been reported to significant increase MDA-MB-231 cell invasion and proliferation compared to cells treated with propranolol [13], suggesting that β2-ARs play a role in breast cancer cell migration and growth. However, in the opposite way, the treatment of the same breast cancer cells, MDA-MB-231, with pirbuterol (10 μM) and salbutamol (1 μM), also selective β2 agonists,

Table 2. Characteristics of included clinical studies.

Study	- Data source name - Data source type - Country	Study population with TNBC	Exclusion criteria	Study design	Exposure	- Matching criteria - Covariates used for statistical adjustment	Outcome and effect size
Melhem-Bertrandt, 2011 [16]	- Breast Cancer Management System Database - Medical chart and pharmacy data Texas (US)	Women with invasive TNBC treated with neoadjuvant anthracylines and taxane from January 1995 to May 2007 n=377	BB after neoadjuvant chemotherapy, unknown receptors expression status, incomplete records longer than 9 months between neoadjuvant chemotherapy initiation and surgery, bilateral BC	Retrospective cohort study	Use of beta blockers during neoadjuvant therapy	- none - age, stage, race, BMI, metformin use, diabetes, hypertension, ACE/ ARBs	- RFS: HR=0.3 (95%CI 0.1-0.87; p=0.027) - OS: HR=0.35 (95%CI 0.12-1.00; p=0.05)
Botteri, 2013 [60]	- Breast Cancer and Cardiology Division Databases Of the European Institute of Oncology of Milan - Disease registries - Milan, Italy	Postmenopausal women diagnosed and operated for early primary TNBC (stage I–III) between 1997 and 2008 n=800	history of invasive cancer or metastatic disease	Retrospective cohort study	BB use at time of diagnoses	- none - age, tumor stage, peritumoral vascular invasion, use of other antihypertensive drugs, antithrombotics and statins, use of and response to neoadjuvant chemotherapy, and loco-regional BC treatment	- BC-related events HR=0.52 (95%CI 0.28–0.97) - Metastases: HR=0.32 (95%CI 0.12–0.90) - BC death: HR=0.42 (95%CI 0.18–0.97)
Spera, 2017 (1) [61]	- ROSE/TRIO-012 - data from a randomized, double blind clinical trial - multicenter	Women with advanced TNBC (n=n.r.)		Retrospective cohort study	BB use either during the active treatment phase and/or within 30 days prior to randomization	- none - n.r	- PFS: HR=0.52 (95%CI 0.34–0.79; P=.002) - OS: HR=0.87 (95%CI 0.58–1.31 p=0.504)
Spera, 2017 (2) [61]	- BCIRG-005 - data from a randomized clinical trial - multicenter	women with node positive operable TNBC (n=n.r.)		Retrospective cohort study	BB use either during the active treatment phase and/or within 30 days prior to randomization	- none - development of treatment emergent hypertension, hormone receptor status, geographic area, and treatment arm	- RFS: HR=0.69 (95%CI 0.35–1.34; p=0.269) - OS: HR=0.73 (95%CI 0.35–1.48; p=0.384)

The table shows the characteristics of the three clinical studies included: data source type and name, study population with TNBC, exclusion criteria, study design, exposure matching criteria, covariates used for statistical adjustment and finally outcomes assessed. BC: breast cancer; TNBC: triple negative breast cancer; BB: beta-blockers; RFS: recurrence free survival; OS: overall survival; ACEIs: angiotensin converting enzyme inhibitors; ARBs: angiotensin receptor blockers; n.r. = not reported. BC-related recurrences, and BC deaths were counted as events, while contralateral tumors, non-breast primary tumors, and deaths from other causes were considered as competing events. Included metastasis and death from BC while loco-regional events, contralateral tumors, non-breast primary tumors, and deaths from other causes were considered as competing events. Deaths from other causes as competing events. For the cumulative incidence non-BC deaths, we considered deaths from BC as competing events.

was reported to decrease cell proliferation, and cell migration and invasion, respectively [18, 48].

***In vivo* studies.** *In vivo* results showed that isoproterenol [20, 26, 29, 44], metaproterenol (nonselective β agonist) [23] and formoterol (β2 agonist) [31] significantly stimulated breast cancer cell metastasis. On the contrary, Perez Pinero et al. have showed a significant inhibition of tumor growth in xenograft models of TNBC by salbutamol [45]. Similarly, and in agreement with the *in vitro* results, pirbuterol decreased breast tumor volume in *in vivo* xenograft models [18].

