**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** |   |  |  | AtenololButoxamineICI-118,551MetaprotenerolNadolol | 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  |  |  | AtenololICI-118,551IsoproterenolPropranolol   |  |  |
| 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) |   |  |  | IsoprotenerolPropranolol | Physical and psychological stress | Ablation of the sympathetic nerve function  |
| Chiesa et al. 2008 | in vitro |  | **MDA-MB-231;**MCF-7 |  |  | CatecholestrogensRauwolscineYohimbine |  |  |
| 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 |  |  | CarvedilolNorephinephrine |  |  |
| Drell et al. 2003 | in vitro |  | **MDA-468** |  |  | BombesinDopamineNorepinephrineMet-enkephalinSubstance 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 | EphinephrineIsoprotenerol |  | 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 | IsoprotenerolPropranololSalmeterol |  | 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 |  |  | AtenololICI 118, 551 Norephinephrine, EphinephrinePropranolol |  |  |
| Lavon et al. 2018 | in vivo | **MADB106** + not breast cancer cell lines |   |  |  | DexmedetomidineYohimbine | Surgery stress, Restraint stress, Wet cage stress |  |
| Le et al. 2015 | in vivo | **MDA-MB-231, 66cl4** |   |  |  | IsoprotenerolPropranolol | Chronic Restraint Stress  |  |
| Madden et al. 2011 | in vitro |  | **MB-231, MB-231BR ;**MCF7, MB-361  |  |  | ICI-118,551IsoproterenolNorepinephrineTerbutaline |  |  |
| 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 |  |  | AdrenalineIsoprenalinePropanololRauwolscineSalbutamol |  |  |
| 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-20712AFormeterolICI 118,551Noradrenaline, AdrenalinePropranololXamoterol | 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** |  |  |  | MetaproterenolNadololPropranolol |  |  |
| Slotkin et al. 2000 | in vitro |  | **MDA-MB-231;**+ not breast cancer cell lines |  |  | IsoprotenerolPropranolol |  | Dexamethasone. Theophilline |
| Strell et al. 2012 | in vitro |  | **MDA-MB-468, MDA-MB-435S, MDA-MB-231**+ not breast cancer cell lines |  |  | NorephinephrinePropranolol |  |  |
| Szewczyk et al. 2012 | in vitro |  | **BT20;**MCF-7 |  |  | BisoprololPropranolol |  |  |
| 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** |  |  | ClenbuterolEpinephrineFormoterolIsoproterenolTerbutaline |  |  |
| Vandewalle et al. 1990 | in vitro |  | **MDA-MB-231;**MCF-7, T47D |  |  | DihydroalprenololIsoproterenolPropranolol |  |  |
| Vazquez et al. 2006 | in vitro |  | **MDA-MB-231, HS-578T;**MCF-7, HBL-100 and MCF-10A , IBH-6, IBH-7 |  |  | EphinephrineClonidineIsoproterenolNorephinephrineOxymetazolinePhenilephrineProzosineRauwolscineYohimbine  |  |  |
| Wang et al. 2015 | in vitro |  | **MDA-MB-231** |  |  | AtenololICI 118, 551 Norephinephrine |  |  |
| Wilson et al. 2015 | in vitro |  | **MDA-MB-231, MDA-MB-468;**HEK293T |  |  | IsoproterenolPropranolol |  |  |
| 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-231MDA-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-435SMDA-MB-231 | 10 µmol/L | With or without norepinephrine | – | Abolish the adehesion mediated by norphinephrine |
| Szewczyk et al. 2012 | BT20 | 0.3 mg/ml2.4 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 | MigrationProliferation |
| 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 | 0.1µM1 µM5 µM | – | – | **Migration****Invasion**Suppressed the Src activation through cAMP/PKA‐Src pathway |
| Bisoprolol | Szewczyk et al. 2012 | BT20 | 0,1 mg/ml0,5 mg/ml | – | DMSO, ethanol | ProliferationLow 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-231HM66cl4 | 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 nM50 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 miceinjected 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 proliferationIncrease 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 miceinjected 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/kg0,2 mg/kg0,5 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/kg1.5 mg/kg6 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/kg0,2 mg/kg0,5 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 communityb) somewhat representative of the average \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ in the community #c) selected group of users eg nurses, volunteersd) 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 withinvasive 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 sourcec) no description of the derivation of the non exposed cohort | # | Drawn from the same community as the exposed cohort |
| Ascertainment of exposurea) secure record (eg surgical records)#b) structured interviewc) written self reportd) 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 studya) 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 reportd) 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 anadequate %) follow up, or description provided of those lost)**#**c) follow up rate < \_\_\_\_% (select an adequate %) and no description of those lostd) 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 communityb) 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, volunteersd) 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 sourcec) no description of the derivation of the non exposed cohort | # | Drawn from the same community as the exposed cohort |
| Ascertainment of exposurea) secure record (eg surgical records)#b) structured interviewc) written self reportd) 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 studya) 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 bytumor characteristics and cancer treatments |
| **Outcome** |  |  |
| 1) Assessment of outcome:a) independent blind assessment**#**b) record linkage**#**c) self reportd) 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 anadequate %) follow up, or description provided of those lost)**#**c) follow up rate < \_\_\_\_% (select an adequate %) and no description of those lostd) 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 communityb) somewhat representative of the average in the community #c) selected group of users eg nurses, volunteersd) 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 sourcec) no description of the derivation of the non exposed cohort | # | drawn from the same clinical trial cohort as the exposed cohort |
| Ascertainment of exposurea) secure record (eg surgical records)#b) structured interviewc) written self reportd) 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 studya) 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 BBintake, development of TEH, hormone receptor status and treatmentarm. “The effect of other concomitant medicationswith 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 reportd) 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 anadequate %) follow up, or description provided of those lost)**#**c) follow up rate < \_\_\_\_% (select an adequate %) and no description of those lostd) 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 communityb) somewhat representative of the average in the community #c) selected group of users eg nurses, volunteersd) 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 sourcec) no description of the derivation of the non exposed cohort | # | Drawn from the same community as the exposed cohort |
| Ascertainment of exposurea) secure record (eg surgical records)#b) structured interviewc) written self reportd) 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 studya) 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 BBintake, development of TEH, hormone receptor status and treatmentarm. “The effect of other concomitant medicationswith 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 reportd) 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 anadequate %) follow up, or description provided of those lost)**#**c) follow up rate < \_\_\_\_% (select an adequate %) and no description of those lostd) no statement |  | No statement |

