40 YEARS OF IGF1

Anti-insulin-like growth factor therapy in breast cancer

Douglas Yee
Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota, USA
Correspondence should be addressed to D Yee: yeeex006@umn.edu
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Abstract
Early preclinical and population data suggested a role for the type I insulin-like growth factor receptor (IGF1R) in the regulation of breast cancer growth and survival. To target this pathway, multiple monoclonal antibodies and tyrosine kinase inhibitors were developed and tested in clinical trials. While some of the early clinical trials suggested a benefit for these drugs, none of the attempts showed improved outcomes when compared to conventional therapy. This failure of the IGF1R inhibitors was pronounced in breast cancer; multiple trials testing IGF1R inhibition in estrogen receptor-positive breast cancer were conducted, none showed benefit. This review will evaluate the rationale for IGF1R inhibition, discuss results of the clinical trials and suggest a path forward.

Introduction
Decreasing mortality rates in breast cancer have been observed since the late 1980s. This decline has been attributed to populationwide screening mammography programs and the greater use of systemic adjuvant therapy (Berry et al. 2005). These clinical results underlie the fact that mortality in breast cancer is driven by metastatic spread of the primary tumor to distant organs. Screening mammography detects disease early before metastatic spread can occur, but it is controversial as to whether this early detection truly results in decreased breast cancer death (Mandelblatt et al. 2009, Biller-Andorno & Juni 2014).

Systemic therapy in early-stage operable breast cancer either eliminates or modulates the behavior of micro-metastatic distant metastases that occurred prior to the clinical detection of the primary breast cancer. In contrast to mammography, it is clear greater use of adjuvant therapy accounts for reduced deaths in women with operable breast cancer (Palmieri & Jones 2012, Peto et al. 2012). Further, even for women with metastatic disease, life expectancy is increasing (Mariotto et al. 2017) presumably due to the development of many new systemic therapies.

Because of this, there has been greater emphasis on development of new strategies to target malignant cells. In this regard, breast cancer has been at the forefront of showing that manipulation of endocrine pathways results in clinical improvements. In 1890, Beatson removed young woman’s ovaries resulting in regression of her locally advanced breast cancer (Beatson 1896). The discovery of the molecular structure of estrogen receptor-α (ER), the identification of molecules disrupting estrogen binding to ER, the inhibition of peripheral conversion of precursors into estradiol by aromatase and the disruption of signaling pathways influencing ER have all resulted in new drugs approved to treat breast cancer.

There was great hope disruption of the type I insulin-like growth factor receptor (IGF1R) could serve as an
effective therapy for breast cancer. Similar to ER, IGF1R is important in normal growth and development, IGF1R initiates signaling events important for cancer cell growth and survival, is frequently expressed in breast cancer cells and has a targetable biochemical function. Further, the first demonstration inhibition of IGF1R could be effective in breast cancer preclinical models (Arteaga et al. 1989), occurred at about the same time the human epidermal growth factor receptor-2 (HER2) targeting monoclonal antibodies were being evaluated in clinical trials (Pegram et al. 1998). Thus, it made eminent sense to develop similar drugs in cancer designed to target another growth factor receptor.

There have been several excellent reviews outlining the preclinical and population science rationale for targeting this receptor (Pollak 2008, 2012). Briefly, the IGF signaling pathway regulates several key aspects of cancer hallmarks (Hanahan & Weinberg 2011). IGF1R stimulation results in proliferation, survival and metastasis. Further, studies from population-based studies suggested IGF1 levels correlated with cancer risk (Chan et al. 1998). Humans with low levels of serum IGF1, because of growth hormone receptor mutation, appear to be refractory to cancer development (Guevara-Aguirre et al. 2011). Thus, targeting IGF1R function could serve to prevent and treat breast cancer.

