

# Combination therapy approaches to target insulin-like growth factor receptor signaling in breast cancer

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## Abstract

Insulin-like growth factor receptor (IGF1R) signaling as a therapeutic target has been widely studied and clinically tested. Despite the vast amount of literature supporting the biological role of IGF1R in breast cancer, effective clinical translation in targeting its activity as a cancer therapy has not been successful. The intrinsic complexity of cancer cell signaling mediated by many tyrosine kinase growth factor receptors that work together to modulate each other and intracellular downstream mediators in the cell highlights that studying IGF1R expression and activity as a prognostic factor and therapeutic target in isolation is certainly associated with problems. This review discusses the current literature and clinical trials associated with IGF-1 signaling and attempts to look at new ways of designing novel IGF1R-directed breast cancer therapy approaches to target its activity and/or intracellular downstream signaling pathways in IGF1R-expressing breast cancers.

## Key Words

- ▶ insulin-like growth factor receptor
- ▶ breast cancer
- ▶ targeted therapies and sphingosine kinase

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## Introduction

A key strategy in precision cancer therapy is the targeting of cell-surface tyrosine kinase receptors that may drive tumor cell survival, proliferation and migration. In some cases, this has been remarkably successful, for example, in the inhibition of the human epidermal growth factor (EGF) receptor-2 (HER2) in women with HER2-positive breast cancer and the blockade of EGF receptor kinase activity in non-small-cell lung cancer. In contrast, the insulin-like growth factor (IGF) type 1 receptor (IGF1R) has, to date, proved to be a much less successful target. The IGF1R has two ligands, IGF-1 and IGF-2, and they are polypeptides of approximately 7.5 kDa that are structurally and functionally related to insulin. Some tumors also produce IGF-2 in larger precursor forms with variable receptor reactivity (Greenall *et al.* 2013). In addition to their high-affinity interaction with IGF1R, the IGFs bind

with much lower affinity (about 1%) to the structurally related insulin receptor (InsR) in its main metabolically active isoform (isoform B), whereas IGF-2 and insulin itself are potent activators of the more mitogenic InsR isoform A (Morcavallo *et al.* 2014). Activation of IGF1R and/or InsR-A has been documented in many cancer types, making these receptors attractive therapeutic targets (Pollak 2012).

IGF-1 and IGF-2 circulate in adults at about 15–30 nM and 50–100 nM, respectively, concentrations high enough to saturate both their anabolic and mitogenic receptors. Their bioactivity is controlled by six IGF-binding proteins (IGFBPs), the most abundant of which is IGFBP-3, the main carrier of IGF-1 and IGF-2 in the circulation. IGFBPs are also found in the pericellular space, where they regulate IGF access to their cell-surface receptors (Baxter 2014).

Cancer therapies that modulate the concentration or activity of any component of this complex system of receptors, binding proteins and ligands are likely to perturb the equilibrium across this system, sometimes in unanticipated ways. This article aims to provide a contemporary overview of the use of IGF1R-directed therapies in breast cancer clinical practice, with particular emphasis on the combination treatments directed toward IGF1R and other cellular targets.

## Breast cancer and therapy resistance

Breast cancer remains the most common form of cancer in women, and GLOBOCAN statistics report ~1.7 million cases and 522,000 deaths worldwide in 2012 (Tao *et al.* 2014). There are predictions that breast cancer rates will reach 3.2 million cases by 2050 (Tao *et al.* 2014). An ongoing clinical problem is the large number of women who do not either respond or develop resistance to current breast cancer therapies. Circumvention of this issue is critical to the management of breast cancer, which urgently requires the development of more sophisticated combinatorial breast cancer therapies that effectively inhibit key cancer-driving signaling pathways (Zanardi *et al.* 2015).

Breast cancer is a highly heterogeneous disease and is treated clinically based on its molecular profile. The different molecular breast cancer subtypes include luminal A/B basal-like and HER2 breast cancers. Within these subtypes, both steroid receptors, including the estrogen receptor (ER) and progesterone receptor (PR), and tyrosine kinase growth factor receptors such as the IGF1R, EGFR and HER2 can be positive or negative in expression and integral to the cells' behavior (Perou *et al.* 2000, 2011). For ER-positive luminal A/B breast cancers, therapies to reduce ER $\alpha$  activity at the receptor level using selective ER antagonists (fulvestrant) and aromatase inhibitors (exemestane, letrozole and anastrozole) to reduce estrogen levels are used. Conversely, triple-negative breast cancers (TNBCs – defined as ER-, PR- and HER2-), that overlap strongly with the basal-like molecular subtype (Lehmann *et al.* 2011), are currently reliant on cytotoxic chemotherapy (e.g. doxorubicin, 5-fluorouracil and docetaxel), which is not reflective of the intrinsic molecular profiles of these cancers (Pal *et al.* 2011). For HER2-amplified breast cancers, a number of HER2-directed therapies, including the monoclonal antibodies (mAbs) trastuzumab and pertuzumab, which inhibit HER2/HER3 dimerization and activity, the dual

tyrosine kinase inhibitor – lapatinib – which inhibits HER2 and EGFR activity and T-DMI (an antibody drug-conjugate of the mAb, trastuzumab and the chemotherapy drug DMI (emtansine)) (Verma *et al.* 2012, Mendes *et al.* 2015), have been developed and are clinically used. Currently, there are no targeted therapies for TNBC, which are widely used although many drugs designed to inhibit the emerging pathways are in clinical development (Kalimutho *et al.* 2015).

Of most concern is that the median overall survival in women diagnosed with metastatic breast cancer for estrogen-targeted therapies is 4 years, for HER2-targeted therapies is 3 years and for cytotoxic chemotherapy and radiotherapy is 12 months (Andre *et al.* 2004, Dawood *et al.* 2010, Pal *et al.* 2011). Despite the impressive clinical improvements associated with current breast cancer-targeted therapies such as the development of the HER2 inhibitor trastuzumab, innovative treatment strategies to manage resistant disease are still required.

## IGF1R-targeted therapies: mechanism of action

Receptor tyrosine kinase inhibitors (TKIs), antireceptor monoclonal antibodies (mAbs) and mAbs against the IGFs have been developed to inhibit IGF1R activity and its downstream signaling (Yang & Yee 2012, King *et al.* 2014). IGF1R tyrosine kinase inhibitors (TKIs) are small molecules that either bind to the receptor competitively with ATP, e.g. PQIP, the forerunner of OSI-906 (Linsitinib) (Ji *et al.* 2007, Mulvihill *et al.* 2009), the structurally-related compound NVP-AEW541 (Brockhoff *et al.* 2012), BMS-754807 (Carboni *et al.* 2009) and BI 885578 (Sanderson 2015) or inhibit in a non-ATP-competitive manner, e.g. picropodophyllin or PPP (Girmita *et al.* 2004). Owing to substantial structural similarity between the IGF1R and the InsR (Lawrence *et al.* 2007), these inhibitors generally show some cross-reactivity with the InsR. Relative potencies, measured as the inhibition of receptor autophosphorylation in living cells, range from about 5% (Garcia-Echeverria *et al.* 2004, Ji *et al.* 2007) to 50–100% (Sanderson *et al.* 2015). Interestingly, inhibitors with a high degree of selectivity in intact cells may still show almost full cross-reactivity between IGF1R and InsR in biochemical kinase assays *in vitro* (Garcia-Echeverria *et al.* 2004, Ji *et al.* 2007), suggesting that receptor conformation in live cells contributes to the specificity. IGF1R TKIs generally do not downregulate the receptor, an exception being PPP (Vasilcanu *et al.* 2008).

mAbs against IGF1R typically inhibit ligand-dependent receptor signaling by binding to the receptor competitively with its ligands IGF-1 and IGF-2 (Cohen *et al.* 2005, Wang *et al.* 2010a). An important component of their mode of action is also receptor downregulation (Burtrum *et al.* 2003, Cohen *et al.* 2005, Wang *et al.* 2010a). As a consequence of receptor blockade, circulating IGF-1 is markedly elevated (Yin *et al.* 2013), although unable to elicit a receptor-mediated response. Because they interact with extracellular receptor sequences rather than the intracellular ATP-binding domain, IGF1R mAbs can be selected to avoid the cross-reactivity with InsR seen for the TKIs (Goetsch *et al.* 2005), while still recognizing IGF1R–InsR hybrids (Cohen *et al.* 2005). However, as IGF-2 is a potent activator of the mitogenic isoform A InsR (Belfiore *et al.* 2009), the concomitant downregulation of InsR together with IGF1R may provide a therapeutic advantage (Sachdev *et al.* 2006). Humanized or fully human IGF1R mAbs include dalotuzumab (MK-0646), figitumumab (CP-751,871), cixutumumab (IMC-A12), robatumumab (SCH 717454), ganitumab (AMG179), AVE1642 and R1507, which inhibit the dimerization of the receptor. At the time of publication, the majority of trials involving these drugs are either completed/terminated or inactive.

A different approach to inhibit IGF1R signaling is by targeting the ligands that activate the receptor – IGF-1 and IGF-2. IGF-1/2 mAbs such as MEDI-573 (Haluska *et al.* 2014) and BI 836845 (Friedbichler *et al.* 2014) act by neutralizing the IGF-1 and IGF-2 ligands, thus potentially blocking the activation of both IGF1R and InsR-A. Such agents greatly increase circulating IGF concentrations, but the majority appear to be complexed to antibody and therefore biologically inert (Mireuta *et al.* 2014). In addition to direct approaches to inhibit IGF1R activation, whether by receptor or ligand blockade, numerous targeted therapies that inhibit IGF1R-driven downstream effectors involved in the regulation of protein translation, such as mTOR inhibitors (i.e. rapamycin, everolimus and temsirolimus), have been developed and are also of clinical interest as discussed in subsequent sections.

Indirectly targeting IGF1R signaling can also be achieved by using somatostatin or its analogues such as pasireotide (SOM230), which is approved as a therapy in the United States and Europe for conditions including Cushing's disease and acromegaly, and it acts to reduce growth hormone production and consequently IGF-1 production and signaling (Kleinberg *et al.* 2011, Lewis *et al.* 2014). In another indirect approach, metformin, a standard treatment for type 2 diabetes, is shown to inhibit

crosstalk between InsR/IGF1R and EGFR and as a result has indirect effects on IGF-1 signaling (Rozenfurt *et al.* 2010, Liu *et al.* 2011).

