**Supplementary material**

**Preclinical and clinical evidence about on betablockers for the treatment of triple negative breast cancer: a systematic review.**

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**Table S1:** Search string; Last search: 31/01/2018

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| **Pathology** |
| (“Breast Cancer”[Title/Abstract] OR “Breast Cancers”[Title/Abstract] OR “Breast Tumor”[Title/Abstract] OR “Breast Tumors”[Title/Abstract] OR “Breast Carcinoma”[Title/Abstract] OR “Breast Carcinomas”[Title/Abstract] OR “Breast Neoplasms”[Title/Abstract] OR (“breast”[MeSH Terms] AND “neoplasms”[MeSH Terms]) OR (Breast[Title] AND (Neoplasm[Title] OR Cancer[Title] OR Carcinoma[Title] OR Tumor[Title] OR Tumour[Title])) OR “Mammary Cancer”[Title/Abstract] OR “Mammary Cancers”[Title/Abstract] OR “Mammary Tumor”[Title/Abstract] OR “Mammary Tumour”[Title/Abstract] OR “Mammary Tumors”[Title/Abstract] OR “Mammary Tumours”[Title/Abstract] OR “Mammary Neoplasms”[Title/Abstract] OR (Mammary[Title] AND Carcinoma[Title]) OR (Triple[Title] AND Negative[Title]) OR ((Animal[Title/Abstract] OR Preclinical[Title/Abstract]) AND Breast[Title/Abstract] AND (Cancer[Title/Abstract] OR Tumours[Title/Abstract])) OR ((Stress[Title/Abstract] OR Stress[Text Word]) AND (Breast[Title/Abstract] OR Mammary[Title/Abstract])) OR(“Stress, Psychological”[Mesh] AND “Breast”[Mesh])) |
| AND |
| **Receptors and drugs** |
| (“Receptors, Adrenergic”[Mesh] OR “Adrenergic Receptor”[Title/Abstract] OR “Adrenergic Receptors”[Title/abstract] OR “Adrenergic Stimulation”[Title/Abstract] OR “Sympathomimetics”[Mesh] OR “Sympatholytics”[Mesh] OR “Adrenergic Agents”[Pharmacological Action] OR “Adrenergic Antagonists”[Pharmacological Action] OR “Adrenergic Agonists”[Pharmacological Action] OR ((Receptor[Title/Abstract]) AND Adrenergic[Title/Abstract] AND (α-2[Title/Abstract])) OR ((Adrenoceptor[Title/Abstract]) AND (α2[Title/Abstract])) OR “Adrenergic alpha-2 Receptor Agonists”[Pharmacological Action] OR “Receptors, Adrenergic, beta-3”[Mesh] OR “beta Adrenergic Receptors”[Title/Abstract] OR (Receptor[Title/Abstract] AND Adrenergic[Title/Abstract] AND β-3[Title/Abstract]) OR ((Adrenoceptor[Title/Abstract] OR Adrenoceptors[Title/Abstract]) AND (beta[Title/Abstract] OR β[Title/Abstract])) OR “Adrenergic beta-Antagonists”[Mesh] OR (Antagonist[Title/Abstract] AND (Adrenergic[Title/Abstract] OR Sympathetic[Title/Abstract]) AND (beta[Title/Abstract] OR β[Title/Abstract])) OR ((Blocker[Title/Abstract] OR Blockers[Title/Abstract] OR “Blocking Agent”[Title/Abstract] OR “Blocking Agents”[Title/Abstract]) AND (β[Title/Abstract] OR β1[Title/Abstract] OR β3[Title/Abstract])) OR Atenolol[Title/Abstract] OR Bisoprolol[Title/Abstract] OR Bufuralol[Title/Abstract] OR Carvedilol[Title/Abstract] OR Metoprolol[Title/Abstract] OR Propanolol[Title/Abstract] OR Talinolol[Title/Abstract]) |