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

**References to supplementary Tables**

1. Abdin AA: S. Effect of propranolol on IL-10, visfatin, Hsp70, iNOS, TLR2, and survivin in amelioration of tumor progression and survival in Solid Ehrlich Carcinoma-bearing mice. *Pharmacological reports : PR* 2014; 66: 1114–21.

2. Anton AH: P. A RELATIONSHIP BETWEEN OVARIAN NOREPINEPHRINE AND BREAST CANCER IN HUMANS. *Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine (New York, NY)* 1963; 114: 145–7.

3. Ashrafi S: S. Immunological consequences of immunization with tumor lysate vaccine and propranolol as an adjuvant: A study on cytokine profiles in breast tumor microenvironment. *Immunology letters* 2017; 181: 63–70.

4. Badino GR: N. Evidence for functional beta-adrenoceptor subtypes in CG-5 breast cancer cell. *Pharmacological research* 1996; 33: 255–60.

5. Barber R: G. Hormone and methylxanthine action on breast epithelial cells. *Life sciences* 1984; 34: 2467–76.

6. Barron TI: C. Beta blockers and breast cancer mortality: a population- based study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2011; 29: 2635–44.

7. Ben-Eliyahu S: S. Suppression of NK cell activity and of resistance to metastasis by stress: a role for adrenal catecholamines and beta-adrenoceptors. *Neuroimmunomodulation* 2000; 8: 154–64.

8. Ben-Eliyahu S: S. Timing within the oestrous cycle modulates adrenergic suppression of NK activity and resistance to metastasis: possible clinical implications. *British journal of cancer* 2000; 83: 1747–54.

9. Borowiec AS: H. IGF-1 activates hEAG K(+) channels through an Akt-dependent signaling pathway in breast cancer cells: role in cell proliferation. *Journal of cellular physiology* 2007; 212: 690–701.

10. Botteri E: M. Therapeutic effect of beta-blockers in triple-negative breast cancer postmenopausal women. *Breast cancer research and treatment* 2013; 140: 567–75.

11. Boudreau DM: Y. Comparative safety of cardiovascular medication use and breast cancer outcomes among women with early stage breast cancer. *Breast cancer research and treatment* 2014; 144: 405–16.

12. Boulay G: M. Loss of Hypermethylated in Cancer 1 (HIC1) in breast cancer cells contributes to stress-induced migration and invasion through beta-2 adrenergic receptor (ADRB2) misregulation. *The Journal of biological chemistry* 2012; 287: 5379–89.

13. Bruzzone A: P. Alpha2-adrenoceptor action on cell proliferation and mammary tumour growth in mice. *British journal of pharmacology* 2008; 155: 494–504.