### Predictive biomarkers of IGF1R therapy response

Although high tumor IGF1R expression is reported to be a poor prognostic indicator in a variety of cancers including gastric (Matsubara *et al.* 2008), colorectal (Takahari *et al.* 2009), renal cell carcinoma (Sichani *et al.* 2010), and in some breast cancer subtypes (Taunk *et al.* 2010, Hartog *et al.* 2011), few proposed markers to predict responsiveness to IGF1R-targeting therapies have progressed beyond preliminary reports. Cell-line studies in sarcoma and neuroblastoma show that sensitivity toward TKI BMS-536924 was associated with high IGF-1, IGF-2 and IGF1R expression, with drug resistance associated with the expression of IGFBP-3 and IGFBP-6 (Huang *et al.* 2009). Other cell studies in breast and lung cancer have similarly pointed to a possible role of high IGF1R expression in predicting both TKI and mAb responsiveness (Gong *et al.* 2009, Litzenburger *et al.* 2011). The glycosylation state of IGF1R has also been found to correlate with CP-751,871 sensitivity in gastric and hepatocellular carcinoma cell lines, possibly related to the formation of IGF1R/InsR hybrid receptors (Kim *et al.* 2012). In a comprehensive screen of CP-751,871 sensitivity across 93 cancer cell lines, gene expression levels of IGF-axis components and MYB were found to have the strongest predictive value (Pavlicek *et al.* 2013). A more complex classifier for OSI-906 responsiveness was developed by integrating gene expression corresponding to drug sensitivity, IGF1R expression by *in situ* hybridization and *K-RAS* status, leading to a test claimed to have 100% predictive accuracy (Pitts *et al.* 2010).

However, despite promising leads from cell biology, measurement of IGF1R and other IGF-axis components has had relatively little success as a predictive marker in clinical studies. In a phase 1 study of patients with prostate cancer treated with CP-751,871 plus docetaxel, IGF1R detectable on circulating tumor cells appeared to correlate with treatment response (measured as PSA decline), although no statistical analysis was presented (de Bono *et al.* 2007). Contrasting with this finding and numerous cell biology studies, IGF1R expression, detected by immunohistochemistry, was unable to predict clinical outcome in patients with bone or soft-tissue sarcomas treated with cixutumumab (IMC-A12) in combination

with the mTOR inhibitor temsirolimus (Schwartz *et al.* 2013). In patients with metastatic pancreatic cancer treated with gemcitabine, increased overall survival in response to ganitumab (AMG179) was predicted by circulating IGF and IGFBP-3 levels – notably high IGF-2 (McCaffery *et al.* 2013). The conclusion to be drawn from these and other clinical studies is that there is currently no clinically well-validated biomarker to predict treatment response for IGF1R pathway blockade, by treatment with either a IGF1R mAb or IGF1R-TKI, that could be applied to breast cancer trials.

### IGF1R-directed combination therapies: specific molecular subtypes of breast cancer

#### Estrogen receptor-positive breast cancer

IGF1R and ER $\alpha$  crosstalk pathways in breast cancer have been widely studied for therapeutic intervention in ER-positive breast cancers (Fagan & Yee 2008). The IGF signaling pathway is intrinsically involved in the regulation of the ER $\alpha$  after phosphorylation of the protein. Given that ER-positive breast cancers are the most common subtype, comprising approximately 70% of all diagnosed breast cancers (Jeselsohn *et al.* 2015), and oncogenic activity in this molecular subtype is contributed to by IGF signaling (Becker *et al.* 2011), many clinical trials have been undertaken to test IGF1R-directed therapies in ER-positive breast cancer. The lack of clinical success of these trials, which did not lead to any improvements in clinical outcomes, highlights that targeting IGF1R in isolation is not sufficient to overcome tumor growth (Yee 2012). There is now re-ignited hope that IGF1R therapies combined with other FDA-approved drug treatments and emerging drug therapies will be more effective and lead to better clinical outcomes by preventing re-activation of IGF1R and/or activation of compensatory signaling pathways that can overcome the drug effects (Beckwith & Yee 2014).

As previously outlined, identification of better predictive biomarkers that reflect IGF1R therapy response is required to determine which individuals will or will not benefit from IGF1R-targeted therapies. Moreover, the complexity of IGF1R signaling dictated by the ability of the cell to either re-activate the IGF1R-signaling pathway and/or drive oncogenesis via the activation of alternative growth factor signaling pathways such as the InsR, EGFR, HER2 or vascular endothelial growth factor receptor (VEGFR), further heightens the difficulty in designing effective IGF1R combination targeted therapies.

**IGF1R and chemotherapy** The role of IGF1R in promoting resistance to chemotherapy is well established (Bohula *et al.* 2003). Cell-line studies using the ER-positive MCF7 and Bcap-37 breast cancer cell lines show that the cell-permeable plant-derived flavonoid wogonin, that is known to possess anti-carcinogenic and anti-inflammatory properties, increases the sensitivity to the chemotherapy agent doxorubicin via the suppression of IGF1R/AKT signaling pathway (Fu *et al.* 2015). Furthermore, *in vitro* studies in MCF7 cells reported that the effectiveness of using the highly potent and selective IGF1R tyrosine kinase inhibitor PQIP, an analogue of OSI-906, in combination with chemotherapy (i.e. gemcitabine) depends on the sequence of drug administration (Khatri *et al.* 2012). Enhancement of the response to the IGF1R mAbs AVE1642 or BMS-754807 by gemcitabine has been demonstrated using established BxPC-3 human pancreatic tumor xenografts (Maloney *et al.* 2003, Awasthi *et al.* 2012), suggesting that these types of IGF1R and chemotherapy combination therapies may also have clinical benefit in other cancers including breast.

The phase 3 NEOZOTAC clinical trial completed in 2013 reported that reduced IGF1R expression during and after neo-adjuvant chemotherapy treatment is associated with improved pathological outcomes in HER2-negative breast cancer (Tables 1 and 2: Ref #16; NCT01099436) (de Groot *et al.* 2016). Similarly, reduced IGF1R expression was predictive of a complete pathologic response in ER-positive breast cancers treated with neoadjuvant chemotherapy (Bhargava *et al.* 2011), highlighting that IGF1R expression modulates the chemotherapy response. Significantly elevated IGF1R protein and mRNA expression have additionally been shown in naturally occurring cisplatin-resistant SKOV3 and HEY ovarian cancer cells compared with those in the more sensitive A2780 and OAW42 ovarian cancer cell lines. Most importantly, chemoresistance was reversed in the early-stage cancer by the addition of the IGF1R inhibitor – small molecule inhibitor PPP. This proves as the evidence that co-treatment with chemotherapy and IGF1R inhibitors has clinical potential (Singh *et al.* 2014b).

A phase I clinical trial investigated the pharmacokinetic (PK) profiles and safety of the chemotherapy agent gemcitabine in conjunction with AMG479 in advanced solid tumors (Table 1: Ref #20, NCT00974896), which has been deemed to be tolerable and safe leading to improved survival outcomes in a placebo-controlled phase 2 trial for metastatic pancreatic cancer (Kindler *et al.* 2012). A combination therapy comprising cisplatin

**Table 1** IGF1R-directed therapy clinical trials/studies for breast cancer and solid tumors. Phase 1, 2 and 3 clinical trials/studies using insulin-like growth factor receptor (IGF1R)-directed therapies either as single-agent therapies or combinatorial therapies for breast cancer, and solid tumors were obtained from the clinical trials U.S. National Institutes of Health website (<https://clinicaltrials.gov>) and listed in reverse chronological order from the estimated completion dates.

Ref #	Clinical trials identifier	Study title	Phase	Estimated completion	Breast cancer grade/molecular subtype	Published drug doses and results
#1	NCT01042379	I-SPY 2 TRIAL: neoadjuvant and personalized adaptive novel agents to treat breast cancer (I-SPY 2) (Barker <i>et al.</i> 2009)	2	Sept-2017	Histologically confirmed invasive cancer of the breast, any tumor ER/PR status, any HER-2/neu status	NA
#2	NCT01446159	Study of MEDI-573 plus standard endocrine therapy for women with hormone-sensitive metastatic breast cancer	1.2	Sept-2017	ER or PR positive, or both; HER2 negative	NA
#3	NCT02045368	Study of IGF-methotrexate conjugate in the treatment of advanced tumors expressing IGF-1R	1	Jan-2017	IGF1R positive	NA
#4	NCT01776008	Akt Inhibitor MK-2206 and anastrozole with or without goserelin acetate in treating patients with stage II-III breast cancer	2	Dec-2016	Clinical stage 2 or 3 PIK3CA mutant ER-positive and HER2-ve invasive breast cancer	NA
#5	NCT0111825	Temsirolimus plus neratinib for patients with metastatic HER2-amplified or triple negative breast cancer	1.2	Dec-2016	Metastatic HER2-amplified or triple negative	NA
#6	NCT01650506	Study of erlotinib and metformin in triple negative breast cancer	1	Sept-2016	Triple-negative breast cancer, prior diagnosis of ER- or PR-positive breast cancer (HER2 negative)	NA
#7	NCT01708161	A phase Ib/II study of the combination of BYL719 plus AMG 479 in adult patients with selected solid tumors	1.2	Jun-2016	PIK3CA mutated or amplified; hormone receptor-positive breast carcinoma	NA
#8	NCT00699491	Cixutumumab and temsirolimus in treating patients with locally recurrent or metastatic breast cancer	1.2	Jun-2016	Metastatic or locally recurrent disease (locally recurrent disease should be stage IV)	NA
#9	NCT01733004	A phase 1 study of MM-141 in patients with advanced solid tumors	1	Dec-2015	Advanced solid tumors	NA
#10	NCT01372618	Breast cancer chemoprevention by SOM230, an IGF-I action inhibitor	2	Oct-2015	ER positive, DCIS	NA
#11	NCT01295632	Safety and tolerability of different dose combinations of ridaforolimus with MK-2206 or MK-0752 for participants with advanced cancer (MK-8669-049) (Gupta <i>et al.</i> 2015, Piha-Paul <i>et al.</i> 2015)	1	Aug-2015	Histologically-confirmed breast cancer: a low RAS-gene signature and a high Ki67 index label if ER positive	Drug doses: MTD: ridaforolimus = 10 mg/day + MK-2206 = 90 mg/week. Results: 2/16 (12.5%) = PR; 2/14 (14.3%) = CR; no clinical response detected in prostate cancer patients. SD for two patients, 416 and 285 days (Gupta <i>et al.</i> 2015). Drug doses: MTD: ridaforolimus = 20 mg/day + MK-0752 = 1800 mg.