**Table S2:** Full text assessed and exclusion motivations

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| **Full text assessed:**  {Abdin, 2014;Anton, 1963 ;Ashrafi, 2017;Badino, 1996;Barber, 1984;Barron, 2011;Ben-Eliyahu, 2000;Ben-Eliyahu, 2000;Borowiec, 2007;Botteri, 2013;Boudreau, 2014;Boulay, 2012;Bruzzone, 2008;Bruzzone, 2011;Cakir, 2002;Campbell, 2012;Cardwell, 2013;Cardwell, 2016;Castillo, 2017;Chang, 2016;Chang, 2016;Charles, 2010;Chen, 2014;Chen, 2018;Chen, 2017;Chen, 2015;Chen, 2006;Chiesa, 2008;Childers, 2015;Choy, 2016;Clavel, 1981;Connor, 2012;Creed, 2015;Cronin-Fenton, 2018;Davis, 2007 ;Devore, 2015;Dezong, 2014;Dhar, 2006;Draoui, 1991;Drell, 2003;Du, 2014;Fitzgerald, 2012;Flint, 2009;Freire-Garabal, 1992;Fryzek, 2006;Ganz, 2011;Garcia-Solis, 2003;Gargiulo, 2014;Gargiulo, 2017;Goldfarb, 2009;Goldfarb, 2011;Goldvaser, 2016;Gomez-Acebo, 2016;Goravanchi, 2012;Hance, 2008;Holmes, 2013;Holmes, 2013;Hong-Fen, 2001;Huang, 2001; Hui, 2008;Kafetzopoulou, 2013; Kang, 2014; Kim, 2016;Kopf, 1996;Lamkin, 2015;Lavon, 2018;Leung, 2015;Li, 2013;Li, 2003;Lindgren, 2013;Liu, 2015;Liu, 2016;Madden, 2011;Madden, 2013;Marchetti, 1989;Marchetti, 1991;Marjamaki, 1992;Marjamaki, 1993;Meier, 2000;Melhem-Bertrandt, 2011;Montoya, 2017;Mulcrone, 2017;Murray, 1989;Nagaraja, 2017;Numbere, 2017;Parada-Huerta, 2016;Parkin, 1976;Pasquier, 2011;Patane, 2015;Perez, 2005;Perez Pinero, 2012;Petty, 2012;Pihlavisto, 1998;Pituskin, 2017;Plummer, 2004 ;Pon, 2016;Powe, 2011;Powe, 2010;Powe, 2011;Qin, 2015;Rains, 2017;Rajamanickam, 2016;Rautio, 2015;Re, 1992;Rivero, 2017;Romeo, 1991;Roy, 2008;Saez Mdel, 2007;Sakellakis, 2014;Shaashua, 2017;Shi, 2011;Shkurnikov, 2014;Shohat, 1975;Singh, 2016;Sloan, 2010;Slotkin, 2000;Smith, 2016;Sorensen, 2013;Spera, 2017;Spina, 2012;Stanojkovic, 2005;Stefanski, 1996;Strell, 2012;Su, 2005;Szewczyk, 2012;Szpunar, 2013;Szpunar, 2016;Talarico, 2016;Tuglu, 2018;Tveit, 1985;Vandewalle, 1990;Vazquez, 2006;Vazquez, 1999;Walters, 2003;Wang, 2015;Wang, 1998;Watanabe, 2017;Weiss, 1980;Weiss, 1986;Wendel, 1996;Williams, 1978;Wilson, 2015;Wishart, 1994;Xia, 2016;Xia, 2016;Yamazaki, 2014;Yamazaki, 2014;Zhou, 2009;Zhou, 2000;Zhou, 2002}[1-150]  **Exclusion motivations:** |
| **1: The study does not analyze TNBC**  {Abdin, 2014; Badino, 1996; Barron, 2011; Boudreau, 2014; Bruzzone, 2008; Bruzzone, 2011; Cardwell, 2013; Cardwell, 2016; Castillo, 2017; Chang, 2016; Charles, 2010; Chen, 2017; Chen, 2015;Cronin-Fenton, 2018;Davis, 2007;Devore, 2015; Draoui, 1991; Fryzek, 2006; Ganz, 2011;Goldvaser, 2016;Gomez-Acebo, 2016;Holmes, 2013; Holmes, 2013; Kopf, 1996; Leung, 2015; Li, 2013; Li, 2003; Liu, 2015; Liu, 2016; Marchetti, 1989; Meier, 2000; Montoya, 2017;Numbere, 2017; Parada-Huerta, 2016;Petty, 2012; Pituskin, 2017;Powe, 2010;Powe, 2011;Rains, 2017; Re, 1992; Sakellakis, 2014; Shaashua, 2017; Shi, 2011; Sloan, 2010; Sorensen, 2013; Stanojkovic, 2005;Su, 2005;Vazquez, 1999;Walters, 2003;Watanabe, 2017; Wendel, 1996}[1,4,6,11,13,14,17,19,21,22,25,26,34,35,36,39,45,46,52,53,56,57,64,67,68,69,71,72,75,79,81,85,86,92, 94,98,101,104,109,110,111,115,118,121,124,133,134,137,140] |
| **2: The study does not consider adrenergic system nor drugs targeting sympathetic system (SNS) or does not analyze SNS receptors expression**  {Anton, 1963;Borowiec, 2007;Dhar, 2006;Madden, 2013; Marjamaki, 1992;Marjamaki, 1993; Rajamanickam, 2016;Rautio, 2015;Roy, 2008;Singh, 2016;Spina, 2012;Wang, 1998;Wishart, 1994;Zhou, 2009;Zhou, 2000;Zhou, 2002}[2,9,38,74,77,78,102,103,107,114,120,136,143,148,149,150] |
| **3: The record is not a clinical or preclinical study**  {Childers, 2015;Clavel, 1981;Fitzgerald, 2012;Goravanchi, 2012;Nagaraja, 2017;Patane, 2015;Perez, 2005;Powe, 2011}[29,31,42,54,84,89,90,97] |
| **4: The study does not directly associate SS and TNBC**  {Chen, 2006;Connor, 2012;Freire-Garabal, 1992;Garcia-Solis, 2003;Hance, 2008;Huang, 2001;Kang, 2014;Marchetti, 1991;Murray, 1989;Parkin, 1976;Pihlavisto, 1998;Plummer, 2004;Romeo, 1991;Saez Mdel, 2007;Smith, 2016;Stefanski, 1996;Szpunar, 2016;Tveit, 1985;Weiss, 1980;Weiss, 1986;Xia, 2016;Yamazaki, 2014;Yamazaki, 2014}[26,32,44,47,55,59,62,76,83,87,93,95,106,108,117,122,127,130,138,139,145,146,147] |
|  |
| **5: Other (no relevant informations)**  {Barber, 1984;Ben-Eliyahu, 2000;Du, 2014;Hong-Fen, 2001;Kafetzopoulou, 2013;Shkurnikov, 2014;Shohat, 1975;Williams, 1978}[5,7,41,58,61,112,112,141] |