Strategies to block IGF1R

The IGF1R is similar in structure to the insulin receptor. IGF1R is a single gene and once transcribed, the protein is processed into two separate chains. The alpha subunit is extracellular and ligand binding. It is covalently bonded to the beta subunit which contains a short extracellular domain, the transmembrane domain and an intracellular tyrosine kinase domain. An alpha-beta subunit is bound to a partner; thus, the functional receptor complex is a heterodimeric structure (Krywicki & Yee 1992). Because of this structure, the extracellular alpha subunits must bind ligand for receptor activation.

The two high-affinity ligands for IGF1R are IGF1 and IGF2. Both IGFs are similar in structure and sequence to insulin. They all share a common structure composed of intra-chain disulfide cross-links. Insulin, but not IGF1 or IGF2, has its internal domain (C-peptide) proteolytically cleaved to form a two chain ligand (Leroith et al. 1993).

IGF1 and -II are expressed by many tissues and can activate IGF1R by endocrine, paracrine and autocrine pathways. In adults, post-pubertal expression of growth hormone (GH) results in increased IGF1 production by the liver. Originally called somatomedin C because of its role in somatic growth, IGF1 interacts with receptors in essentially all normal tissues (Binoux et al. 1986). In mice, IGF2 is a fetal somatomedin. After birth, rodents experience a decline in serum IGF2 levels. In humans, IGF2 levels persist during life. However, in adult humans, a clear physiologic role for IGF2 has still not yet been identified. In serum and extracellular fluids, IGFs are found bound to high-affinity IGF-binding proteins (IGFBPs). Six well-characterized binding proteins have been identified, and each has the ability to affect release of ligand to the receptor (Perks & Holly 2008).

Ligand suppression

Before drugs were developed to suppress estradiol levels, there were surgical methods to suppress ER-expressing breast cancers. Oophorectomy in premenopausal women was a commonly used strategy. In postmenopausal women, both adrenalectomy and hypophysectomy were performed. Since the pituitary is the source of GH, this surgical ablation certainly resulted in lowered levels of IGF1. While clinical benefits of hypophysectomy were seen after adrenalectomy, this clinical benefit cannot be solely attributed to suppression of serum IGF1, although it is possible, this could be the reason for the therapeutic benefits of hypophysectomy after all sources of estradiol were surgically removed (Pearson & Ray 1960, Fracchia et al. 1971).

Just as drugs were created to suppress pituitary regulation of estradiol, pegvisomant was created to disrupt GH production (Yin et al. 2007). Despite preclinical evidence demonstrating efficacy in mouse models (Divisova et al. 2006), this drug was never developed in breast cancer. Somatostatin analogs can also be used to suppress GH expression, but first-generation analogs were not successful in combination with tamoxifen (Ingle et al. 1999, Pritchard et al. 2011).

Ligand neutralization is also a possible strategy to block receptor/ligand interactions. Two drugs have been described and tested in early-stage clinical trials (Friedbichler et al. 2014, Haluska et al. 2014). Both are monoclonal antibodies with high affinity to both IGF1 and IGF2. Currently, xentuzumab is in trial in combination with the CDK4/6 inhibitor abemaciclib in endocrine-resistant breast cancer (NCT03099174).

Tyrosine kinase inhibition

It is well established that the first step in IGF1R signaling is autophosphorylation. As in the development of
HER2-targeted therapies, tyrosine kinase inhibitors were also developed. However, the high homology between IGF1R and insulin receptor (INSR) have made it impossible to make an IGF1R-specific inhibitor. While these kinase inhibitors had activity in early phase clinical trials, they were not pursued because of toxicity (Yee 2015) and in the case of linsitinib, a lack of efficacy in a randomized phase 3 trial of adrenal cortical cancer was also a major reason (Fassnacht et al. 2015).

Monoclonal antibodies

In a pathway parallel to the development of anti-HER2 therapies, multiple monoclonal antibodies were created to bind IGF1R. These drugs shared similar properties; they were either fully human or humanized, they had little binding to the INSR, they resulted in receptor downregulation and they disrupted the negative feedback pathway between GH, IGF1 and insulin. The development of these drugs has been reviewed (Iams & Lovly 2015, Ekyalongo & Yee 2017).