14. Bruzzone A: P. alpha(2)-Adrenoceptors enhance cell proliferation and mammary tumor growth acting through both the stroma and the tumor cells. *Current cancer drug targets* 2011; 11: 763–74.

15. Cakir Y: P. Beta-adrenergic and arachidonic acid-mediated growth regulation of human breast cancer cell lines. *International journal of oncology* 2002; 21: 153–7.

16. Campbell JP: K. Stimulation of host bone marrow stromal cells by sympathetic nerves promotes breast cancer bone metastasis in mice. *PLoS biology* 2012; 10: e1001363.

17. Cardwell CR: C. Beta-blocker usage and breast cancer survival: a nested case-control study within a UK clinical practice research datalink cohort. *International journal of epidemiology* 2013; 42: 1852–61.

18. Cardwell CR: P. Propranolol and survival from breast cancer: a pooled analysis of European breast cancer cohorts. *Breast cancer research : BCR* 2016; 18: 119.

19. Castillo LF: R. Alpha2-adrenoceptor agonists trigger prolactin signaling in breast cancer cells. *Cellular signalling* 2017; 34: 76–85.

20. Chang A: L. beta2-Adrenoceptors on tumor cells play a critical role in stress-enhanced metastasis in a mouse model of breast cancer. *Brain, behavior, and immunity* 2016; 57: 106–115.

21. Chang CH: C. Antihypertensive agents and the risk of breast cancer in women aged 55 years and older: a nested case-control study. *Journal of hypertension* 2016; 34: 558–66; discussion 566.

22. Charles NJ: T. Expression of membrane progesterone receptors (mPR/PAQR) in ovarian cancer cells: implications for progesterone-induced signaling events. *Hormones & cancer* 2010; 1: 167–76.

23. Chen H: L. Adrenergic signaling promotes angiogenesis through endothelial cell-tumor cell crosstalk. *Endocrine-related cancer* 2014; 21: 783–95.

24. Chen H: L. Chronic psychological stress promotes lung metastatic colonization of circulating breast cancer cells by decorating a pre-metastatic niche through activating beta-adrenergic signaling. *The Journal of pathology* 2018; 244: 49–60.

25. Chen L: C. Use of Antihypertensive Medications and Risk of Adverse Breast Cancer Outcomes in a SEER-Medicare Population. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2017; 26: 1603–1610.

26. Chen L: M. Use of Antihypertensive Medications Not Associated with Risk of Contralateral Breast Cancer among Women Diagnosed with Estrogen Receptor-Positive Invasive Breast Cancer. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2015; 24: 1423–6.

27. Chen ZB: L. Effects of tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) on the activation of ERK1/2 MAP kinases and the proliferation of human mammary epithelial cells. *Environmental toxicology and pharmacology* 2006; 22: 283–91.

28. Chiesa IJ: C. Contribution of alpha2-adrenoceptors to the mitogenic effect of catecholestrogen in human breast cancer MCF-7 cells. *The Journal of steroid biochemistry and molecular biology* 2008; 110: 170–5.

29. Childers WK: H. beta-Blockers Reduce Breast Cancer Recurrence and Breast Cancer Death: A Meta-Analysis. *Clinical breast cancer* 2015; 15: 426–31.

30. Choy C: R. Inhibition of beta2-adrenergic receptor reduces triple-negative breast cancer brain metastases: The potential benefit of perioperative beta-blockade. *Oncology reports* 2016; 35: 3135–42.

31. Clavel F: L. [Breast cancer and use of antihypertensive drugs and oral contraceptives: results of a case-control study (author’s transl)]. *Bulletin du cancer* 1981; 68: 449–55.

32. Connor A: B. ADRB2 G-G haplotype associated with breast cancer risk among Hispanic and non-Hispanic white women: interaction with type 2 diabetes and obesity. *Cancer causes & control : CCC* 2012; 23: 1653–63.

33. Creed SJ: L. beta2-adrenoceptor signaling regulates invadopodia formation to enhance tumor cell invasion. *Breast cancer research : BCR* 2015; 17: 145.

34. Cronin-Fenton D: L. Concurrent new drug prescriptions and prognosis of early breast cancer: studies using the Danish Breast Cancer Group clinical database. *Acta oncologica (Stockholm, Sweden)* 2018; 57: 120–128.

35. Davis S: M. Medication use and the risk of breast cancer. *European journal of epidemiology* 2007; 22: 319–25.

36. Devore EE: K. Antihypertensive medication use and incident breast cancer in women. *Breast cancer research and treatment* 2015; 150: 219–29.

37. Dezong G: Z. Carvedilol suppresses migration and invasion of malignant breast cells by inactivating Src involving cAMP/PKA and PKCdelta signaling pathway. *Journal of cancer research and therapeutics* 2014; 10: 998–1003.