**Table S3.** Preclinical characteristics TNBC studies

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| **Reference: (Author - year)** | **Type** | **In vivo** | **In vitro** |  |  | **Exposure (Adrenergic stimulation or inhibition)** | |  |
|  | **in vivo/in vitro** | **Cell line injected** | **Cell line (bold if TNBC)** | **Genetic modifications** | **Knock-down** | **Drugs of interest** | **Stress** | **Other interventions** |
| Ashrafi et al. 2017 | in vivo | **4T1** |  |  |  | Propranolol |  |  |
| Ben-Eliyahu et al. 2000 | in vivo | **MADB106** |  |  |  | Atenolol  Butoxamine  ICI-118,551  Metaprotenerol  Nadolol | Swim stress | Adrenal demedullation |
| Boulay et al. 2012 | in vitro |  | **MDA-MB-231**;  + not breast cancer cell lines |  | Beta 2 adrenergic receptors | Isoprotenerol |  | Gene silencing |
| Cakir et al. 2002 | in vitro |  | **MDA-MB-435, MDA-MB-468**;  MCF-7, ZR-75, MDA-MB-361, MDA-MB-453 |  |  | Atenolol  ICI-118,551  Isoproterenol  Propranolol |  |  |
| Campbell et al. 2012 | in vivo/in vitro | **MDA-MB-231 VU** | **MDA-231 VU, 4T1-592** | Highly  bone metastatic clone |  | Isoprotenerol (in vivo/in vitro)  Propanolol (in vivo) | Chronic Immobilization Stress |  |
| Carie et al. 2007 | in vivo/in vitro | **MDA-MB-231** | **MDA-MB-231**  + not breast cancer cell lines |  |  | Pirbuterol (in vivo/in vitro) |  |  |
| Chang et al. 2016 | in vivo/in vitro | **MDA-MB-231HM; MDA-MB-231HM**  (Beta2 deficient) | **MDA-MB-231HM** |  | Beta 2 adrenergic receptors | ICI-118,551 (in vivo)  Isoprotenerol (in vitro) | Chronic Stress | Gene silencing |
| Chen et al. 2014 | in vivo/in vitro | **4T1** | **MDA-MB-231;**  MCF-7,MDA-453 |  | Beta 2 adrenergic receptors | Atenolol (in vitro)  Isoprotenerol (in vivo/in vitro)  ICI-118,551 (in vitro)  Propanolol (in vitro) |  | Gene silencing |
| Chen et al. 2018 | in vivo | **4T1;**   B16F10 (Melanoma) |  |  |  | Isoprotenerol  Propranolol | Physical and psychological stress | Ablation of the sympathetic nerve function |
| Chiesa et al. 2008 | in vitro |  | **MDA-MB-231;**  MCF-7 |  |  | Catecholestrogens  Rauwolscine  Yohimbine |  |  |
| Choy et al 2016 | in vivo/in vitro | **MDA-MB-231Br**  (brain-trophic derivative); | **MDA-MB-231, MDA-MB-231Br** (brain-trophic derivative);  COH-BBM3 (BBM3), primary Her2+ breast cancer SkBr3 cell line,  low passage Her2-amplified brain metastasis cell lines COH-BBM1 (BBM1), COH-BBM2 (BBM2) |  |  | Propranolol (in vivo/in vitro)  Terbutaline (in vitro) |  |  |
| Creed et al. 2015 | in vivo/in vitro |  | **MDA-MB-231HM, 66cl4** |  |  | CGP-20712A (in vitro)  Formoterol (in vivo)  ICI-118,551 (in vitro)  Xamoterol (in vitro) |  |  |
| Dezong et al. 2014 | in vitro |  | **MDA-MB-231;**  MCF-7 |  |  | Carvedilol  Norephinephrine |  |  |
| Drell et al. 2003 | in vitro |  | **MDA-468** |  |  | Bombesin  Dopamine  Norepinephrine  Met-enkephalin  Substance P |  |  |
| Flint et al. 2009 | in vitro |  | **MDA-MB-231** |  |  | Norephinephrine, Ephinephrine |  | Paclitaxel, Cortisol |
| Gargiulo et al. 2014 | in vitro |  | **MDA-MB-231;**  MCF-10A, MCF-7  HBL-100 |  | Beta 2 | Ephinephrine (in vitro)  Isoprotenerol (in vivo/in vitro) |  | Gene silencing, Beta AR overxepression |
| Gargiulo et al. 2017 | in vivo/in vitro |  | MCF-7 , MCF 10 A |  | Beta 2 | Ephinephrine  Isoprotenerol |  | Gene silencing, Beta AR overxepression, ovariectomy |
| Goldfarb et al. 2009 | in vivo | **MADB106** |  |  |  | Metaproterenol | Physiological stress | Laparotomy |
| Goldfarb et al. 2011 | in vivo | **MADB106 ;**  B16F10.9 (Melanoma) |  |  |  | Propranolol |  | Laparotomy |
| Hui et al. 2008 | in vitro |  | **MDA-MB-231;**  MCF-7, MCF12A (normal breast lines) |  |  | Doxazosin |  |  |
| Kim et al. 2016 | in vitro |  | **MDA-MB-231HM**  + not breast cancer cell lines | Knock out Beta 2 adrenergic receptors | Beta 2 adrenergic receptors | Isoprotenerol  Propranolol  Salmeterol |  | Gene silencing, gene knock out |
| Lamkin et al. 2015 | in vivo/in vitro | **MDA-MB-231HM** | **MDA-MB-231HM** |  |  | Efaroxan (in vivo)  Fentolamina (in vivo/in vitro)  Norephinephrine, Ephinephrine (in vitro)  Propranolol (in vivo)  Prazosin (in vivo) | Restraint stress |  |