Early phase clinical trials

Tyrosine kinase inhibitors in breast cancer

The tyrosine kinase inhibitor linsitinib was evaluated in phase I clinical trials in two different dosing schedules: continuous and intermittent (Jones et al. 2015, Puzanov et al. 2015). Despite evidence of single agent activity in these studies and preclinical evidence the IGF1R and INSR pathways played a role in endocrine-resistant breast cancer (Fox et al. 2011); this drug was not further developed. BMS-754807 has a similar mechanism of action, but the drug sponsor discontinued a randomized phase II clinical trial comparing BMS-754807 alone or in combination with the aromatase inhibitor letrozole (NCT01225172).

Randomized clinical trials of IGF1R inhibitors in ER+ breast cancer

Given the preclinical evidence suggesting IGF1R signaling-enhanced ER function (Becker et al. 2011), many trials were conducted in patients with ER+ breast cancer. Almost all trials included women who had previously been treated with endocrine therapy; by definition, these tumors were endocrine resistant.

Ganitumab in combination with the aromatase inhibitor exemestane or the selective estrogen receptor modulator fulvestrant (Robertson et al. 2013) was reported. This trial demonstrated no evidence of benefit for the combination; if anything, the combination of ganitumab with endocrine targeting suggested inferior outcomes. Overall survival was worse in the patients who received ganitumab. The major toxicity of this combination was hyperglycemia, and this toxicity might explain the results.

A reason for these worse outcomes was suggested by Gradishar and coworkers (2016) when receptor levels were measured in the patient’s tumors. In this trial, the antibody cixutumumab was given with or without an aromatase inhibitor in patients who had already progressed through first-line endocrine therapy. A trial arm of cixutumumab alone was also studied. Similar to the trial of ganitumab, cixutumumab offered no significant benefit when added to endocrine therapy or by itself.

A novel aspect of this trial included the evaluation of tumor expression of IGF1R and INSR expression by RT-qPCR. These authors showed the level of INSR far exceeded the level of IGF1R, the intended therapeutic target. Further, the two isoforms of INSR were measured. The fetal isoform, INSR-A was expressed at higher levels than the adult isoform, INSR-B. This is a significant finding as INSR-A has been shown to have high affinity for the ligand IGF2 (Sciacca et al. 1999).

The downregulation of IGF1R was demonstrated in both preclinical and clinical data of endocrine-resistant breast cancers. We showed cells selected for resistance to tamoxifen had little IGF1R (Fagan et al. 2012). Drury and coworkers demonstrated a loss of tumor IGF1R expression after patients progressed through tamoxifen (Drury et al. 2011).

Thus, it is not surprising any drug targeting the IGF1R receptor, expressed at very low levels in these endocrine-resistant breast cancers, had any possibility of working. Further, the elevation of serum insulin has been described as a ‘class effect’ of IGF1R monoclonal antibodies (Haluska et al. 2007). This induction of hyperinsulinemia is thought to be secondary to the disruption of a negative feedback to the pituitary, which controls IGF1 levels via GH. By blocking IGF1 sensing in the brain, GH levels become elevated resulting in increased free fatty acid output from the liver resulting in hyperglycemia and compensatory hyperinsulinemia (Yee 2015). This hyperinsulinemia likely diminished any beneficial effect of IGF1R inhibition. Indeed, Gradishar and coworkers suggested this as a possibility in their trial. As shown by Gradishar and coworkers, the tumors with the top quartile of IR expression had the poorest progression-free survival (Gradishar et al. 2016). While this does not negate the possibility that anti-IGF1R strategies have
some therapeutic value, it clearly suggests tumors with high IR levels do poorly, perhaps because of the increased levels of serum insulin seen after administration of IGF1R moAbs (Haluska et al. 2007). Certainly, in most clinical trials, the reporting of grade 2 hyperglycemia by Common Terminology Criteria for Adverse Events (fasting glucose greater than 160) is an underestimation of the stability of glucose homeostasis.