38. Dhar MS: P. Protein expression of G-protein inwardly rectifying potassium channels (GIRK) in breast cancer cells. *BMC physiology* 2006; 6: 8.

39. Draoui A: V. Beta-adrenergic receptors in human breast cancer: identification, characterization and correlation with progesterone and estradiol receptors. *Anticancer research* 1991; 11: 677–80.

40. Drell TL th: J. Effects of neurotransmitters on the chemokinesis and chemotaxis of MDA-MB-468 human breast carcinoma cells. *Breast cancer research and treatment* 2003; 80: 63–70.

41. Du Y: Z. Association of alpha2a and beta2 adrenoceptor expression with clinical outcome in breast cancer. *Current medical research and opinion* 2014; 30: 1337–44.

42. Fitzgerald PJ. Beta blockers, norepinephrine, and cancer: an epidemiological viewpoint. *Clinical epidemiology* 2012; 4: 151–6.

43. Flint MS: K. Stress hormones mediate drug resistance to paclitaxel in human breast cancer cells through a CDK-1-dependent pathway. *Psychoneuroendocrinology* 2009; 34: 1533–41.

44. Freire-Garabal M: N. Effects of amphetamine on the development of MTV-induced mammary tumors in female mice. *Life sciences* 1992; 51: PL37-40.

45. Fryzek JP: P. A cohort study of antihypertensive medication use and breast cancer among Danish women. *Breast cancer research and treatment* 2006; 97: 231–6.

46. Ganz PA: H. Examining the influence of beta blockers and ACE inhibitors on the risk for breast cancer recurrence: results from the LACE cohort. *Breast cancer research and treatment* 2011; 129: 549–56.

47. Garcia-Solis P: A. 5’Deiodinase in two breast cancer cell lines: effect of triiodothyronine, isoproterenol and retinoids. *Molecular and cellular endocrinology* 2003; 201: 25–31.

48. Gargiulo L: C. Differential beta(2)-adrenergic receptor expression defines the phenotype of non-tumorigenic and malignant human breast cell lines. *Oncotarget* 2014; 5: 10058–69.

49. Gargiulo L: M. A Novel Effect of beta-Adrenergic Receptor on Mammary Branching Morphogenesis and its Possible Implications in Breast Cancer. *Journal of mammary gland biology and neoplasia* 2017; 22: 43–57.

50. Goldfarb Y: B. CpG-C oligodeoxynucleotides limit the deleterious effects of beta-adrenoceptor stimulation on NK cytotoxicity and metastatic dissemination. *Journal of immunotherapy (Hagerstown, Md : 1997)* 2009; 32: 280–91.

51. Goldfarb Y: S. Improving postoperative immune status and resistance to cancer metastasis: a combined perioperative approach of immunostimulation and prevention of excessive surgical stress responses. *Annals of surgery* 2011; 253: 798–810.

52. Goldvaser H: R. The Association between Angiotensin Receptor Blocker Usage and Breast Cancer Characteristics. *Oncology* 2016; 91: 217–223.

53. Gomez-Acebo I: D-S. The Use of Antihypertensive Medication and the Risk of Breast Cancer in a Case-Control Study in a Spanish Population: The MCC-Spain Study. *PloS one* 2016; 11: e0159672.

54. Goravanchi F: K. A case series of thoracic paravertebral blocks using a combination of ropivacaine, clonidine, epinephrine, and dexamethasone. *Journal of clinical anesthesia* 2012; 24: 664–7.

55. Hance MW: D. G-Protein Inwardly Rectifying Potassium Channel 1 (GIRK1) Knockdown Decreases Beta-Adrenergic, MAP Kinase and Akt Signaling in the MDA-MB-453 Breast Cancer Cell Line. *Breast cancer : basic and clinical research* 2008; 1: 25–34.

56. Holmes MD: H. Beta blockers and angiotensin-converting enzyme inhibitors’ purported benefit on breast cancer survival may be explained by aspirin use. *Breast cancer research and treatment* 2013; 139: 507–13.

57. Holmes S: G. Antihypertensive medications and survival in patients with cancer: a population-based retrospective cohort study. *Cancer epidemiology* 2013; 37: 881–5.

58. Hong-Fen L: W. The effects of a Chinese herb formula, anti-cancer number one (ACNO), on NK cell activity and tumor metastasis in rats. *International immunopharmacology* 2001; 1: 1947–56.

59. Huang XE: H. Possible association of beta2- and beta3-adrenergic receptor gene polymorphisms with susceptibility to breast cancer. *Breast cancer research : BCR* 2001; 3: 264–9.