| Lang et al. 2004 | in vitro |  | **MDA-MB-468;**  + not breast cancer cell lines |  |  | Atenolol  ICI 118, 551  Norephinephrine, Ephinephrine  Propranolol |  |  |
| Lavon et al. 2018 | in vivo | **MADB106**  + not breast cancer cell lines |  |  |  | Dexmedetomidine  Yohimbine | Surgery stress, Restraint stress, Wet cage stress |  |
| Le et al. 2015 | in vivo | **MDA-MB-231, 66cl4** |  |  |  | Isoprotenerol  Propranolol | Chronic Restraint Stress |  |
| Madden et al. 2011 | in vitro |  | **MB-231, MB-231BR ;**  MCF7, MB-361 |  |  | ICI-118,551  Isoproterenol  Norepinephrine  Terbutaline |  |  |
| Mulcrone et al. 2017 | in vivo | **MDA-MB-231** |  |  |  | Isoprotenerol  Norepinephrine | Chronic immobilization Stress | B2 receptor knockout mice |
| Pasquier et al. 2011 | in vivo/in vitro | **MDA-MB-231 (orthotopic xenograft model )** | **MDA-MB-231;**  MCF-7, SKBR3  + not breast cancer cell lines |  |  | Propranolol (in vivo/in vitro) |  | 5-Fluoro Uracil; Paclitaxel |
| Perez Pinero et al. 2012 | in vivo/in vitro | **MDA-MB-231;**  IBH-4 IBH-6 4-HD;  CC4-3-HI mouse mammary tumours. | **MDA-MB-231;**  IBH-4 IBH-6,  MC4-L5 |  |  | Adrenaline  Isoprenaline  Propanolol  Rauwolscine  Salbutamol |  |  |
| Pon et al. 2016 | in vivo/in vitro | **MDA-MB-231HM;** 66cl4 murine mammary adenocarcinoma cells (unclear if triple negative) | **MDA-MB-231HM**; 66cl4 murine mammary adenocarcinoma cells  (unclear if triple negative) |  | Beta 1 ; Beta 2 | CGP-20712A  Formeterol  ICI 118,551  Noradrenaline, Adrenaline  Propranolol  Xamoterol | Chronic Stress |  |
| Qin et al. 2015 | in vivo/in vitro | **4T1** | **4T1** |  | Beta 2 | Ephinephrine (in vitro)  Phenotolamine (in vitro)  Propranolol (in vitro) | Chronic Stress  (social isolation) | Gene silencing |
| Reeder et al. 2015 | in vivo/in vitro | **MDA-MB-231** | **MDA-MB-231m MDA-MB-436;**  MCF-7 + several breast cancer cell lines |  |  | Norephinephrine (in vitro)  Propranolol (in vitro) | Restraint stress | Paclitaxel |
| Rivero et al. 2017 | in vivo/in vitro | **MDA-MB-231;**  IBH-6 | **MDA-MB-231;**  IBH-6, |  |  | Norephinephrine (in vitro)  Propranolol (in vitro)  Salbutamol (in vitro/ in vivo) |  |  |
| Sastry et al. 2006 | in vitro |  | **MDA-MB-231;**  + not breast cancer cell lines |  |  | Ephinephrine |  |  |
| Shakhar et al. 1998 | in vivo | **MADB106** |  |  |  | Metaproterenol  Nadolol  Propranolol |  |  |
| Slotkin et al. 2000 | in vitro |  | **MDA-MB-231;**  + not breast cancer cell lines |  |  | Isoprotenerol  Propranolol |  | Dexamethasone. Theophilline |
| Strell et al. 2012 | in vitro |  | **MDA-MB-468, MDA-MB-435S, MDA-MB-231**  + not breast cancer cell lines |  |  | Norephinephrine  Propranolol |  |  |
| Szewczyk et al. 2012 | in vitro |  | **BT20;**  MCF-7 |  |  | Bisoprolol  Propranolol |  |  |
| Szpunar et al. 2013 | in vivo/in vitro | **4T1** | **4T1; MDA-MB-231** |  |  | Desipramine (in vivo)  Dexmedetomidine (in vivo/in vitro)  Isoproterenol (in vivo/in vitro)  Norepinephrine (in vitro)  Phenilephrine (in vivo/in vitro) |  |  |
| Talarico et al. 2016 | in vivo/in vitro | **MDA-MB-436 (orthotopic xenograft model )** | **MDA-MB-436 ;**  ZR-75-1 |  |  | Atenolol (in vivo/in vitro) |  | Metformin |
| Tuglu et al. 2018 | in vitro |  | **MDA‐MB‐231,MDA‐MB‐468** |  |  | Clenbuterol  Epinephrine  Formoterol  Isoproterenol  Terbutaline |  |  |
| Vandewalle et al. 1990 | in vitro |  | **MDA-MB-231;**  MCF-7, T47D |  |  | Dihydroalprenolol  Isoproterenol  Propranolol |  |  |
| Vazquez et al. 2006 | in vitro |  | **MDA-MB-231, HS-578T;**  MCF-7, HBL-100 and MCF-10A , IBH-6, IBH-7 |  |  | Ephinephrine  Clonidine  Isoproterenol  Norephinephrine  Oxymetazoline  Phenilephrine  Prozosine  Rauwolscine  Yohimbine |  |  |
| Wang et al. 2015 | in vitro |  | **MDA-MB-231** |  |  | Atenolol  ICI 118, 551  Norephinephrine |  |  |
| Wilson et al. 2015 | in vitro |  | **MDA-MB-231, MDA-MB-468;**  HEK293T |  |  | Isoproterenol  Propranolol |  |  |
| Xia (M. J.) et al. 2016 | in vivo/in vitro | **MDA-MB-231** | **MDA-MB-231** |  |  | Dexdemetodine (in vivo/in vitro) |  |  |