One trial has been reported to overcome the issues noted earlier. The IGF1R monoclonal antibody figitumumab was tested in a randomized phase II trial in previously untreated patients with metastatic ER+ breast cancer. These patients received exemestane alone or exemestane with figitumumab. This patient population might have had higher levels of IGF1R as they had not yet been exposed to endocrine therapy. While these data were only presented in abstract form, there were potentially important observations made in this trial (Ryan et al. 2011). First, in the overall population, there was no benefit to adding figitumumab to the aromatase inhibitor exemestane. Second, a subpopulation of women with a hemoglobin A1C less than 5.7% suggested potential benefit for adding figitumumab. While this subgroup of patients was too small to draw firm statistical conclusions (hazard ratio=0.60, CI: 0.32–1.1), the data suggested women with normal glucose homeostasis prior to drug therapy had benefit from the combination. Unfortunately, this drug is no longer being pursued.

Early phase clinical trials of IGF1R inhibitors with chemotherapy in breast cancer

Compared to trials in endocrine therapy of breast cancer, there has been little evaluation of IGF1R inhibitors with cytotoxic chemotherapy. To date, only the I-SPY 2 trial has reported such a combination. The I-SPY 2 trial evaluates the combination of paclitaxel with investigational agents in the neoadjuvant therapy of breast cancer (Yee et al. 2012). Patients enrolled on this trial have high-risk tumors as measured by tumor size (>2.5 cm). If ER+, then they also must have high genomic risk scores as determined by the 70-gene assay (Cardoso et al. 2016). The primary endpoint of the trial is pathological complete response, no invasive tumor in breast or lymph nodes, after completion of chemotherapy.

In this trial, ganitumab was tested in combination with paclitaxel. Because of the known hyperglycemia induced by ganitumab, patients were also given metformin (850 mg po BID) to manage glucose. Therapy was 12 weeks in combination with paclitaxel and ganitumab, followed by 4 cycles (given every 2 or 3 weeks) of doxorubicin and cyclophosphamide. There was no benefit for adding ganitumab to conventional cytotoxic chemotherapy in this trial as pathologic complete response rates were similar in the ganitumab-treated group compared to the control group (Yee et al. 2017). However, a preliminary analysis of the data suggests metformin did not maintain glucose homeostasis. Hemoglobin A1C increased after ganitumab therapy. Thus, as seen with therapies in ER+ patients, elevation of insulin levels might be an alternative pathway to activate similar signaling pathways to those activated by the IGFs.

Is there a future for IGF1R inhibitors as cancer therapies?

The disappointing results of the first generation of IGF1R inhibitors in breast cancer, as well as in other cancers, have decreased interest in the further commercial development of this class of receptor targeting drugs. However, there were also important lessons learned from these first clinical trials that might be used for further development of inhibitors of this pathway. There are several ways in which the next generation of clinical trials targeting IGF signaling could be improved.

Predictive biomarkers for IGF1R-driven tumors should be used for future clinical trials

The biology of IGF signaling is complex; it is clear some of the functions of IGF1R activation are not easily observable in clinical trials. It is well-recognized IGF1R activation may enhance cell motility and metastasis, independent of enhancement of proliferation (Sachdev et al. 2004). The two adaptor proteins, INSR substrate (IRS) -1 and -2 function differently in breast cancer cells (Nagle et al. 2004, Dearth et al. 2006, Ma et al. 2006, Mardilovich & Shaw 2009). It is also evident that mRNA expression profiling could be used to identify IGF-driven tumors (Creighton et al. 2008, Becker et al. 2016).

Serum levels of IGF ligands were also associated with response as demonstrated in a trial using ganitumab in pancreas cancer (McCaffery et al. 2013). In this trial, patients with higher levels of IGF1 and IGF2 benefited from the addition of IGF1R inhibition to cytotoxic chemotherapy.