60. Hui H: F. The alpha1-adrenergic receptor antagonist doxazosin inhibits EGFR and NF-kappaB signalling to induce breast cancer cell apoptosis. *European journal of cancer (Oxford, England : 1990)* 2008; 44: 160–6.

61. Kafetzopoulou LE: B. Biomarker identification in breast cancer: Beta-adrenergic receptor signaling and pathways to therapeutic response. *Computational and structural biotechnology journal* 2013; 6: e201303003.

62. Kang F: M. Propranolol inhibits glucose metabolism and 18F-FDG uptake of breast cancer through posttranscriptional downregulation of hexokinase-2. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* 2014; 55: 439–45.

63. Kim TH: G. Cancer cells become less deformable and more invasive with activation of beta-adrenergic signaling. *Journal of cell science* 2016; 129: 4563–4575.

64. Kopf I: H. Vascular cross-sectional area in rat mammary tumours; influence of noradrenaline. *Anticancer research* 1996; 16: 1161–4.

65. Lamkin DM: S. alpha2-Adrenergic blockade mimics the enhancing effect of chronic stress on breast cancer progression. *Psychoneuroendocrinology* 2015; 51: 262–70.

66. Lavon H: M. Dexmedetomidine promotes metastasis in rodent models of breast, lung, and colon cancers. *British journal of anaesthesia* 2018; 120: 188–196.

67. Leung HW: H. Long-Term Use of Antihypertensive Agents and Risk of Breast Cancer: A Population-Based Case-Control Study. *Cardiology and therapy* 2015; 4: 65–76.

68. Li CI: D. Use of antihypertensive medications and breast cancer risk among women aged 55 to 74 years. *JAMA internal medicine* 2013; 173: 1629–37.

69. Li CI: M. Relation between use of antihypertensive medications and risk of breast carcinoma among women ages 65-79 years. *Cancer* 2003; 98: 1504–13.

70. Lindgren ME: F. Beta-blockers may reduce intrusive thoughts in newly diagnosed cancer patients. *Psycho-oncology* 2013; 22: 1889–94.

71. Liu D: D. A Her2-let-7-beta2-AR circuit affects prognosis in patients with Her2-positive breast cancer. *BMC cancer* 2015; 15: 832.

72. Liu D: Y. beta2-AR signaling controls trastuzumab resistance-dependent pathway. *Oncogene* 2016; 35: 47–58.

73. Madden KS: S. beta-Adrenergic receptors (beta-AR) regulate VEGF and IL-6 production by divergent pathways in high beta-AR-expressing breast cancer cell lines. *Breast cancer research and treatment* 2011; 130: 747–58.

74. Madden KS: S. Early impact of social isolation and breast tumor progression in mice. *Brain, behavior, and immunity* 2013; 30 Suppl: S135-41.

75. Marchetti B: S. Beta-adrenergic receptors in DMBA-induced rat mammary tumors: correlation with progesterone receptor and tumor growth. *Breast cancer research and treatment* 1989; 13: 251–63.

76. Marchetti B: S. A potential role for catecholamines in the development and progression of carcinogen-induced mammary tumors: hormonal control of beta-adrenergic receptors and correlation with tumor growth. *The Journal of steroid biochemistry and molecular biology* 1991; 38: 307–20.

77. Marjamaki A: A-U. Stable expression of recombinant human alpha 2-adrenoceptor subtypes in two mammalian cell lines: characterization with [3H]rauwolscine binding, inhibition of adenylate cyclase and RNase protection assay. *Biochimica et biophysica acta* 1992; 1134: 169–77.

78. Marjamaki A: L. Use of recombinant human alpha 2-adrenoceptors to characterize subtype selectively of antagonist binding. *European journal of pharmacology* 1993; 246: 219–26.

79. Meier CR: D. Angiotensin-converting enzyme inhibitors, calcium channel blockers, and breast cancer. *Archives of internal medicine* 2000; 160: 349–53.

80. Melhem-Bertrandt A: C-M. Beta-blocker use is associated with improved relapse-free survival in patients with triple-negative breast cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2011; 29: 2645–52.

81. Montoya A: A. Use of non-selective beta-blockers is associated with decreased tumor proliferative indices in early stage breast cancer. *Oncotarget* 2017; 8: 6446–6460.

82. Mulcrone PL: C. Skeletal Colonization by Breast Cancer Cells Is Stimulated by an Osteoblast and beta2AR-Dependent Neo-Angiogenic Switch. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* 2017; 32: 1442–1454.

83. Murray E: M. Rapid, simple identification of individual osteoblastic cells and their specific products by cell blotting assay. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* 1989; 4: 831–8.