**Bold**: Triple negative cell line

**Table S4.** In vitro and in vivo evidence of BBs in TNBC models

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| **In vitro evidence on BBS** | | | | | | |
| Drugs | Study | TNBC Cell Line | Dose | Associate drugs or interventions | Comparator | Anticancer activity\* |
| Propranolol | Cakir et al. 2002 | MDA-MB-435 MDA-MB-468 | 1 µM | – | – | **Proliferation (inhibition DNA synthesis)** |
| Chen et al. 2014 | MDA-MB-231 | 10 µM | – | – | Strong inhibition of norepinephrine-induced Jagged 1 transcription and protein expression |
| Choy et al 2016 | MDA-MB-231 MDA-MB-231 Br(brain-trophic derivative) | 33.3 µM | With or without terbutaline | Saline | **Migration (with terbutaline);**  Migration (without terbutaline);  **Proliferation (with or without terbutaline);**  **Invasion (under terbutaline stimulation );**  Invasion (without terbutaline stimulation) |
| Creed et al. 2015 | MDA-MB-231 HM 66cl4 | 0.05 µM | With or without isoproterenol | Vehicle, isoproterenol alone | **Invasion (under isoproterenol stimulation);**  Invasion (alone) |
| Kim et al. 2016 | MDA-MB-231 HM | 100 nM | Isoproterenol | Vehicle | **Migration (induced by isoproterenol);**  Prevent cell deformability;  Invasion (induced by isoproterenol) |
| Lang et al. 2004 | MDA-MB-468 | – | Norepinephrine | – | Migration (induced by norepinephrine) |
| Pasquier et al. 2011 | MDA-MB-231 | 50-100 µM | With or without 5-Fluorouracil or Paclitaxel | Saline | Proliferation  **Pseudo angiogenesis (with or without 5-Fluorouracil or Paclitaxel)** |
| Qin et al. 2015 | 4T1 | 10 µM | Chronic stress /Adrenaline treatment | TNS (supernatant of T41 cells) | Reduce the number of M2 macrophages |
| Reeder et al. 2015 | MDA-MB-231  MDA-MB-436 | 10 µM | Paclitaxel, cortisol, or norepinephrine |  | Diminished presence of nuclei in cells before exposure to corresponding stress hormones Cort and NE |
| Rivero et al. 2017 | MDA-MB-231 | 10 µM |  | Saline | **Migration** |
| Slotkin et al. 2000 | MDA-MB-231 | 10 µM | With isoproterenol | – | Block DNA synthesis inhibition mediated by Isoproterenol |
| Strell et al. 2012 | MDA-MB-468, MDA-MB-435S  MDA-MB-231 | 10 µmol/L | With or without norepinephrine | – | Abolish the adehesion mediated by norphinephrine |
| Szewczyk et al. 2012 | BT20 | 0.3 mg/ml  2.4 mg/ml | – | DMSO, ethanol | **Proliferation (2.4mg/ml)**  Citotoxic effect (0.3 mg/ml) |
| Wilson et al. 2015 | MDA-MB-231,  MDA-MB-468 | 0.1 µM -50 µM | – | PBS | Migration  Proliferation |
| Atenolol | Cakir et al. 2002 | MDA-MB-435, MDA-MB-468 | – | – | – | **Proliferation** |
| Chen et al. 2014 | MDA-MB-231 | 10 µM | – | – | No effect on the norepinephrine-induced Jagged 1 transcription and protein expression |
| Lang et al. 2004 | MDA-MB-468 | – | Norepinephrine | – | Migration (induced by norepinephrine) |
| Talarico et al. 2016 | MDA-MB-436 | 0.1 mg/ml | With or without Metformin | – | Did not or marginally increased the frequency of apoptotic BC cells when compared to control;  Inhibits complex I of the respiratory chain (with metformin) |
| Wang et al. 2015 | MDA-MB-231 | 10 µM | – | – | Had a little impact on the reduction of CXCR4 expression mediated by norepinephrine |
| Carvedilol | Dezong et al. 2014 | MDA-MB-231 | 0.1µM  1 µM  5 µM | – | – | **Migration**  **Invasion**  Suppressed the Src activation through cAMP/PKA‐Src pathway |
| Bisoprolol | Szewczyk et al. 2012 | BT20 | 0,1 mg/ml  0,5 mg/ml | – | DMSO, ethanol | Proliferation  Low cytotoxicity |