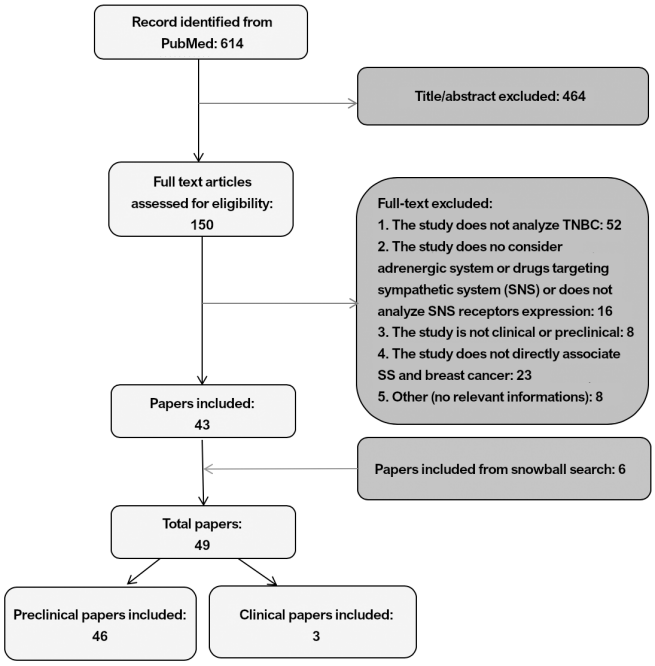

Figure 1. Flow chart. The figure shows the number of records identified from PubMed. The number of full text articles assessed for eligibility and the total number of papers included. Exclusions criteria were listed.

Evidence about β-blockers in TNBC

***In vitro* studies.** A total of 5 studies reported higher β2 adrenergic receptor expression in TNBC cell lines than other cell lines [13, 21, 27, 45, 48]. Propranolol, a nonselective β antagonist, inhibited migration [13, 40, 48, 58] and proliferation [12–14, 51, 58] of TNBC cell lines (surrogate for cancer progression). Propranolol also inhibited cell invasion promoted by adrenergic stimulation [31, 40]. Similarly, Carvedilol, a nonselective β and selective-α1 antagonist and atenolol, a selective β1 antagonist, showed a significant inhibition of cell migration and invasion [32] and cell proliferation [12], respectively.

***In vivo* studies.** In *in vivo* xenograft models of TNBC, propranolol inhibited metastasis formation and growth [15, 20, 26, 27], and lymphatic vessel density in tumors, promoted by adrenergic stimulation [20]. Further, Pasquier et al. demonstrated that propranolol increased the survival of animals bearing TNBC following treatment with paclitaxel or fluorouracil [14]. Interestingly, propranolol also restored natural killer cells activity [23] that was reduced by adrenergic stimulation [37]. Evidence retrieved on BBs in TNBC models are reported on Table S4.

Evidence about alpha adrenergic stimulation in TNBC

***In vitro* studies.** In *in vitro* studies, dexmedetomidine and clonidine, two presynaptic selective alpha-2 adrenoceptor agonist, significantly stimulated migration, invasion [59] and proliferation [52]. In particular, one study reported that dexmedetomidine activate the α2-adrenergic receptors/ERK signaling pathway in breast cancer cell lines [59].

***In vivo* studies.** Two drugs, dexmedetomidine and desimipramine, block the reuptake of norepinephrine and serotonin in the presynaptic neuronal membrane and appears to downregulate beta-adrenergic receptors and serotonin receptors, have reported to significantly stimulate tumor growth in xenograft model of TNBC [52].

Clinical studies. All the three papers included in this review concerned retrospective observational cohort studies. The characteristics of the three studies are reported in more details in Table 2. Melhem-Bertrandt et al. [16] analyzed medical chart and pharmacy data from the Breast Cancer Management System Database of Texas. The study reported a favorable effect of BBs in a sub-cohort of 377 women with invasive TNBC treated with neoadjuvant medications with respect to Recurrence Free Survival (RFS) (HR=0.3; 95%CI 0.1–0.87; p=0.027) and overall survival (OS) (HR=0.35; 95%CI 0.12–1.00; p=0.05). The second study from Botteri et al. [60] reported a beneficial effect of BBs with respect to breast cancer-related events (HR=0.52; 95%CI 0.28–0.97), metastases (HR=0.32; 95%CI 0.12–0.90) and breast cancer death (HR=0.42 95%CI 0.18–0.97) in a cohort of 800 postmenopausal women diagnosed and operated for early primary TNBC which was drawn from the Breast Cancer and Cardiology Division Databases of the European Institute of Oncology of Milan, Italy. The third paper from Spera et al. [61] reported two post-hoc analyses from two distinct clinical trials, i.e. ROSE/TRIO-012 and BCIRG-005. The authors analyzed the two cohorts of BC patients separately by applying the same study design and statistical analysis. Information on the association between BBs intake and clinical outcomes was respectively provided in a subgroup of patients with TNBC. Within the sub-cohort of patients with advanced TNBC drawn from ROSE/TRIO-012, BB use was associated with a beneficial effect on PFS (HR=0.52; 95%CI 0.34–0.79; p=0.002), but not on OS (HR=0.87; 95%CI 0.58–1.31 p=0.504) and RFS (HR=0.69; 95%CI 0.35–1.34; p=0.269). Among women with node positive operable TNBC from the clinical trial BCIRG-005, RFS (HR=0.69; 95%CI 0.35–1.34; p=0.269) and OS (HR=0.73; 95%CI 0.35–1.48; p=0.384) were not significantly associated with BBs intake.