84. Nagaraja AS: D. Adrenergic-mediated increases in INHBA drive CAF phenotype and collagens. *JCI insight*; 2. Epub ahead of print 17 August 2017. DOI: 10.1172/jci.insight.93076.

85. Numbere B: F. Adrenergic blockers and the risk for common solid cancers: a case-control study. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP)* 2017; 26: 86–93.

86. Parada-Huerta E: A-D. Metastasis Risk Reduction Related with Beta-Blocker Treatment in Mexican Women with Breast Cancer. *Asian Pacific journal of cancer prevention : APJCP* 2016; 17: 2953–7.

87. Parkin R: N. The effect of isoprenaline on induction of tumours by methyl nitrosourea in the salivary and mammary glands of female wistar rats. *British journal of cancer* 1976; 34: 437–43.

88. Pasquier E: C. Propranolol potentiates the anti-angiogenic effects and anti-tumor efficacy of chemotherapy agents: implication in breast cancer treatment. *Oncotarget* 2011; 2: 797–809.

89. Patane S. Re: association of alpha2a and beta2 adrenoceptor expression with clinical outcome in breast cancer. *Current medical research and opinion* 2015; 31: 333–4.

90. Perez DG: L. Newer antidepressants and other nonhormonal agents for the treatment of hot flashes. *Comprehensive therapy* 2005; 31: 224–36.

91. Perez Pinero C: B. Involvement of alpha2- and beta2-adrenoceptors on breast cancer cell proliferation and tumour growth regulation. *British journal of pharmacology* 2012; 166: 721–36.

92. Petty A: M. A small molecule agonist of EphA2 receptor tyrosine kinase inhibits tumor cell migration in vitro and prostate cancer metastasis in vivo. *PloS one* 2012; 7: e42120.

93. Pihlavisto M: S. Modulation of agonist binding to recombinant human alpha2-adrenoceptors by sodium ions. *Biochimica et biophysica acta* 1998; 1448: 135–46.

94. Pituskin E: M. Multidisciplinary Approach to Novel Therapies in Cardio-Oncology Research (MANTICORE 101-Breast): A Randomized Trial for the Prevention of Trastuzumab-Associated Cardiotoxicity. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2017; 35: 870–877.

95. Plummer HK. Expression of inwardly rectifying potassium channels (GIRKs) and beta-adrenergic regulation of breast cancer cell lines. *BMC cancer* 2004; 4: 93.

96. Pon CK: L. The beta2-adrenoceptor activates a positive cAMP-calcium feedforward loop to drive breast cancer cell invasion. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 2016; 30: 1144–54.

97. Powe DG: E. Targeted therapies: Using beta-blockers to inhibit breast cancer progression. *Nature reviews Clinical oncology* 2011; 8: 511–2.

98. Powe DG: V. Beta-blocker drug therapy reduces secondary cancer formation in breast cancer and improves cancer specific survival. *Oncotarget* 2010; 1: 628–38.

99. Powe DG: V. Alpha- and beta-adrenergic receptor (AR) protein expression is associated with poor clinical outcome in breast cancer: an immunohistochemical study. *Breast cancer research and treatment* 2011; 130: 457–63.

100. Qin JF: J. Adrenergic receptor beta2 activation by stress promotes breast cancer progression through macrophages M2 polarization in tumor microenvironment. *BMB reports* 2015; 48: 295–300.

101. Rains SL: A. Beta-adrenergic receptors are expressed across diverse cancers. *Oncoscience* 2017; 4: 95–105.

102. Rajamanickam S: P. Inhibition of FoxM1-Mediated DNA Repair by Imipramine Blue Suppresses Breast Cancer Growth and Metastasis. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2016; 22: 3524–36.

103. Rautio J: K. Amino acid ester prodrugs conjugated to the alpha-carboxylic acid group do not display affinity for the L-type amino acid transporter 1 (LAT1). *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences* 2015; 66: 36–40.

104. Re G: B. Effects of a beta 2-agonist (clenbuterol) on cultured human (CG-5) breast cancer cells. *Pharmacological research* 1992; 26: 377–84.

105. Rivero EM: P. The beta 2-Adrenergic Agonist Salbutamol Inhibits Migration, Invasion and Metastasis of the Human Breast Cancer MDA-MB- 231 Cell Line. *Current cancer drug targets* 2017; 17: 756–766.

106. Romeo HE: C. Slower growth of tumours in sympathetically denervated murine skin. *Journal of the autonomic nervous system* 1991; 32: 159–64.

107. Roy J: V. Pharmacological separation of hEAG and hERG K+ channel function in the human mammary carcinoma cell line MCF-7. *Oncology reports* 2008; 19: 1511–6.