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| ICI-118,551 | Cakir et al. 2002 | MDA-MB-435,  MDA-MB-468 | 1 µM | – | – | **Proliferation** |
| Chang et al. 2016 | MDA-MB-231HM | 1 µM | Isoproterenol | DMEM | Abolish the effect of isoproterenol on MMP2 expression;  Reduced MMP2 expression in isoproterenol-treated β2AR- deficient cells to baseline levels |
| Chen et al. 2014 | MDA-MB-231; | 10 µM | – | – | Strong inhibition of norepinephrine-induced Jagged 1 transcription and protein expression;  Inhibition of norepinephrine-induced angiogenesis by HUVECs cocultured with MDA-231 or MDA-453 cells |
| Creed et al. 2015 | MDA-MB-231HM  66cl4 | 0.05 µM | With or without isoproterenol | Vehicle, isoproterenol alone | **Invasion (under isoproterenol stimulation)** |
| Lang et al. 2004 | MDA-MB-468; | – | Norepinephrine | – | Migration (induced by norepinephrine) |
| Pon et al. 2016 | MDA-MB-231HM;  66cl4 | 100 nM  50 nM | Stress condition | Vehicle | **Invasion (under stress condition)** |
| Wang et al. 2015 | MDA-MB-231 | 10 µM | – | – | Eliminated the impact of norepinephrine on CXCR4 expression |

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| **In vivo evidence on BBs** |

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| **Dugs** | **Study** | **Animal** | **Dose** | **Associate drug/ intervention** | **Comparator** | **Anticancer activity\*** |

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| Propranolol | Ashrafi et al. 2017 | Balb/C mice  injected with 4T1 | 3 mg/kg | 100 µg of tumor lysate vaccine | PBS (with or without 100µg of tumor lysate vaccine) | **Inhibit tumor growth and reduce tumor volume**  Increase lymphocyte proliferation  Increase the concentration of IL-12, IL-17, IL-2 and IFN- cytokines in tumor microenvironment |
| Campbell et al. 2012 | Propranolol | 0.5 g/L | With or without chronic stress | PBS | **Metastasis (with stress condition)**  Reduce metastasis formation (without stress condition)  **Reduce lesion number and lesion area (under stress condition**)  Reduce lesion number and lesion area (without stress condition) |
| Chen et al. 2018 | Balb/c mice,  C57BL/6j mice,  Balb/C nude mice,  NOD scid gamma (NSG) mice,  MMTV-PyMT mice  injected with 4T1 | 2 mg/kg | – | – | **Metastasis** |
| Lamkin et al. 2015 | NU-Foxn1n nu/nu mice injected with MDA-MB-231HM | 2 mg/kg | With or without phentolamine | Placebo | Tumor growth (alone or in combination with phentolamine)  Tumor volume (alone or in combination with phentolamine)  Metastasis (alone or in combination with phentolamine) |
| Le et al. 2015 | BALB/c nu/ nu  BALB/cJAsm MMTV-PyMT C57Bl/6 injected with MDA-MB-231, 66cl4 | 5 mg/kg | Stress | Vehicle | **Metastasis (under stress condition)**  Blocked chronic stress from increasing tumour LYVE-1 + LVD and reduced metastasis to lymph node |
| Pasquier et al. 2011 | NMRI nude mice injected with MDA-MB-231 (orthotopic xenograft model ) | 10 mg/kg | Alone or with Paclitaxel (20mg/kg), or 5 Fluorouracile | Saline | **Increase survival (in combination)**  Tumor growth (in combination)  Potentiate the anti-angiogenic effects of chemotherapy |
| Perez Pinero et al. 2012 | Balb/c mice, N : NIH(S)-nu (athymic nude mice) injected with MDA-MB-231 | 1 mg/kg | – | Saline | Tumor growth |
| Shakhar et al. 1998 | Fischer F344 injected with MADB106 | 0,1 mg/kg  0,2 mg/kg  0,5 mg/kg | Metaproterenol | Saline | Block NK cells suppression mediated by metaproterenol |
| Atenolol | Ben-Eliyahu et al. 2000 | Fisher 344 rats injected with MADB106 | 1 mg/kg  1.5 mg/kg  6 mg/kg | With or without butoxamine, stress | Saline | **Metastatasis (with or without butoxamine under stress condition)** |
| Talarico et al. 2016 | NOD SCID IL2RG null  immune-competent FVB injected with MDA-MB-436 (orthotopic xenograft model ) | – | With or without metformin | – | **Tumor growth (with metformin)**  **Metastasis (with metformin)**  Target the endothelial compartment of BC vessels and generated dysplastic vessels |
| Nadolol | Ben-Eliyahu et al. 2000 | Fisher 344 rats injected with MADB106 | 0.4 mg/kg | Stress | Saline | **Metastasis (under stress condition)** |
| Shakhar et al. 1998 | Fischer F344 injected with MADB106 | 0,1 mg/kg  0,2 mg/kg  0,5 mg/kg | Metaproterenol | Saline | Metastasis (reverse metaproterenol effects)  Block NK cells suppression |