Results from the quality assessment of the included clinical studies were reported in Table S5. In general, the study quality was low. The lowest score was attributed to the two cohort studies from Spera et al. [61]: reporting of study characteristic was poor (e.g. the size of the cohort of patients with TNBC was not even reported) and the two TNBC sub-cohorts were unlikely to be representative of any relevant patient group in the community since data were drawn from two multi-center clinical trials. In addition, the two studies assessed BB use either during the active treatment phase and/or within 30 days prior to randomization, thus likely intro-

ducing an immortal-time bias, [62] which might have artificially inflated the beneficial effect of BBs use on the study outcomes.

Discussion

Beta blockers (BBs), also called beta-adrenergic blocking agents, are a class of medications that treat a variety of conditions including abnormal heart rhythms, hypertension and migraines. In the present systematic literature review we collected evidence from preclinical and clinical studies on the role of adrenergic receptors, BBs and other sympathetic agents in the treatment of triple negative breast cancer (TNBC).

We showed that *in vitro* beta activation increases migration and invasion [25, 27, 31, 40], while Pirbuterol and Salbutamol, beta agonists as well, decreased cell migration and invasion [18, 48]. These results appeared to be controversial despite the authors used the same TNBC cell lines. Other authors have already noted these discrepancies regarding the effects of β2-adrenergic receptor agonism on cancer cells: Choy et al. [13] and Thaker et al. [63] suggested that this could be attributed to pathway specificity (i.e. pirbuterol can inhibit the Ras/Raf-1/Mek-1/Erk1/2 pathway while other agonists may inhibit the cAMP/PKA pathway).

Contrary to beta agonists, evidence from preclinical studies clearly suggest a favorable role of BB in the treatment of cellular models of TNBC. *In vitro* studies showed a high expression of β2-adrenergic receptors in TNBC cell lines [13, 21, 27, 45, 48], which significantly responded to propranolol by reducing their proliferation, migration and invasion [12–14, 31, 40, 48, 51, 58]. Similarly, carvedilol (β1-2 and α1 antagonist) is reported to possess an anti-migration and anti-invasion property on TNBC cell lines [32]. *In vivo* studies, in models of breast cancer xenografts, propranolol was found to reduce metastasis and tumor growth [15, 26, 27], to increase survival following the treatment with chemotherapy [14] and to restore natural killer cells activity [23]. These results were in line with other preclinical studies on BB effects in cancer: propranolol showed anti-proliferative effects in "*in vitro models*" also on colorectal cancer cells [64], on pancreatic cancer cells [65, 66] and on melanoma cell lines [67]. In *in vivo* studies demonstrated that propranolol reduced invasion of prostate cancer cells mediated by adrenaline [68, 69]. According to these studies, which suggest further pre-clinical and clinical evaluations for cancer patients treated with BB, our results demonstrate that BB may have a beneficial role in the treatment of TNBC; among all studied drugs propranolol seems to be the more promising.

Contrary to preclinical studies, evidence coming from clinical studies on the effect of BB on TNBC is still scarce. No random clinical trials (RCT) are currently available. Only three papers, providing a total of four distinct observational studies on TNBC and BBs use, were found, and the results from all the studies retrieved suggested that BBs in association with chemotherapy could have a beneficial effect on survival, disease progression and recurrence of TNBC. However, considering the low number and quality of the collected studies, together with the observational nature of the latter, no definite conclusion can be drawn. In fact, the sample size of the study cohorts was small and evidence on TNBC and BB use derived from subgroup analysis [16, 60, 61]. Further, patients' characteristics, outcome definitions and data sources used were heterogeneous between studies, as well as exposure definition to BB and assessment. For all the reasons mentioned above, we decided to avoid any quantitative synthesis of reported effect sizes.

Recommendations for clinical studies: given the overall low quality of the evidence coming from available clinical studies, further observational and interventional investigation on the role of BB in TNBC treatment are needed.

As for observational studies, large scale, well-designed and high quality, retrospective studies should be performed in order to quickly confirm or disprove the evidence collected here. These types of studies are usually less reliable than RCT and methodologically challenging. However, they are less time and resource consuming and allow to include large, unselected population from the real world experience. Although they do not require *ad hoc* data collection, the choice of the most adequate data source remains fundamental [70]. For such type of study, the linking of different source of information is expected to be necessary: beyond medical records collecting clinical information, administrative data might be required to ensure more comprehensive follow-up and survival data, as well as inpatient and outpatient drug registry [71].

Nevertheless, RCT remains the gold standard for assessing drug efficacy [72]. When results from the completion of such a study could be finally available, they will finally shed light on the role on BB in TNBC treatment.

In conclusion, the evidence collected in the present systematic review suggests that BBs could potentially improve disease progression, recurrence and survival in TNBC patients and might be further investigated as anticancer repurposing drugs. Although no definite conclusion can be drawn from the available data, the results from the preclinical evidence and from the clinical studies support the role of adrenergic stimulation on TNBC progression and the potential benefit of using BBs in TNBC treatment. Well-designed and high quality, retrospective observational studies should be performed in order to quickly confirm or disprove the evidence collected here. Moreover, randomized clinical studies testing the effect of BBs should be conducted to clarify their efficacy for the treatment of TNBC.

Supplementary information is available in the online version of the paper.

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