(Continued)

Table 1 Continued.

Ref #	Clinical trials identifier	Study title	Phase	Estimated completion	Breast cancer grade/molecular subtype	Published drug doses and results
#12	NCT00728949	A study for safety and effectiveness of IMC-A12 by itself or combined with antiestrogens to treat breast cancer (Gradishar <i>et al.</i> 2015)	2	Feb-2015	ER and/or PR positive	Results: 2/15 (13%) responded; one PR and one CR. SD for one patient >6 months (Piha-Paul <i>et al.</i> 2015). Drug doses: Arm 1 (n=62): CIX = 10 mg/kg + anti-estrogen; Arm B (n=31): CIX = 10 mg/kg. CIX well tolerated. Results: DCR: Arm A = 40.3% (95% CI: 28.1, 53.6); Arm B = 51.6% (95% CI: 33.1, 69.8). Low IR, IR-A and IR-B mRNA expression associated with significant increase in PFS and OS. NA
#13	NCT01225172	Study of BMS-754807 combined with letrozole or BMS-754807 alone in patients with hormone receptor-positive breast cancer and resistance to non-steroidal aromatase inhibitors	2	Nov-2014	Postmenopausal women with HR-positive and HER-2-negative locally advanced or metastatic breast cancer	NA
#14	NCT01245205	Akt inhibitor MK2206 in combination with lapatinib ditosylate in patients with advanced or metastatic solid tumors or breast cancer	1	Aug-2014	Advanced and unresectable or metastatic HER2-positive breast cancer	NA
#15	NCT00684983	Capecitabine and lapatinib with or without cixutumumab in treating patients with previously treated HER2-positive stage IIIB, stage IIIC, or stage IV breast cancer	2	Jan-2014	HER2-positive stage IIIB-IV breast cancer	NA
#16	NCT01099436	Neo-adjvant chemotherapy (TAC) with or without zoledronic acid in treating HER2-negative breast cancer patients (NEO-ZOTAC) (de Groot <i>et al.</i> 2016)	3	Sept-2013	HER2-negative large resectable or locally advanced breast cancer	Drug doses: TAC chemotherapy = 75mg/m <sup>2</sup> docetaxel; 50 mg/m <sup>2</sup> doxorubicin and 500 mg/m <sup>2</sup> cyclophosphamide ± 4 mg zoledronic acid. Results: 47.2% significant reduction in IGF1R expression after TAC; better pathological response to TAC in patients with reduced IGF1R (P=0.006; during therapy) and (P=0.020; after therapy) and polymorphism (P=0.032), multivariate analysis. Drug doses: TAC chemotherapy = 75mg/m <sup>2</sup> docetaxel; 50 mg/m <sup>2</sup> doxorubicin and 500 mg/m <sup>2</sup> cyclophosphamide ± 4 mg zoledronic acid. Results: 47.2% significant reduction in IGF1R expression after TAC; better pathological response to TAC in patients with reduced IGF1R (P=0.006; during therapy) and (P=0.020; after therapy) and polymorphism (P=0.032), multivariate analysis.
#17	NCT00739453	A phase 1 dose-escalation study of OSI-906 and erlotinib (Tarceva) (Maccaulay <i>et al.</i> 2016)	1	Dec-2011	Advanced solid tumors	Drug doses: linsitinib schedules (S): S1 = 50–600 mg/QD; S2 = 50–400 mg/QD; S3 = 100/150 mg/BID in conjunction with 100–150 mg/QD erlotinib. Linsitinib/erlotinib combination was well tolerated. Results: 5/75 (7%) = PR and 38/75 (51%) = DCR.

#18	NCT00963547	A study of MK2206 in combination with trastuzumab and lapatinib for the treatment of HER2+ solid tumors (2206-015) (Hudis <i>et al.</i> 2013)	1	Dec-2011	Locally advanced or metastatic HER2-positive solid tumor	Drug doses: trastuzumab = 8 mg/kg day 1 and 6 mg/kg every 3 weeks in conjunction with MK-2206 = 45 or 60 mg QOD (two cohorts) or 135 and 200 mg dose (two cohorts). Trastuzumab and MK-2206 in general was well tolerated. Results: 1/31 = PR; 1/31 = CR and 5/31 = SD (up to 4 months). Drug doses: pasireotide = 600 µg/BID. Results: AH lesions = reduced proliferation (3.6 ± 2.6% to 1.3 ± 1.2%) and increased apoptosis (0.3 ± 0.2% to 1.3 ± 0.6%). DCIS response was similar. IGF1R, ERK1/2 and AKT phosphorylation were significantly reduced.
#19	NCT01372644	Breast cancer chemoprevention by SOM230, an IGF-I action inhibitor: a proof of principle trial (Singh <i>et al.</i> 2014a)	1.2	Jun-2011	Atypical ductal hyperplasia, lobular carcinoma in situ, and/or atypical lobular hyperplasia	NA
#20	NCT00974896	Study of AMG 479 with biologics or chemotherapy for subjects with advanced solid tumors	1	Apr-2011	Advanced solid tumors	NA
#21	NCT01205685	Endocrine therapy + OSI-906 with or without erlotinib for hormone-sensitive metastatic breast cancer	2	Jul-2011	Clinical stage IV invasive mammary carcinoma, ER- and/or PR-positive	NA; terminated
#22	NCT00372996	Study of CP-751,871 in combination with exemestane in postmenopausal women with hormone receptor positive advanced breast cancer	2	May-2011	HR-positive advanced breast cancer	NA, terminated
#23	NCT00730379	A combination study with ridaforolimus (MK8669) and dalotuzumab (MK0646) in patients with advanced cancer (8669-004) (Di Cosimo <i>et al.</i> 2015)	1	Nov-2010	Advanced cancer	Drug doses: ridaforolimus = 10–40 mg/day and dalotuzumab = 10 mg/kg/week or 7.5 mg/kg every other week. Results: PR = 6/87 (7%), 40/87 (46%) = SD and 27/87 (31%) = PD. 10/23 (43%) breast cancer patients showed anti-tumor activity of which 6/11 (55%) were ER+/KI67 high expressing. NA, Terminated
#24	NCT00774878	Study of AVE1642 (IGF-1R/CD221) in combination with fulvestrant (Faslodex) in postmenopausal patients with advanced hormone-dependent breast cancer	2	Nov-2010	Advanced hormone-dependent breast cancer, HER2-positive excluded	NA
<b>Other solid cancers</b>						
#25	NCT00955305	Paclitaxel, carboplatin, and bevacizumab with or without cixutumumab in treating patients with stage IV or recurrent non-small cell lung cancer	2	Dec-2015	NA	(Continued)

Table 1 Continued.

Ref #	Clinical trials identifier	Study title	Phase	Estimated completion	Breast cancer grade/molecular subtype	Published drug doses and results
#26	NCT00887159	Cisplatin and etoposide with or without vismodegib or cixutumumab in treating patients with extensive-stage small cell lung cancer (Belani <i>et al.</i> 2013)	2	Sept-2014	NA	Drug doses: CE alone ((75 mg/m <sup>2</sup> D1 and E 100 mg/m <sup>2</sup> D1-3) Arm A) combined with either V ((150 mg/day) Arm B) or CIX ((6 mg/kg weekly IV) Arm C). Results: no significant improvement in PFS was detected for CE combined with either V or CIX, compared with CE alone.
#27	NCT00906373	A study of IMC-A12 in combination with sorafenib in patients with advanced cancer of the liver	2	May-2014	NA	NA
#28	NCT00617708	S0727 gemcitabine hydrochloride and erlotinib hydrochloride with or without monoclonal antibody therapy in treating patients with metastatic pancreatic cancer that cannot be removed by surgery	1/2	Feb-2014	NA	NA

DCIS, ductal carcinoma *in situ*; ER, estrogen receptor; HER, human epidermal receptor; PIK3CA, phosphatidylinositol 3-kinase; PR, progesterone receptor; Ref, reference; TNBC, triple negative breast cancer; AH, atypical hyperplasia; BID, twice daily; CIX, cixutumumab; CR, complete response; DCR, disease control rate; MITD, maximum tolerated dose; NA, not available; NR, not relevant; OS, overall survival; PD, progressive disease; PFS, progression free survival; PR, partial response; QD, once daily; QOD, every other day; QW, once weekly; SD, stable disease; TAC, (T – Docetaxel (also called Taxotere), A – Doxorubicin (originally called Adriamycin) and C – Cyclophosphamide).

and etoposide (CE) in conjunction with the IGF1R inhibitor cixutumumab has been evaluated in a phase 2 trial for small-cell lung carcinoma (Table 1: Ref #26, NCT00887159), but was reported not to be associated with any clinical improvements in progression-free survival (PFS) compared with CE therapy alone (Belani *et al.* 2013). Nonetheless, given that IGF1R inhibition has been shown to positively influence the chemotherapy response, a therapy approach of this nature may still have benefit for breast cancer and is worth further investigation possibly with other FDA-approved therapies.

**IGF1R and anti-estrogens** Early studies using MCF7 and T47D breast cancer cell lines and mouse xenograft studies using MCF7 cells provide evidence that IGF1R inhibitors including the TKI, NVP-AEW541 and the mAb CP-751,871 in combination with ER-targeted therapies such as letrozole or tamoxifen lead to more effective anti-tumor responses compared with the single agents (Cohen *et al.* 2005, Lisztwan *et al.* 2008). Similarly, *in vitro* and *in vivo* studies using BMS-754807 in combination with either of the hormonal agents: 4-hydroxytamoxifen, fulvestrant or letrozole have also demonstrated a strong synergistic effect on tumor inhibitory responses in ER-positive breast cancer (Table 2) (Hou *et al.* 2011). As IGF-1 can regulate ER phosphorylation and activity in breast cancer (Becker *et al.* 2011, Ochnik *et al.* 2016) and the indirect IGF-1-inhibitor (somatostatin analogue) SOM230 has been shown to reduce proliferation and increase apoptosis via an IGF1R-mediated effect in pre-malignant breast lesions (i.e. atypical hyperplasia) in a phase 1 proof-of-principle trial (Table 1: Ref #19, NCT01372644) (Singh *et al.* 2014a), an additional treatment phase 1 study was completed to determine whether SOM230 can also be used as chemoprevention for early-stage ER-positive ductal carcinoma *in situ* (Table 1: Ref #10, NCT01372618).