\*Anticancer activities marked in bold were statistically significant

**Table S5.** Risk of bias assessment according to the New-Castle Ottawa scale

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Study** | | | |
|  | **#1** | **#2** | **#3** | **#4** |
| **Selection**  **(max 4 stars)** | **# # #** | **###** | **##** | **##** |
| **Comparability**  **(max 2 stars)** | **#** | **##** | **-** | **-** |
| **Outcome**  **(max 3 stars)** | **#** | **##** | **##** | **-** |

**Study #1 (Melhem-Bertrandt, 2011)**

|  |  |  |
| --- | --- | --- |
| **Assessment Items** |  | **Comments** |
| **Selection** |  |  |
| Representativeness of the exposed cohort:  a) truly representative of the average \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ (describe) in the community  b) somewhat representative of the average \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ in the community #  c) selected group of users eg nurses, volunteers  d) no description of the derivation of the cohort |  | The study cohort was drawn from “The Breast Cancer Management System Database at The University of Texas MD Anderson Cancer Center was searched, and 1,449 patients with  invasive breast cancer who were treated with anthracylines and taxane-based neoadjuvant chemotherapy”  Notably, 33 patients exposed to BB after neoadjuvant were excluded from the study cohort: it is not specified whether those were was in the exposed arm (102 patients) , which would make them a relevant portion. In principle, it could be that those exposed to BB after neoadjuvant therapy were exactly those with a worse prognosis. |
| Selection of the non exposed cohort:  a) drawn from the same community as the exposed cohort#  b) drawn from a different source  c) no description of the derivation of the non exposed cohort | # | Drawn from the same community as the exposed cohort |
| Ascertainment of exposure  a) secure record (eg surgical records)#  b) structured interview  c) written self report  d) no description | # | Information on medication use was retrieved from review of the patient medical and pharmacy records. Patients were asked about their medications during their first clinic visit and follow-up, this information is then updated in their medical record. |
| Demonstration that outcome of interest was not present at start of study  a) yes#  b) no | # | Yes, by definition, i.e. Recurrence Free Survival (RFS) and overall survival (OS) |
| **Comparability** |  |  |
| 1) Comparability of cohorts on the basis of the design or analysis:  a) study controls for *age, stage, race, BMI, metformin use, diabetes, hypertension, ACE/ ARBs* (select the most important factor)**#**  b) study controls for any additional factor# (This criteria could be modified to indicate specific control for a second important factor.) | # | Other factors that could affect breast cancer relapse may also be confounding this study. These include aspirin use, alcohol intake, dietary factors, and lack of exercise.  In addition, although age and age related characteristics were considered for statistical adjustment they were strongly imbalanced across the two treatment groups. |
| **Outcome** |  |  |
| 1) Assessment of outcome:  a) independent blind assessment**#**  b) record linkage**#**  c) self report  d) no description | # | From medical records. Specimens were reviewed by pathologists |
| 2) Was follow-up long enough for outcomes to occur?  a) yes (select an adequate follow up period for outcome of interest)**#**  b) no |  | No, 3 years of follow-up might not be sufficient in such a population, especially to compare the overall survival rate. |
| 3) Adequacy of follow up of cohort:  a) complete follow up - all subjects accounted for#  b) subjects lost to follow up unlikely to introduce bias - small number lost - > \_\_\_\_ % (select an  adequate %) follow up, or description provided of those lost)**#**  c) follow up rate < \_\_\_\_% (select an adequate %) and no description of those lost  d) no statement |  | No statement |

**Study #2 Botteri, 2013**

|  |  |  |
| --- | --- | --- |
| **Assessment Items** |  | **Comments** |
| **Selection** |  |  |
| Representativeness of the exposed cohort:  a) truly representative of the average \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ (describe) in the community  b) somewhat representative of the average of *post-menopausal patients with primary TNBC grade I-III* in the community #  c) selected group of users eg nurses, volunteers  d) no description of the derivation of the cohort | # | All consecutive postmenopausal women diagnosed and operated for early primary TNBC (grade I-III) between 1997 and 2008 at the European Institute of Oncology in Milan. |
| Selection of the non exposed cohort:  a) drawn from the same community as the exposed cohort#  b) drawn from a different source  c) no description of the derivation of the non exposed cohort | # | Drawn from the same community as the exposed cohort |
| Ascertainment of exposure  a) secure record (eg surgical records)#  b) structured interview  c) written self report  d) no description |  | Any BB at the moment of their diagnosis of TNBC (medical records).  No information on BB use before and after the diagnosis of TNBC. |
| Demonstration that outcome of interest was not present at start of study  a) yes#  b) no | # | Yes, by definition, i.e. BC-related events, Metastases, BC death |
| **Comparability** |  |  |
| 1) Comparability of cohorts on the basis of the design or analysis:  a) study controls for *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* (select the most important factor)**#**  b) study controls for any additional factor# (This criteria could be modified to indicate specific control for a second important factor.) | ## | BB users and non-users were uniformly distributed by  tumor characteristics and cancer treatments |
| **Outcome** |  |  |
| 1) Assessment of outcome:  a) independent blind assessment**#**  b) record linkage**#**  c) self report  d) no description | # | Retrospective analysis of patients’ medical records |
| 2) Was follow-up long enough for outcomes to occur?  a) yes (select an adequate follow up period for outcome of interest)**#**  b) no | # | Yes, up to 7 years |
| 3) Adequacy of follow up of cohort:  a) complete follow up - all subjects accounted for#  b) subjects lost to follow up unlikely to introduce bias - small number lost - > \_\_\_\_ % (select an  adequate %) follow up, or description provided of those lost)**#**  c) follow up rate < \_\_\_\_% (select an adequate %) and no description of those lost  d) no statement |  | No statement |