108. Saez Mdel C: B. Exercise-induced stress enhances mammary tumor growth in rats: beneficial effect of the hormone melatonin. *Molecular and cellular biochemistry* 2007; 294: 19–24.

109. Sakellakis M: K. beta-Blocker Use and Risk of Recurrence in Patients with Early Breast Cancer. *Chemotherapy* 2014; 60: 288–9.

110. Shaashua L: S-S. Perioperative COX-2 and beta-Adrenergic Blockade Improves Metastatic Biomarkers in Breast Cancer Patients in a Phase-II Randomized Trial. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2017; 23: 4651–4661.

111. Shi M: L. The beta2-adrenergic receptor and Her2 comprise a positive feedback loop in human breast cancer cells. *Breast cancer research and treatment* 2011; 125: 351–62.

112. Shkurnikov MY: G. On statistical relationship between ADRA2A expression and the risk of breast cancer relapse. *Bulletin of experimental biology and medicine* 2014; 157: 454–8.

113. Shohat B: K. The effect of L-dopa, noradrenalin and adrenalin on P-388 mouse leukemia, B-16 mouse melanoma and E 0771 mammary carcinoma. *Experientia* 1975; 31: 110–1.

114. Singh NS: B. Selective GPR55 antagonism reduces chemoresistance in cancer cells. *Pharmacological research* 2016; 111: 757–766.

115. Sloan EK: P. The sympathetic nervous system induces a metastatic switch in primary breast cancer. *Cancer research* 2010; 70: 7042–52.

116. Slotkin TA: Z. Beta-adrenoceptor signaling and its control of cell replication in MDA-MB-231 human breast cancer cells. *Breast cancer research and treatment* 2000; 60: 153–66.

117. Smith TA: P. Effects of Administered Cardioprotective Drugs on Treatment Response of Breast Cancer Cells. *Anticancer research* 2016; 36: 87–93.

118. Sorensen GV: G. Use of beta-blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and risk of breast cancer recurrence: a Danish nationwide prospective cohort study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2013; 31: 2265–72.

119. Spera G: F. Beta blockers and improved progression-free survival in patients with advanced HER2 negative breast cancer: a retrospective analysis of the ROSE/TRIO-012 study. *Annals of oncology : official journal of the European Society for Medical Oncology* 2017; 28: 1836–1841.

120. Spina A: DM. cAMP Elevation Down-Regulates beta3 Integrin and Focal Adhesion Kinase and Inhibits Leptin-Induced Migration of MDA-MB-231 Breast Cancer Cells. *BioResearch open access* 2012; 1: 324–32.

121. Stanojkovic TP: Z. Inhibition of proliferation on some neoplastic cell lines-act of carvedilol and captopril. *Journal of experimental & clinical cancer research : CR* 2005; 24: 387–95.

122. Stefanski V: B-E. Social confrontation and tumor metastasis in rats: defeat and beta-adrenergic mechanisms. *Physiology & behavior* 1996; 60: 277–82.

123. Strell C: N. Norepinephrine promotes the beta1-integrin-mediated adhesion of MDA-MB-231 cells to vascular endothelium by the induction of a GROalpha release. *Molecular cancer research : MCR* 2012; 10: 197–207.

124. Su F: O. Psychological stress induces chemoresistance in breast cancer by upregulating mdr1. *Biochemical and biophysical research communications* 2005; 329: 888–97.

125. Szewczyk M: R. A retrospective in vitro study of the impact of anti-diabetics and cardioselective pharmaceuticals on breast cancer. *Anticancer research* 2012; 32: 2133–8.

126. Szpunar MJ: B. Sympathetic innervation, norepinephrine content, and norepinephrine turnover in orthotopic and spontaneous models of breast cancer. *Brain, behavior, and immunity* 2016; 53: 223–233.

127. Szpunar MJ: B. The antidepressant desipramine and alpha2-adrenergic receptor activation promote breast tumor progression in association with altered collagen structure. *Cancer prevention research (Philadelphia, Pa)* 2013; 6: 1262–72.

128. Talarico G: O. Aspirin and atenolol enhance metformin activity against breast cancer by targeting both neoplastic and microenvironment cells. *Scientific reports* 2016; 6: 18673.

129. Tuglu MM: B. The role of dualspecificity phosphatase 1 and protein phosphatase 1 in beta2adrenergic receptormediated inhibition of extracellular signal regulated kinase 1/2 in triple negative breast cancer cell lines. *Molecular medicine reports* 2018; 17: 2033–2043.

130. Tveit E: H. Effects of noradrenaline on interstitial fluid pressure in induced rat mammary tumours. *Cancer letters* 1985; 27: 249–53.