A phase 2 clinical trial completed in 2015, testing IMC-A12 alone or in combination with anti-estrogen therapies in women who had progressed on anti-estrogen therapy, deemed the combination to be safe and tolerable, but the dual therapy did not show any significant clinical outcomes compared with single therapies (Table 1: Ref #12, NCT00728949) (Gradishar *et al.* 2015). BMS-754807 has been given in combination with the aromatase inhibitor, letrozole, in ER-positive breast cancers with acquired resistance to non-steroidal aromatase inhibitors in a phase 2 trial completed during late 2014 (Tables 1 and 2; Ref #13, NCT01225172). Furthermore, a phase 1/2 trial, for an estimated completion in 2017, has been designed to test MEDI-573 in combination with



**Table 2** Current and future directions of IGF1R-targeted breast cancer therapies. The table summarizes the preclinical studies and clinical testing of the various IGF1R-targeted – direct and/or indirect (i.e. IGF1R-downstream mediators) – as detailed in this review based on ER-positive, ER-negative, HER2-positive, BRCA-mutated and PI3K-amplified/mutated molecular subtypes.

Breast cancer molecular subtype	IGF1R-targeted (direct or indirect) combination treatments
ER+	IGF1R and chemotherapy Wogonin plus chemotherapy (Fu <i>et al.</i> 2015) IGF1Ri plus chemotherapy (IGF1R polymorphisms) (Ref #16 – NCT010994436) (de Groot <i>et al.</i> 2016) IGF1R and anti-estrogens BMS-754807 plus letrozole (Ref #13 – NCT01225172) or 4-hydroxytamoxifen or fulvestrant (Hou <i>et al.</i> 2011) MEDI-573 plus aromatase inhibitor (Ref #2 – NCT01446159) MEK inhibitor and anti-estrogens (Periyasamy-Thandavan <i>et al.</i> 2012) IGF1R and other <sup>a</sup> IGF1Ri and SphK1i plus anti-estrogens (luminal A) (Fig. 2) (Pitson <i>et al.</i> 2003, Granata <i>et al.</i> 2007).
ER–	IGF1R and chemotherapy BMS-754807 plus chemotherapy (Litzenburger <i>et al.</i> 2011) IGF1R and EGFR <sup>a</sup> IGF1R/EGFR bispecific antibodies (Dong <i>et al.</i> 2011, Croasdale <i>et al.</i> 2012) <sup>a</sup> PI3K-inhibitor (i.e. SF1126) plus EGFR inhibitor (gefitinib/erlotinib) (basal) (Deng <i>et al.</i> 2015) Temsirolimus plus gefitinib/erlotinib (basal) (Madden <i>et al.</i> 2014) Metformin and erlotinib (Ref #6 – NCT01650506) AG1024 plus gefitinib (mesenchymal) (Camirand <i>et al.</i> 2005) OSI-906 plus erlotinib (Ref #17 NCT00739453) (Macaulay <i>et al.</i> 2016) Cixutumumab, gemcitabine and erlotinib (Ref #28 – NCT00617708) <sup>a</sup> Selumetinib and gefitinib (Maiello <i>et al.</i> 2015) IGF1R and VEGF Chemotherapy plus cixutumumab ± bevacizumab (Ref #25 – NCT00955305) R1507 or AMG479 plus bevacizumab (Ref #20 – NCT00974896) (Mahadevan <i>et al.</i> 2014) IGF2 and other <sup>a</sup> MAB292 plus NVP-AEW541 (Mancini <i>et al.</i> 2014) <sup>a</sup> ERβ antagonist, PHTPP plus bevacizumab and/or erlotinib (Mancini <i>et al.</i> 2014) IGF1R and FAK <sup>a</sup> FAK/ROCK inhibitors plus chemotherapy (mesenchymal) (Kurio <i>et al.</i> 2011, Taliaferro-Smith <i>et al.</i> 2015) IGF1R and other AMG479 plus metformin (Ref #1 – NCT01042379) <sup>a</sup> IGF1Ri, SphK1i plus chemotherapy (basal) (Fig. 2) (Wang <i>et al.</i> 2010, Fu <i>et al.</i> 2015, de Groot <i>et al.</i> 2016)
HER2+	IGF1R, HER2 and chemotherapy <sup>a</sup> MM-141 plus chemotherapy (Fitzgerald <i>et al.</i> 2014) <sup>a</sup> Trastuzumab or lapatinib plus PI3K inhibitors (Chakrabarty <i>et al.</i> 2012) <sup>a</sup> Figitumumab, trastuzumab and neratinib (Chakraborty <i>et al.</i> 2015) Capecitabine and lapatinib ± cixutumumab (Ref #15 – NCT00684983) AKT inhibitor, MM-2206, trastuzumab ± paclitaxel (Ref #1 – NCT01042379) (Chien <i>et al.</i> 2016) MK-2206 plus lapatinib (Ref #14 – NCT01245205) or trastuzumab (Ref #18 – NCT00963547) (Hudis <i>et al.</i> 2013) Temsirolimus and neratinib (Ref #5 – NCT01111825) IGF1R, Src and HER2 Sorafenib, IMC-A12 plus trastuzumab/lapatinib (Ref #27 – NCT00906373) (Browne <i>et al.</i> 2012)
BRCA mutated	PARP, chemotherapy and IGF1R <sup>a</sup> Olaparib/veliparib, chemotherapy plus BMS-536924 (Amin <i>et al.</i> 2015) Veliparib plus chemotherapy (NCT02595905)
PI3K amplified and/or mutated	IGF1R and PI3K <sup>a</sup> AMG479 plus BYL719 (Ref #7 – NCT01708161) PI3K and EGFR <sup>a</sup> SF1126/PI-103 plus EGFR inhibitor (Yi <i>et al.</i> 2013, Deng <i>et al.</i> 2015) AKT and other MK-2206 plus goserelin acetate (pre-menopausal) or anastrozole (post-menopausal) (Ref #4 – NCT01776008)

(Continued)

Table 2 Continued.

Breast cancer molecular subtype	IGF1R-targeted (direct or indirect) combination treatments
Not specified	IGF1R and other Ridaforolimus, MK-2206 and MK-0752 (Ref #11 – NCT01295632) Ridaforolimus and MK0646 (Ref #1 – NCT00730379) (Di Cosimo et al. 2015) <sup>a</sup> Cixutumumab, gemcitabine and erlotinib (Mahadevan et al. 2014) <sup>a</sup> Cixutumumab, paclitaxel and bevacizumab (Mahadevan et al. 2014) <sup>a</sup> Cixutumumab, carboplatin and etoposide (Mahadevan et al. 2014) <sup>a</sup> Cixutumumab, mFOLFOX6 and bevacizumab (Mahadevan et al. 2014) <sup>a</sup> Cixutumumab, capecitabine and trastuzumab (Mahadevan et al. 2014) <sup>a</sup> Cixutumumab and sorafenib (Mahadevan et al. 2014)

<sup>a</sup>IGF1R-combination therapies that have preclinical potential and could be priority therapies for future clinical testing.

an aromatase inhibitor (AI) vs AI alone in relation to its safety, tolerability and anti-tumor ability in metastatic breast cancers (Tables 1 and 2: Ref #2, NCT01446159).

A phase 2 clinical trial to investigate the effects of the endocrine therapy letrozole plus OSI-906 with and without erlotinib in breast cancer was terminated in 2011 as all the patients experienced severe toxicities and tumor progression (Table 1: Ref #21, NCT01205685). Moreover, a phase 2 clinical trial to test a combination of exemestane with CP-751,871 (AVE1642) for women diagnosed with ER-positive advanced breast cancer was terminated in 2011 as the manufacturer discontinued the production of AVE1642 (Table 1: Ref #22, NCT00372996). A similar study was also terminated based on the discontinuation of AVE1642 (Table 1: Ref #24, NCT00774878). These terminated studies serve to highlight that there are toxicity issues associated with some IGF-directed targeted combination therapies and demonstrate the intrinsic problems with translation of preclinical findings into effective clinical practice.

### Triple-negative breast cancers

The need to develop new therapies for TNBC currently reliant on chemotherapy and radiotherapy, and to better identify prognostic markers and therapeutic targets, is an ongoing clinical issue. IGF signaling has been shown to be a direct therapeutic target in TNBC (Creighton et al. 2008, Bhargava et al. 2011, Davison et al. 2011, Taliaferro-Smith et al. 2015). Briefly, IGF1R was shown to be expressed and activated by phosphorylation in TNBC (Lerma et al. 2007, Law et al. 2008), high *IGF1R* mRNA expression leads to poorer overall survival in basal/triple-negative groups (Peiro et al. 2011) and rare high-level amplification of *IGF1R* has been detected in some basal-like breast cancers (Adelaide et al. 2007). Collectively, these studies highlight that IGF1R is an oncogenic driver and therapeutic target in

TNBC. It has been suggested that the lack of clinical trials testing IGF1R therapies in TNBC has been attributed to insufficient preclinical data demonstrating effectiveness. The following sections give an overview of the preclinical evidence of IGF-targeted therapies in TNBC and combinatorial approaches that show clinical potential.

**IGF1R and chemotherapy** Owing to the lack of effective targeted therapies, chemotherapy is the most widely used treatment for TNBC and based on evidence that IGF1R decreases sensitivity and response to chemotherapy (Wang et al. 2010b, Beckwith & Yee 2014), chemo- and IGF1R-targeted combination therapies have been of clinical interest. Specifically, the timing and sequence of administering an IGF1R therapy with chemotherapy have been shown to be important in its effectiveness (Zeng et al. 2009). A preclinical study aimed at investigating the combined effects of the anti-IGF1R/InsR inhibitor, BMS-754807 in conjunction with chemotherapy in TNBC, has shown a complete regression response *in vivo* using the MC1 tumor-graft model of TNBC compared with either agent alone, in parallel with reversal in an IGF gene expression signature (Table 2) (Litzenberger et al. 2011). Given this evidence in the literature that IGF1R inhibitors and chemotherapy may be beneficial, further preclinical and clinical testing with other breast cancer therapies is of interest.