**Study #3 Spera, 2017(1)**

|  |  |  |
| --- | --- | --- |
| **Assessment Items** |  | **Comments** |
| **Selection** |  |  |
| Representativeness of the exposed cohort:  a) truly representative of the average \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ (describe) in the community  b) somewhat representative of the average in the community #  c) selected group of users eg nurses, volunteers  d) no description of the derivation of the cohort |  | Patients were selected from the study cohort of a multicenter double blind RCT.  No further description of the derivation of the cohort. |
| Selection of the non exposed cohort:  a) drawn from the same community as the exposed cohort#  b) drawn from a different source  c) no description of the derivation of the non exposed cohort | # | drawn from the same clinical trial cohort as the exposed cohort |
| Ascertainment of exposure  a) secure record (eg surgical records)#  b) structured interview  c) written self report  d) no description |  | Patients were allocated to the BB use group measuring exposure either at baseline or during follow-up, thus likely introducing an immortal time bias favouring the BB use category. |
| Demonstration that outcome of interest was not present at start of study  a) yes#  b) no | # | Yes, by definition (PSF, OS, RFS). |
| **Comparability** |  |  |
| 1) Comparability of cohorts on the basis of the design or analysis:  a) study controls for \_\_\_\_\_\_\_\_\_\_\_\_\_ (select the most important factor)**#**  b) study controls for any additional factor# (This criteria could be modified to indicate specific control for a second important factor.) |  | Variables considered for the analysis only included BB  intake, development of TEH, hormone receptor status and treatment  arm. “The effect of other concomitant medications  with known potential anti-cancer effect including ACE inhibitors,  metformin and non-steroidal agents was not explored. Neither interactions between BB and other anti-hypertensive drugs were examined” |
| **Outcome** |  |  |
| 1) Assessment of outcome:  a) independent blind assessment**#**  b) record linkage**#**  c) self report  d) no description | # | Data came from a double blind RCT |
| 2) Was follow-up long enough for outcomes to occur?  a) yes (select an adequate follow up period for outcome of interest)**#**  b) no | # | Yes, up to 4 years of follow-up might be enough in such a population uf patients with advanced disease stage. |
| 3) Adequacy of follow up of cohort:  a) complete follow up - all subjects accounted for#  b) subjects lost to follow up unlikely to introduce bias - small number lost - > \_\_\_\_ % (select an  adequate %) follow up, or description provided of those lost)**#**  c) follow up rate < \_\_\_\_% (select an adequate %) and no description of those lost  d) no statement |  | No statement |

**Study #4 Spera, 2017(2)**

|  |  |  |
| --- | --- | --- |
| **Assessment Items** |  | **Comments** |
| **Selection** |  |  |
| Representativeness of the exposed cohort:  a) truly representative of the average \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ (describe) in the community  b) somewhat representative of the average in the community #  c) selected group of users eg nurses, volunteers  d) no description of the derivation of the cohort |  | Patients were selected from the study cohort of a multicenter RCT.  No further description of the derivation of the cohort. |
| Selection of the non exposed cohort:  a) drawn from the same community as the exposed cohort#  b) drawn from a different source  c) no description of the derivation of the non exposed cohort | # | Drawn from the same community as the exposed cohort |
| Ascertainment of exposure  a) secure record (eg surgical records)#  b) structured interview  c) written self report  d) no description |  | Patients were allocated to the BB use group measuring exposure either at baseline or during follow-up, thus likely introducing an immortal time bias favouring the BB use category. |
| Demonstration that outcome of interest was not present at start of study  a) yes#  b) no | # | Yes, by definition (OS, RFS). |
| **Comparability** |  |  |
| 1) Comparability of cohorts on the basis of the design or analysis:  a) study controls for \_\_\_\_\_\_\_\_\_\_\_\_\_ (select the most important factor)**#**  b) study controls for any additional factor# (This criteria could be modified to indicate specific control for a second important factor.) |  | Variables considered for the analysis only included BB  intake, development of TEH, hormone receptor status and treatment  arm. “The effect of other concomitant medications  with known potential anti-cancer effect including ACE inhibitors,  metformin and non-steroidal agents was not explored. Neither interactions between BB and other anti-hypertensive drugs were examined” |
| **Outcome** |  |  |
| 1) Assessment of outcome:  a) independent blind assessment**#**  b) record linkage**#**  c) self report  d) no description |  | No description. |
| 2) Was follow-up long enough for outcomes to occur?  a) yes (select an adequate follow up period for outcome of interest)**#**  b) no |  | No description |
| 3) Adequacy of follow up of cohort:  a) complete follow up - all subjects accounted for#  b) subjects lost to follow up unlikely to introduce bias - small number lost - > \_\_\_\_ % (select an  adequate %) follow up, or description provided of those lost)**#**  c) follow up rate < \_\_\_\_% (select an adequate %) and no description of those lost  d) no statement |  | No statement |

Details from the application of the Newcastle-Ottawa scale for risk of bias assessment.

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