131. Vandewalle B: R. Functional beta-adrenergic receptors in breast cancer cells. *Journal of cancer research and clinical oncology* 1990; 116: 303–6.

132. Vazquez SM: M. Human breast cell lines exhibit functional alpha2-adrenoceptors. *Cancer chemotherapy and pharmacology* 2006; 58: 50–61.

133. Vazquez SM: P. Alpha2-adrenergic effect on human breast cancer MCF-7 cells. *Breast cancer research and treatment* 1999; 55: 41–9.

134. Walters MR: S. Cross-talk between beta-adrenergic stimulation and estrogen receptors: isoproterenol inhibits 17beta-estradiol-induced gene transcription in A7r5 cells. *Journal of cardiovascular pharmacology* 2003; 42: 266–74.

135. Wang LP: J. Norepinephrine attenuates CXCR4 expression and the corresponding invasion of MDA-MB-231 breast cancer cells via beta2-adrenergic receptors. *European review for medical and pharmacological sciences* 2015; 19: 1170–81.

136. Wang S: M. Evidence for an early G1 ionic event necessary for cell cycle progression and survival in the MCF-7 human breast carcinoma cell line. *Journal of cellular physiology* 1998; 176: 456–64.

137. Watanabe M: O. Effect of beta-agonist on the dexamethasone-induced expression of aromatase by the human monocyte cells. *Endocrine connections* 2017; 6: 82–88.

138. Weiss L: H. Blood flow and reactivity to noradrenaline in DMBA-induced rat mammary neoplasia. *Cancer letters* 1980; 9: 293–8.

139. Weiss L: T. Vascular reactivity to norepinephrine of 7,12-dimethylbenz(a)anthracene-induced rat mammary tumors and normal tissue as studied in vitro. *Cancer research* 1986; 46: 3254–7.

140. Wendel V: V. Prazosin and stress effect on tumoral growth of 7,12-dimethylbenz[A]anthracene-induced rat mammary tumors. *Acta physiologica, pharmacologica et therapeutica latinoamericana : organo de la Asociacion Latinoamericana de Ciencias Fisiologicas y [de] la Asociacion Latinoamericana de Farmacologia* 1996; 46: 277–85.

141. Williams RR: F. Case-control study of antihypertensive and diuretic use by women with malignant and benign breast lesions detected in a mammography screening program. *Journal of the National Cancer Institute* 1978; 61: 327–35.

142. Wilson JM: L. beta-Adrenergic receptors suppress Rap1B prenylation and promote the metastatic phenotype in breast cancer cells. *Cancer biology & therapy* 2015; 16: 1364–74.

143. Wishart GC: B. Quinidine as a resistance modulator of epirubicin in advanced breast cancer: mature results of a placebo-controlled randomized trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 1994; 12: 1771–7.

144. Xia M: J. Dexmedetomidine regulate the malignancy of breast cancer cells by activating alpha2-adrenoceptor/ERK signaling pathway. *European review for medical and pharmacological sciences* 2016; 20: 3500–6.

145. Xia M: T. Tramadol inhibits proliferation, migration and invasion via alpha2-adrenoceptor signaling in breast cancer cells. *European review for medical and pharmacological sciences* 2016; 20: 157–65.

146. Yamazaki S: M. Quercetin-3-O-glucuronide inhibits noradrenaline-promoted invasion of MDA-MB-231 human breast cancer cells by blocking beta(2)-adrenergic signaling. *Archives of biochemistry and biophysics* 2014; 557: 18–27.

147. Yamazaki S: S. Quercetin-3-O-glucronide inhibits noradrenaline binding to alpha2-adrenergic receptor, thus suppressing DNA damage induced by treatment with 4-hydroxyestradiol and noradrenaline in MCF-10A cells. *The Journal of steroid biochemistry and molecular biology* 2014; 143: 122–9.

148. Zhou L: S. The effect of breast cancer resistance protein and P-glycoprotein on the brain penetration of flavopiridol, imatinib mesylate (Gleevec), prazosin, and 2-methoxy-3-(4-(2-(5-methyl-2-phenyloxazol-4-yl)ethoxy)phenyl)propanoic acid (PF-407288) in mice. *Drug metabolism and disposition: the biological fate of chemicals* 2009; 37: 946–55.

149. Zhou Q: M. Control of mammary tumor cell growth in vitro by novel cell differentiation and apoptosis agents. *Breast cancer research and treatment* 2002; 75: 107–17.

150. Zhou Q: M. Rapid induction of histone hyperacetylation and cellular differentiation in human breast tumor cell lines following degradation of histone deacetylase-1. *The Journal of biological chemistry* 2000; 275: 35256–63.