**IGF1R and EGFR** EGFR inhibitors have been widely tested in various malignancies such as breast, lung, pancreatic and head and neck cancers (Mendelsohn & Baselga 2006, Subramaniam et al. 2015). EGFR is overexpressed in up to 70% of basal-like TNBC (Livasy et al. 2006), but EGFR monotherapies such as gefitinib and erlotinib have to date not been successful for breast cancer (Masuda et al. 2012), potentially owing to modulation and crosstalk with other growth factors including IGF1R (i.e. EGFR/IGF1R dimer formation) (Morgillo et al. 2006,

Guix *et al.* 2008). There are several studies now suggesting that IGF1R and EGFR combinatorial therapies are worth investigating further for cancer and given there is crosstalk between the two growth factors, combinations have the potential to be more effective than targeting each factor in isolation (Guix *et al.* 2008, Saxena *et al.* 2008, Castano *et al.* 2013, Wong *et al.* 2014).

A recent study identified that levels of the *IGF1* and *EGF* ligands were significantly higher in the stromal micro-environment and bone marrow in TNBC tumors, which were otherwise inactive before being exposed to the active stimuli (Castano *et al.* 2013). The study demonstrated that high IGF-1 and EGF expression levels lead to an induction of the transcription factors *Oct4*, *c-Myc* and *Zeb1* as a trigger for subsequent tumor recurrence and metastasis. Interestingly, IGF1R and EGFR were shown to be expressed in the tumor cells suggesting that the paracrine interplay between stromal and epithelial cells is essential for the maintenance of tumor growth. Importantly, the authors demonstrated that IGF1R activation itself was not sufficient enough to initiate tumor growth and that EGFR activation was also required.

Inhibition of HER-1 (EGFR) and IGF1R using HER-1-418 and IGF-1R-56 peptide mimics has been shown to promote increased anti-tumor responses in the TNBC MDA-MB-231 cell line (Overholser *et al.* 2015). An *in vitro* study using MDA-MB-231 and MDA-MB-468 TNBC cells also reported that co-treatment with gefitinib and the IGF1R inhibitor, AG1024, was effective in producing additive-to-synergistic effects on tumor cell growth (Table 2) (Camirand *et al.* 2005). Lastly, bispecific antibodies such as XGFR2, XGFR3, XGFR4 and EI-04 have been developed, which target both IGF1R and EGFR activity and have been shown to have potent anti-tumor activity that may be a more effective alternative approach to dual-targeting IGF1R and EGFR in breast cancer than using single-targeted therapies (Table 2) (Dong *et al.* 2011, Croasdale *et al.* 2012).

A phase 1 clinical trial completed in 2011 reports that co-treatment with OSI-906 and erlotinib is tolerable leading to effective IGF-1R/InsR phosphorylation reduction in advanced solid cancers such as non-small-cell lung cancer, pancreatic, colorectal and prostate (Tables 1 and 2; Ref #17, NCT00739453) (Macaulay *et al.* 2016). Based on the indirect effects of metformin on IGF1R/EGFR activity (Rozengurt *et al.* 2010, Liu *et al.* 2011), there may be value in testing novel metformin combination therapies. In line with this therapeutic approach, a phase 1 trial that commenced in 2012 has been designed to

test metformin in combination with erlotinib for TNBC (Tables 1 and 2; Ref #6, NCT01650506).

In breast cancer, a number of trials for ER-negative/TNBC cancer have tested EGFR inhibitors in combination with chemotherapy and other targeted therapies (Clinical Trials Gov. Identifiers: NCT00744408, NCT00834678, NCT00239343, NCT00491816 and NCT00998036). In light of the fact that EGFR- and IGF1R-directed combination therapies have shown some preclinical success, and EGFR inhibitors have entered into clinical trials in combination with other drug therapies such as chemotherapy, VEGF-inhibitors and metformin in TNBC, the further addition of IGF1R-inhibitors may also be beneficial to these treatment regimens. In line with this thinking, the I-SPY 2 Trial includes the testing of AMG479 plus metformin for locally advanced breast cancers in addition to a standard neoadjuvant chemotherapy regimen with or without trastuzumab as determined by the HER2 status (Tables 1 and 2; Ref #1, NCT01042379) (Barker *et al.* 2009). A triple combination therapy, comprising cixutumumab, gemcitabine and erlotinib has also been tested in a phase 1 trial as a first-line therapy for pancreatic cancer and may also be an effective approach for TNBC (Tables 1 and 2; Ref #28, NCT00617708).

**IGF1R and VEGF** In the search for better prognostic biomarkers in TNBC, a recent study has demonstrated that VEGF-A, IGF-1 and IGF1R serum levels were significantly higher in women with TNBC than those with other breast cancer types and were also associated with poorer prognosis (Bahhnassy *et al.* 2015a). In a separate study, elevated tissue expression of *IGF1R*, IGF-1 and VEGF-A mRNA and protein was identified in TNBC vs non-TNBC; however, only IGF-1 was found to be an independent prognostic factor (Bahhnassy *et al.* 2015b). IGF1R and VEGF-A are both client proteins of heat-shock protein (Hsp)90, a molecular chaperone involved in the maturation and protein folding of many oncogenic factors, and therapies inhibiting Hsp90 activity in breast cancer cell lines have shown preclinical potential (Jensen *et al.* 2008, Terwisscha van Scheltinga *et al.* 2014). Interestingly, downregulation of IGF1R and VEGF-A secretion after Hsp90 inhibition by NVP-AUY922 *in vivo* was evident in both the MCF7 and MDA-MB-231 cell lines. Importantly, the study provides new evidence that loss of IGF1R and VEGF-A is involved in mediating an anti-tumor response, and they are potential biomarkers of effective Hsp90 therapeutic inhibition (Terwisscha van Scheltinga *et al.* 2014).

In a phase 1 trial, R1507, a humanized IGF1R mAb, has been shown to be tolerable in combination with chemotherapy plus the VEGF-A inhibitor bevacizumab (Table 2) (Mahadevan *et al.* 2014). Similarly, a phase 2 trial tested the safety and PK of AMG479 in conjunction with bevacizumab for advanced solid tumors (Tables 1 and 2: Ref #20, NCT00974896). Currently, there are no specific clinical trials testing IGF1R and VEGF combination therapies in TNBC, but a phase 2 trial completed in 2015 tested chemotherapy in conjunction with cixutumumab with and without bevacizumab for recurrent non-small-cell lung carcinoma (Tables 1 and 2: Ref #25, NCT00955305). Clearly, there is scope for the use of IGF1R and VEGF dual therapies and future testing is likely to have value for the improvement of available TNBC therapies.

**IGF1R and IGF2** There is evidence that non-genomic activation of MAPK/ERK/ phosphoinositide 3-kinase (PI3K) and mTOR pathways by estrogen receptor beta (ER $\beta$ ) in conjunction with IGF-2 is involved in regulating cancer growth in TNBC (Hamilton *et al.* 2015). This is proposed to occur via the activation of growth factor pathways such as VEGF and EGFR in addition to metabolic pathways. Specifically, a study involving shRNA knockdown of ER $\beta$  and treatment with the ER $\beta$  antagonist (PHTPP and ER $\beta$  agonist (DPN)) demonstrated that ER $\beta$  is involved in stimulating the proliferation of the MDA-MB-231 cell line (Hamilton *et al.* 2015). It has also been shown that high ER $\beta$  is expressed in TNBC and associated with worse prognostic outcomes (Hamilton *et al.* 2015). A combination therapy comprising the IGF-2 sequestering antibody MAB292 and topotecan to inhibit the hypoxia-inducible factor-1 enhances the anti-migratory effect of NVP-AEW541 in the TNBC MDA-MB-231 cell line (Mancini *et al.* 2014).

### HER2 amplified breast cancer

In recent years, IGF1R activity has been shown to play a role in driving HER2 therapy trastuzumab resistance, and co-targeting HER2 and IGF1R is thought to be a viable therapeutic approach to overcome resistance (Browne *et al.* 2012). Mechanisms associated with IGF1R-driven HER2 therapy resistance are not well defined; however, there are studies suggesting that it is mediated via de-regulation of the PTEN/PI3K/AKT signaling pathway (Gallardo *et al.* 2012). Although PI3K activity is involved in HER2-therapy resistance, it has been proposed that HER2-overexpressing cancers treated with PI3K inhibitors

should also be combined with HER2-targeted therapies such as trastuzumab or lapatinib to overcome the AKT-mediated activation of other tyrosine kinase growth factor receptors including IGF1R, InsR and HER3 as a result of PI3K inhibitor treatment (Table 2) (Chakrabarty *et al.* 2012). In addition, it has been shown that IGF1R-dependent cell motility in trastuzumab resistance occurs via the stimulation of FAK signaling and Forkhead box protein M1 (FoxM1), and co-targeting HER2 and IGF1R is seen to be beneficial mostly in trastuzumab-resistant cells (Sanabria-Figueroa *et al.* 2015).

A combination of figitumumab plus the HER2-targeted therapies, trastuzumab and neratinib (HER2 TKI), was shown to elicit a synergistic effect on enhancing cell apoptosis and reducing cell proliferation in the BT474 and MCF7 breast cancer cells lines (Table 2) (Chakrabarty *et al.* 2015). Indirectly targeting IGF1R overexpression in HER2-amplified breast cancers by the introduction of miR-630 into the cell can restore the sensitivity of the cell to different HER2 inhibitors and may have clinical benefit to prevent IGF1R-driven HER2-therapy resistance (Corcoran *et al.* 2014). A separate study has similarly demonstrated that overexpression of miR-375 can restore the sensitivity of SKBR3 HER2-amplified cells to targeted HER2 therapy via an IGF1R-mediated mechanism (Ye *et al.* 2014).

Identifying better clinical biomarkers of HER2 therapy response is essential due to the high rate of HER2 therapy-resistant breast cancers that develop in individuals. Micro-RNAs (miRNAs) have been identified as short (18–25) nucleotide non-coding RNAs, which in effect can act as tumor suppressors in the cell by inhibiting gene expression. Most importantly, some miRNAs are known to be decreased in cancer vs normal cells and therefore re-introduction of miRNA back into the cell has therapeutic implications (Zhang *et al.* 2007). Specifically, overexpression of miR-630 has been shown to increase the degradation of both IGF1R mRNA and protein in pancreatic cancer cells (Farhana *et al.* 2013). In relation to IGF1R activity in HER2-expressing breast cancers, indirectly targeting IGF1R overexpression in HER2-amplified breast cancers by the introduction of miR-630 into the cell can restore the sensitivity of the cell to different HER2 inhibitors and may have clinical benefit to prevent IGF1R-driven HER2 therapy resistance (Corcoran *et al.* 2014). A separate study has similarly demonstrated that overexpression of miR-375 can restore the sensitivity of SKBR3 HER2-amplified cells to targeted HER2 therapy via an IGF1R-mediated mechanism (Ye *et al.* 2014). miR-630 is also reduced in the HER2 therapy-resistant

MDA-MB-435 cell line compared with that in the HER2 therapy-sensitive SKBR3 cell line and in HER2-expressing tumors compared with matched peritumors, suggesting that miRNAs can be used as diagnostic and predictive markers of HER2 therapy response (Corcoran *et al.* 2014).

Similar to the bispecific antibodies that target both IGF1R and EGFR (Dong *et al.* 2011, Croasdale *et al.* 2012), a tetravalent bispecific antibody, MM-141, has been developed that targets both IGF1R and erbB3 (HER3), preventing IGF-1 and IGF-2 ligand binding to IGF1R and heregulin binding to erbB3, subsequent ligand-induced receptor phosphorylation, and p-AKT downstream signaling (Fitzgerald *et al.* 2014). MM-141 is more effective in reducing p-AKT than antibodies that target IGF1R and erbB3 individually and can enhance the anti-tumor effects of the chemotherapeutic agent, docetaxel, in DU145 prostate cancer xenografts (Table 2) (Fitzgerald *et al.* 2014). Given the potential clinical benefits of using MM-141 as a dual-targeting drug, a phase 1 clinical trial was completed in 2015, which tested the dose levels and frequency of MM-141 in patients with advanced solid cancers (Table 1: Ref #9, NCT01733004). Using this type of therapy approach in combination with chemotherapy and/or other targeted therapies may be an effective strategy in preventing IGF1R-driven trastuzumab resistance in breast cancer and requires further clinical testing. A phase 2 trial completed in 2014, in women with HER2-positive breast cancers tested capecitabine and lapatinib with or without cixutumumab (Table 1: Ref #15, NCT00684983).

One study has demonstrated that high overexpression of the IGF1R-driven tyrosine kinase activated the phosphorylated form of Src (pY416), and is correlated with poorer overall survival (OS) and disease-free survival (DFS) in adjuvant trastuzumab therapy treated HER2-positive/hormone receptor negative breast cancers (Peiro *et al.* 2014). Thus, co-targeting strategies using Src inhibitors in conjunction with HER2-targeted therapies may be clinically effective for HER2-amplified cancers to eliminate IGF1R-mediated HER2 therapy resistance (Browne *et al.* 2012). At present, there are no clinical trials testing this type of combination therapy for breast cancer; however, a phase 2 trial testing the safety of IMC-A12 and the Src inhibitor, sorafenib, in combination for advanced liver cancer may give some further insight into the potential of combination therapy (Tables 1 and 2: Ref #27, NCT00906373). Given that there is some progress in targeting Src activity in cancer, combination therapies such as IMC-A12 and sorafenib in addition to trastuzumab may be effective as a prevention of IGF1R-driven HER2

therapy resistance. There is certainly future potential for investigating the involvement of IGF1R in HER2-driven cancers, and this is reflected in the progression of clinical trials in this therapeutic area of interest.

### BRCA-mutated breast cancer

**IGF1R and DNA-damaging agents** BRCA-mutated breast cancers are susceptible to the impairment of DNA damage repair by homologous recombination and interestingly are known to have high IGF-1 protein expression (Hudelist *et al.* 2007). Specific therapies aimed at targeting DNA repair factors include poly ADP-ribose polymerase (PARP) inhibitors (e.g. olaparib). Most important to the development of more effective IGF1R-targeted therapies, PARP regulation in the ER-positive MCF7 and HER2-positive BT474 cell lines is mediated via IGF1R (Kim *et al.* 2015). Preclinical support for the use of dual IGF1R and PARP inhibitors is highlighted by a study that showed an increased sensitivity to olaparib by the addition of the IGF1R inhibitor, BMS-536924, in BRCA1-mutated (MDA-MB-436 and HCC1937) compared with BRCA1 wild-type (BT20 and MDA-MB-231) TNBC cell lines (Table 2) (Amin *et al.* 2015). There is also compelling evidence that IGF1R is involved in DNA damage repair, highlighting the need for further investigation of the role of IGF-1 signaling in the DNA repair process in breast cancer (Lin *et al.* 2014). There are no current clinical trials testing PARP inhibitors in conjunction with IGF1R-inhibitors; however, there is a phase 2 trial that commenced in 2016 designed to test the PARP inhibitor, veliparib, with chemotherapy agents cisplatin and vinorelbine ditartrate in TNBC, BRCA-mutated breast cancers (Clinical Trials Gov. Identifier; NCT02595905, Table 2) and a phase 1 trial that was completed in 2015 for recurrent and/or metastatic breast cancer (Clinical Trials Gov. Identifier; NCT01104259).

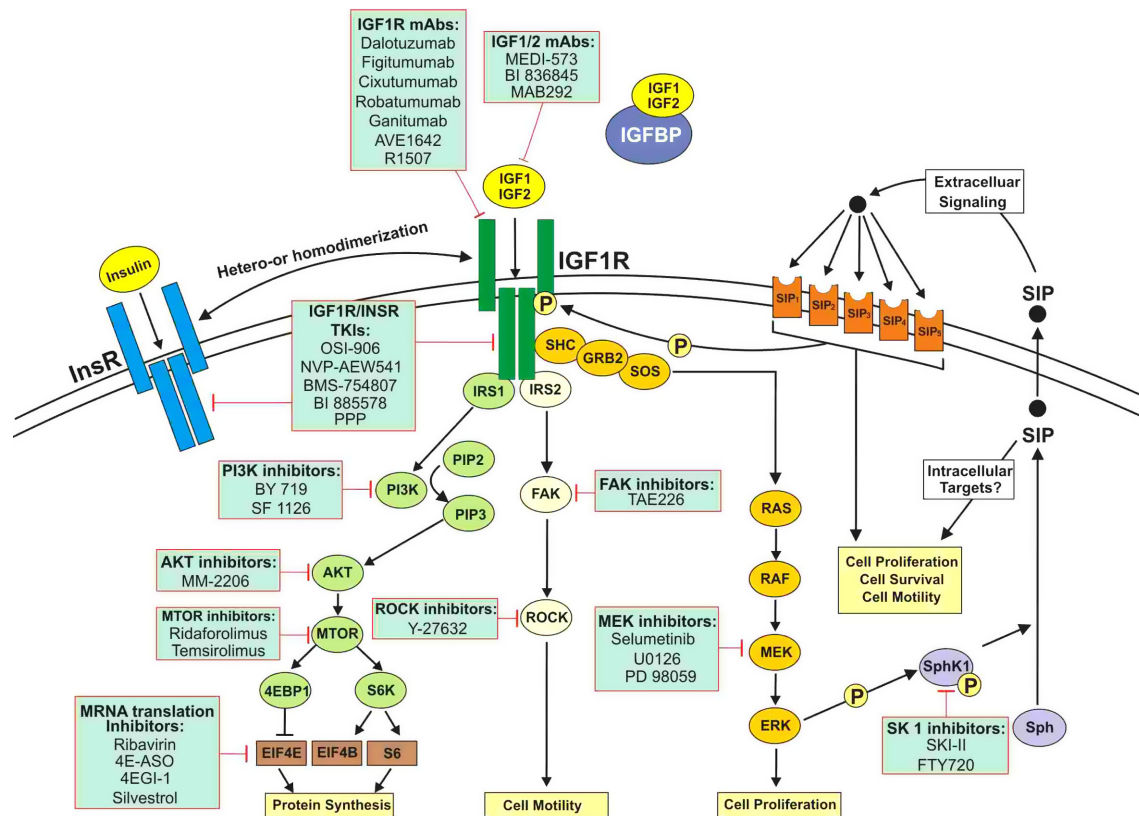
### Downstream mediators of IGF1R signaling: potential therapeutic targets

Targeting IGF1R signaling is intrinsically difficult because it is not a singular pathway but comprises various downstream mediators that promote oncogenesis. Additionally, a number of other signaling receptors can also regulate many IGF1R downstream mediators, which make it difficult to attribute all the tumorigenic effects to IGF1R. The disappointing success of IGF1R-directed therapies is most certainly in part attributable to the

complex interplay of growth factor signaling that exists in the cancer cell to provide for sustainable growth, migration and survival. As a number of growth factors can activate AKT/PI3K and MAPK signaling pathways, inhibition of IGF1R signaling may in fact be more effective by targeting its 'activity' (i.e. downstream mediator-targeted therapies inhibition) rather than IGF1R 'expression' or both as discussed in the following sections (Fig. 1).

IGF1R signaling is directly involved in the regulation of downstream mediators involved in protein synthesis via activation of AKT/PI3K signaling (e.g. mammalian target of rapamycin complex 1 (mTORC1) and 4E-binding protein 1 (4E-BP1)), as well as apoptosis (e.g. BAD), cellular proliferation (i.e. Ras, MEK and ERK), altered

integrin expression/cell motility (i.e. Src-focal adhesion kinase (FAK), RhoA/Rho-associated kinase (ROCK) and metabolic pathways (i.e. xCT (*SLC7A11*) and pyruvate kinase M2 (Yang & Yee 2014, Salani *et al.* 2015)). A variety of combinatorial therapies targeting IGF1R (NVP-AEW541), PI3K (NVP-BKM120), mTORC (KU0063794) and MEK (PD0325901) in the MDA-MB-231 cell line have shown synergistic effects *in vitro* by a reduction in cell growth (i.e. combination index (CI) < 1) in parallel with an increase in cell apoptosis and G<sub>0</sub>/G<sub>1</sub> cell cycle arrest (Ayub *et al.* 2015). Importantly, these combinations have a potential to prevent the re-activation of these tyrosine kinase pathways and reduce toxicity by the administration of lower drug doses.



**Figure 1**

Schematic representation of therapeutic targeting of IGF1R expression and activity in breast cancer. Insulin-like growth factor receptor (IGF1R) signaling is mediated after the formation of homo- and heterodimers of IGF1R and the insulin receptor (InsR) in response to the binding of their respective ligands (i.e. IGF1 and IGF2 to IGF1R and insulin to InsR). In the cytoplasm, IGF1/IGF2 form a complex with insulin growth factor-binding proteins (IGFBPs) to reduce the pool of available ligand that can bind to IGF1R. IGF1R activation leads to downstream cell signaling of effector molecules that regulate PI3K/AKT, FAK and MAPK/MEK intracellular signaling pathways. SphK1 is phosphorylated and activated by ERK leading to both intracellular and extracellular activity of the bioactive lipid molecular sphingosine 1 phosphate (SIP) (Pitson *et al.* 2003, Granata *et al.* 2007). IGF1R can be activated after SIP<sub>1</sub> and SIP<sub>2</sub> receptor-mediated phosphorylation (Martin *et al.* 2009). Targeted therapies have been developed to inhibit IGF1R expression including IGF1R monoclonal antibodies (mAbs), IGF1/2 mAbs and IGF1R/InsR tyrosine kinase inhibitors. IGF1R-directed cancer therapies that inhibit IGF1R downstream effector molecules have also been developed including PI3K; AKT; mTOR; mRNA translation; FAK; MEK and SphK1. Novel combination therapies aimed at targeting IGF1R expression and activity are developing this area of breast cancer therapeutics. Reprinted by permission from the American Association for Cancer Research: Iams WT & Lovly CM, Molecular pathways: clinical applications and future direction of insulin-like growth factor-1 receptor pathway blockade, *Clinical Cancer Research*, 30 Sep 2015, volume 21 issue 19, pages 4270 to 4277, doi:10.1158/1078-0432.ccr-14-2518.

### PI3K inhibitors

PI3K signaling is upregulated in an estimated 70% of breast cancers (Leroy *et al.* 2016), whether as a consequence of increased IGF1R (or EGFR) pathway activation, or secondary to oncogenic mutations in *PIK3CA*, reported to occur in about 25% of all breast cancers (Zardavas *et al.* 2014). IGF1R pathway activation is known to confer resistance to PI3K inhibition (Leroy *et al.* 2016), suggesting the possible utility of combined IGF1R/PI3K inhibition. A phase 1/2 clinical trial that commenced in 2012 has tested the effectiveness of a dual therapy comprising AMG479 and the PI3K-inhibitor, BYL719, in *PIK3CA*-mutated or -amplified solid cancers (Tables 1 and 2: Ref #7, NCT01708161).

Using the MDA-MB-231 and MDA-MB-436 TNBC cell lines, a combination therapy comprising the PI3K inhibitor, SF1126 plus gefitinib, has shown some promising preclinical results (Table 2) (Deng *et al.* 2015). Dual inhibition of PI3K/AKT activity using PI-103 and EGFR activity by gefitinib or erlotinib showed enhanced combined drug effects on reducing cell viability and expression of anti-apoptotic proteins in basal-like SUM149PT and MDA-MB-468 breast cancer cell lines, but not for mesenchymal stem-like Hs578T and MDA-MB-231 cell lines (Table 2) (Yi *et al.* 2013). Further complexity in understanding how to therapeutically target IGF1R and its downstream mediators is highlighted by the ability of IGF1R to induce basal and estrogen-induced phosphorylation of the stress-activated protein kinase c-Jun (JNK) via PI3K activation (Fan *et al.* 2015). Whether co-treatment with IGF1R plus JNK-inhibitors such as SP600125 would be effective in overcoming anti-estrogen therapy resistance remains to be further tested (Fan *et al.* 2015).

### AKT inhibitors

Given that PI3K is integral to downstream IGF1R-mediated AKT activity, targeting AKT as an alternative to PI3K has logically been pursued as a therapeutic target. A phase 1b study has demonstrated the clinical potential of the AKT inhibitor, MM-2206, combined with paclitaxal and trastuzumab in HER2-driven cancers (Table 2) (Chien *et al.* 2016). Moreover, the I-SPY 2 Trial has included the testing of the AKT inhibitor, MK-2206, with and without the HER-2 inhibitor, trastuzumab (Tables 1 and 2: Ref #1, NCT01042379). Further, a phase 2 trial commenced in 2013 to test whether the co-therapies comprising MK-2206, anastrozole (post-menopausal women) and goserelin acetate (a gonadotropin-releasing hormone agonist; premenopausal women) is more effective in suppressing estrogen production

in *PI3KA* mutant, ER-positive or HER2-negative breast cancers (Tables 1 and 2: Ref #4, NCT01776008).

Completed clinical trials to test various AKT inhibitor combination therapies in breast cancer include phase 1 trials testing the maximum tolerated doses (MTD) and dose-limiting toxicities (DLT) of MK-2206 combined with the dual HER2/EGFR inhibitor, lapatinib ditosylate (Tables 1 and 2: Ref #14, NCT01245205), or trastuzumab which was shown to be associated with good safety and clinical outcomes (Tables 1 and 2: Ref #18, NCT00963547) (Hudis *et al.* 2013). At present, there are no clinical trials testing IGF1R and AKT dual inhibition, which may be more effective than just targeting the downstream effects of IGF1R activity.

### FAK inhibitors

The IGF signaling pathway is well established to regulate cell migration in breast cancer via the activation of FAK and RhoA/Rho-associated kinase (ROCK), as further discussed in subsequent sections; therefore, co-targeting IGF1R and FAK activation is potentially a viable therapeutic approach (Zhang *et al.* 2005, Taliaferro-Smith *et al.* 2015). As with many other oncogenic signaling pathways, there is often crosstalk and/or reciprocal regulation that has been reported for IGF1R and FAK in TNBC (Taliaferro-Smith *et al.* 2015). Interestingly, inhibiting both IGF1R and FAK has shown effective anti-tumor effects in a number of different cancers including esophageal adenocarcinoma and glioma (Liu *et al.* 2007, Watanabe *et al.* 2008). The specific ATP-competitive tyrosine kinase small molecule inhibitor TAE226 that targets both IGF1R and FAK has been used to inhibit MDA-MB-231 breast cancer bone metastasis (Kurio *et al.* 2011). There is evidence from MDA-MB-231 cells that IGF-1 stimulation leads to the cellular re-distribution of FAK from focal adhesion proteins to regulate cell motility, which can be blocked using the ROCK inhibitor, Y-27632 (Zhang *et al.* 2005). Moreover, a recent study supports the potential benefit of using IGF1R and FAK co-targeted therapies in TNBC by the effective suppression of epithelial-to-mesenchymal transition, cell migration and invasion (Table 2) (Taliaferro-Smith *et al.* 2015). Currently, there are no clinical trials testing IGF1R and FAK inhibitor dual therapies for breast cancer, yet given the literature, there is certainly some worth in pursuing this therapy approach.

### mTOR inhibitors/mRNA translation inhibitors

Targeting IGF1R downstream activators involved in the regulation of mRNA translation using therapies such as

the anti-viral drug ribavirin, the antisense oligonucleotide 4E-ASO, the small molecule inhibitor, 4EGI-1, and the natural inhibitor, silvestrol (Silvera *et al.* 2010, Pettersson *et al.* 2011) is an emerging therapeutic window of opportunity, which can interrogate many converging oncogenic signaling pathways and is undergoing extensive preclinical and clinical testing (Clinical Trials Gov. Identifier; NCT01309490) (Drygin *et al.* 2011, Devlin *et al.* 2015).

IGF-1 signaling downstream mediators contribute to cancer growth via the regulation of cap-dependent mRNA translation primarily by the activation of mTOR and S6K1 to initiate the activation of the translational modulators 4EBP1 and eIF4E (Sonenberg & Hinnebusch 2009).

Recently published novel findings highlight that the IGF-1 downstream signaling human oncogene, amplified in breast cancer 1 (*AIB1*), possesses cancer-promoting effects potentially via positively regulating global polyribosome mRNA recruitment in ER-positive MCF7L and ER-negative, MDA-MB-231 and MDA-MB-435/LCC6 breast cancer cells (Anzick *et al.* 1997, Ochnik *et al.* 2016). Importantly, these findings highlight the need for a better identification of potential therapeutic targets in 'IGF-expressing' breast cancers. In relation to therapeutically inhibiting mTOR and suppressing IGF-induced mRNA translation, it has been shown that basal-like TNBC breast cancers respond synergistically (i.e.  $CI < 1$ ) to co-treatment of the mTOR inhibitor, temsirolimus and gefitinib by reduced phosphorylation of the translational mediator, eIF4B (Table 2) (Madden *et al.* 2014). Briefly, the *in vitro* study demonstrated, using a luciferase-based reporter assay system, that cap-dependent mRNA translation was reduced by the co-treatment with temsirolimus and gefitinib compared with the individual drugs (Madden *et al.* 2014).

A phase 1/2 clinical trial aims to test the associated side effects and MTD of temsirolimus plus cixutumumab in metastatic or locally recurring breast cancer (Table 1: Ref #8, NCT00699491) in addition to survival outcomes of temsirolimus plus the tyrosine kinase inhibitor (neratinib) in triple-negative, *HER2*-amplified breast cancers (Tables 1 and 2: Ref #5, NCT01111825). A phase 1 breast cancer trial has been completed testing ridaforolimus in conjunction with either MK-2206 or the notch inhibitor, MK-0752 (Tables 1 and 2: Ref #11, NCT01295632) (Gupta *et al.* 2015, Piha-Paul *et al.* 2015). The development of these dual-targeting therapies is advancing the knowledge required to eventually underpin therapies that are working and therapies that, despite the promising basic science findings, do not show the same clinical benefit.

## MEK inhibitors

Targeting the anti-apoptotic actions of the IGF-1 downstream effector, MEK-1, using small molecule inhibitors including PD98059 and U0126 in breast cancer in conjunction with anti-hormonal therapies has been shown to be effective in blocking IGF-dependent survival in ER-positive cells (Table 2) (Periyasamy-Thandavan *et al.* 2012). Dual blockade of MEK and EGFR using the MEK inhibitor, selumetinib and gefitinib, has been shown to be potentially of value for a subset of TNBC (Table 2) (Maiello *et al.* 2015). Future studies investigating these types of dual therapies have merit; yet, the authors note that without sufficient biomarkers of efficacy, there will be many individuals who will not respond. Conversely, the WNT pathway element, DVL3, can potentially suppress the response of anti-IGF1R-targeted drugs via MEK-ERK regulation in cancer (Gao *et al.* 2014). The study reports improved the clinical outcomes for individuals expressing low-absent DVL3 expression after figitumumab or AVE164 therapy and highlights that there is a need for positive and negative IGF1R therapy response biomarkers to be identified (Gao *et al.* 2014).

## All breast cancer molecular subtypes

The safety and tolerability of the IGF1R monoclonal antibody R1507 in combination with several standard oncology regimens have been shown to be manageable in a variety of advanced solid malignancies including breast (Table 2) (Mahadevan *et al.* 2014). Different combination therapies including cixutumumab have been studied in a phase 1b trial to test the tolerability in solid cancers with the following therapies: (1) gemcitabine plus erlotinib; (2) paclitaxel plus bevacizumab; (3) carboplatin plus etoposide; (4) mFOLFOX6 (combination chemotherapy) plus bevacizumab; (5) capecitabine plus trastuzumab and (6) sorafenib. Based on the findings that they were well tolerated, with some individuals presenting with a lasting response for a median of 29 weeks, the combination treatments are potentially viable for a subset of cancers (Mahadevan *et al.* 2014).

For advanced, previously treated cancers that are known to overexpress IGF1R, a phase 1 study that commenced in 2014 has been designed to evaluate the disease response and toxicities of the chemotherapy drug IGF-methotrexate, which binds specifically to the IGF1R (Table 1: Ref #3, NCT02045368) (McTavish *et al.* 2009). mTOR inhibition using ridaforolimus in conjunction with the IGF1R-inhibitor MK0646 in a phase 1 trial for



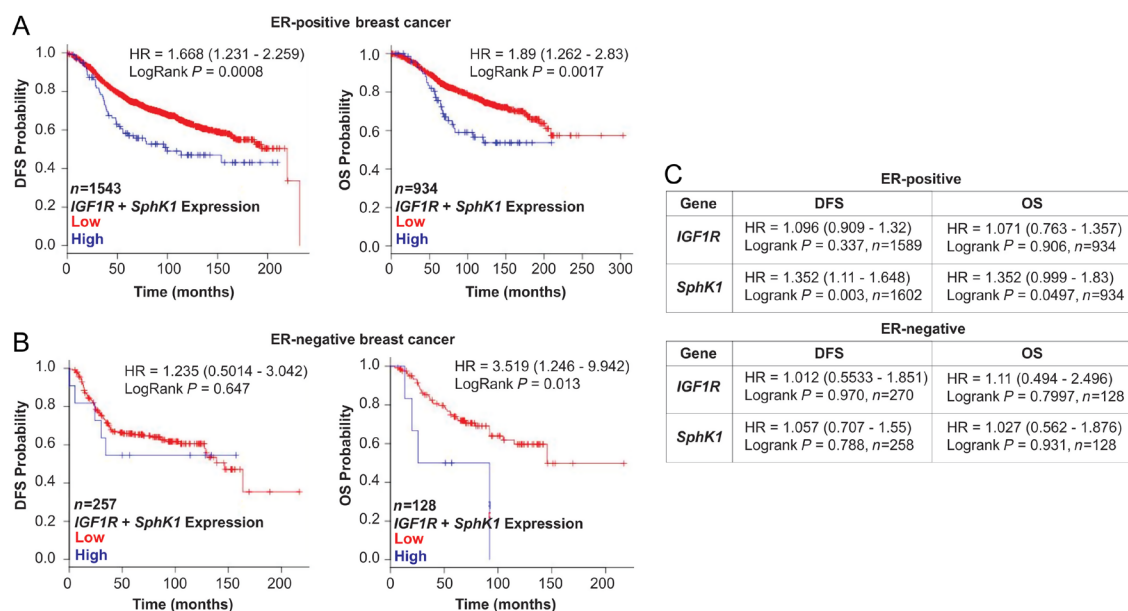
advanced cancer has also shown promising results and again highlights the need for further novel approaches to target IGF1R expression and/or its activity (Tables 1 and 2: Ref #23, NCT00730379) (Di Cosimo *et al.* 2015).

### Emerging IGF1R co-related oncogenic pathway: sphingosine kinase signaling

IGFBP-3 in breast tumors has the potential to influence IGF1R-dependent survival pathways because it has been shown to potentiate IGF1R signaling, through a mechanism involving the activation of the oncogenic lipid kinase, sphingosine kinase 1 (SphK1) and generation of the intracellular second messenger pro-survival lipid, sphingosine 1-phosphate (SIP) (Martin *et al.* 2009). SIP has both intracellular activity, the targets of which are not well defined, and extracellular oncogenic activity after binding to SIP receptors 1–5 in the cell membrane to activate various G-protein-responsive pathways involved in cell proliferation, migration and survival (Hannun & Obeid 2008). It has been reported that activation of ERK by growth factors such as IGF-1 leads to SphK1 phosphorylation on residue Ser225 and in effect enhances intracellular production of SIP (Pitson *et al.* 2003, Granata *et al.* 2007). As IGF-1 signaling can regulate SphK1

via phosphorylation, it may even be considered as a downstream mediator of IGF1R activity (Fig. 1).

Both sphingolipid signaling (Wang *et al.* 2010b) and IGF1R activation (Beckwith & Yee 2014) have been shown in many studies to contribute to the resistance of a variety of cancer chemotherapies and increase sensitivity/response to doxorubicin (Fu *et al.* 2015, de Groot *et al.* 2016), yet in-depth studies identifying co-dependence of IGF1R and sphingolipid signaling to therapy responsiveness are lacking. In contrast, co-treatment strategies targeting SphK1 and EGFR have shown exciting preclinical success and are currently being investigated for TNBC (Martin *et al.* 2014). Recently, *SphK1* mRNA and protein expression have been shown to be elevated in TNBC compared with non-adjacent non-cancerous breast tissue as well as in the MDA-MB-231, Hs578T and MDA-MB-436 TNBC compared with non-TNBC cell lines (Li *et al.* 2016). Not only was SphK1 expression higher in TNBC; the increased serum levels of SIP were also evident in breast cancer patients compared with healthy donor individuals (Li *et al.* 2016). *SphK1* siRNA knockdown in the MDA-MB-231 and Hs578T cell lines is effective in reducing cell proliferation, migration and apoptosis *in vitro*, potentially via the downregulation of PI3K/AKT signaling (Li *et al.* 2016). In terms of the prognostic significance of IGF1R



**Figure 2**

Prognostic significance of *IGF1R* and *SphK1* co-expression in ER-positive and ER-negative breast cancers. Kaplan–Meier (KM) mRNA survival analysis was performed as follows in: (A) estrogen receptor (ER)-positive and (B) ER-negative, lymph node-negative breast cancers after the stratification for high *IGF1R* and *SphK1* mRNA co-expression to determine disease-free survival (DFS) and overall survival (OS) probability (<http://glados.ucd.ie/BreastMark/>). (C) Table includes hazard ratio (HR) and log-rank P values for DFS and OS of *IGF1R* and *SphK1* individual gene KM analysis.

and SphK1, poorer DFS and OS are evident in women with both ER-positive and ER-negative breast cancers expressing high *IGF1R* and *SphK1* mRNA compared with single-gene analysis, highlighting these kinases as dual therapeutic targets (Fig. 2 and Table 2).

A S1P receptor modulator that also shows SphK1 inhibitory activity (Novartis; fingolimod, FTY-720) has been FDA approved as an immunosuppressive agent for multiple sclerosis, and has also been suggested to exhibit anticancer activity (Pitman *et al.* 2012). Specifically in relation to cancer, a number of different SphK inhibitors have been developed and shown to elicit anticancer effects including SKI-II, ABC294640 and MP-A08 (Pitman & Pitson 2010, Pitman *et al.* 2015, McNaughton *et al.* 2016). Currently, SphK1 inhibitors are not clinically in testing for breast cancer, but the preclinical and prognostic findings of a relationship between IGF1R and SphK1 expression provide evidence that there is a need to learn more about the potential clinical benefits of IGF1R and SphK1 as novel dual therapeutic targets (Fig. 1).

#### Future IGF-targeted therapies: where to now

This review has aimed to provide a comprehensive assessment of the current testing of IGF1R-directed therapies for breast cancer based on the different molecular subtypes. In some instances, preclinical studies and clinical trials that have tested IGF1R-therapies in other solid cancers and cell lines have been discussed as a platform to potentially translate through as therapies that may also be beneficial to IGF1R-expressing breast cancers. Since IGF-targeted therapies have led to a large percentage of negative clinical trial results, there is now a greater need for the next wave of IGF1R-directed clinical trials to be better designed based on preclinical findings and current clinical trials.

It is increasingly clear that IGF-signaling plays a cancer-promoting role not only in the most widely tested ER-positive breast cancers but also in some TNBC, HER2-positive and *BRCA*-mutated breast cancers, and all are potentially important in terms of anti-IGF1R combination therapy clinical testing. As there has been a very low success rate for anti-IGF1R monotherapies, it is difficult to further predict the success of many of the preclinical and current clinical trials. However, a list of the preclinical and clinical IGF1R-targeted combination therapies discussed in this review based on breast cancer molecular subtype has now been summarized as a guide and suggestion for future clinical testing directions based on breast cancer subtype (Table 2). In terms of combination therapies

that should be prioritized, we have made note of the preclinical studies that are of particular interest and worth for possible future clinical testing.

#### Conclusion

The vast array of potential ways of combining IGF1R-targeted therapies with other existing and emerging therapies is developing rapidly and likely to be clinically important. In light of the high degree of heterogeneity that exists in breast cancer, between and within molecular subtypes, it is becoming increasingly difficult to design effective targeted therapies with broad applicability. The complexity of cancer cell signaling pathways, and how their re-activation can occur to overcome the inhibitory actions of breast cancer therapies, has in effect led to new molecular forms of treatment-resistant breast cancer. Given that we are now learning more about the potential pitfalls associated with many therapeutic approaches for breast cancer that lead to therapy resistance, the next wave of research aims to consolidate this information to ultimately underpin more effective ways of targeting key oncogenic signaling pathways to prevent and/or treat therapy resistance.

Multiple lines of evidence indicate that IGF1R-directed therapies are important and the vast amount of literature supporting the integral role of IGF-1 signaling in breast cancer should not be ignored despite the lack of clinical success of IGF1R-targeted monotherapies. In essence, this lack of success only serves to highlight that IGF1R signaling is more complicated than initially understood, and most certainly requires concurrent combination therapies targeted to other growth factor pathways (e.g. HER2/HER3, EGFR, VEGF, and InsR), its downstream mediators (e.g. PI3K, mTOR, eIF4E, FAK) and/or co-related signaling pathways (e.g. SphK1), to be clinically effective. Current and future clinical trials will work toward providing more effective IGF-directed targeting strategies.

#### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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