Thus, using an unselected group of patients to study IGF1R inhibitors will not be successful if only a subgroup of patients has ‘IGF1R-driven’ tumors. Further, identifying key downstream molecules associated with enhanced...
survival or unlimited proliferation by measuring serum ligands, expression of key adaptor proteins or expression profiles may identify the subgroup of patients who could benefit from IGF1R inhibition.

**Specific subtypes of breast cancer should be included in clinical trials**

As discussed earlier, the largest numbers of clinical trials included women with ER+ tumors that were resistant to endocrine therapy. Other preclinical data suggesting the activity of IGF1R inhibitors were most effective in ER-negative breast cancers, and HER2-negative breast cancers were largely ignored (Litzenburger et al. 2011). In this work, the tyrosine kinase inhibitor BMS-754807 was most effective in the ‘triple-negative’ breast cancer cell lines. Moreover, the IGF1R-activated gene expression signature was completely reversed by the TKI in these cell lines.

Further data in breast cancer show IGF1R signals to ATM/ATR and sensitize breast cancer cells to DNA damage caused by cisplatin (O’Flanagan et al. 2016). In triple-negative breast cancer cells, inhibition of IGF1R in combination with phosphatidyl-inositol 3-kinase inhibitors has also been demonstrated (de Lint et al. 2016).

Thus, the numerous clinical trials conducted in ER+ tumors might have been the wrong strategy. As noted below, there are additional reasons why endocrine-resistant ER+ tumors were likely the wrong subtype of breast cancer to target with IGF1R inhibitors.

**INSR is also a target in breast cancer**

As noted earlier, Gradishar and coworkers showed endocrine-resistant ER+ breast cancers had very little expression of IGF1R, yet, had much higher levels of INSR (Gradishar et al. 2016). This observation by itself might not have been a problem; however, the reflex hyperinsulinemia seen with IGF1R inhibition should have been addressed (Haluska et al. 2007). In this clinical scenario, the ‘therapeutic’ drug was harmful for two reasons. First, the actual target, IGF1R was not expressed in these tumors. Second, the disruption of glucose homeostasis could have resulted in hyperinsulinemia, stimulation of tumor INSR and enhanced proliferation, exactly the opposite of the intended therapeutic effect.

An important role for INSR in breast cancer is clear. In a mouse model of hyperinsulinemia, insulin alone is enough to drive tumor progression (Fierz et al. 2010). Further, this acceleration of breast cancer progression occurs independently of IGF1R (Gallagher et al. 2013).

While the IGF1R and INSR tyrosine kinase inhibitor BMS-53692 inhibited tumor progression in this mouse model of breast cancer (Novosyadlyy et al. 2010), the more specific inhibitor of INSR S961 was also effective (Rostoker et al. 2015). These data argue strongly for a specific role for INSR in breast cancer progression.

As mentioned, INSR exists in two isoforms A and B with the fetal A isoform as the predominant INSR species in breast cancer. Our data show endocrine-resistant tumors become more sensitive to insulin due to a relative lack of IGF1R (Fagan et al. 2012). In human tumors, IGF1R resistant to tamoxifen also has lowered levels of this receptor (Drury et al. 2011). While these authors did not study levels of INSR in these tamoxifen resistant breast cancers, there is little evidence to suggest INSR is regulated by ER in breast cancer. In contrast, the regulation of IGF1R by estradiol is well characterized in ER+ breast cancer cells (Oesterreich et al. 2001). Presumably, in tamoxifen-resistant cells, the drug continues to suppress IGF1R expression while INSR is unaffected. Inhibition of INSR results in diminished growth of tamoxifen-resistant breast cancer cells in vitro (Chan et al. 2016, 2017).

Taken together, these data suggest an important role for both IGF1R and INSR. While suppression of elevated insulin levels after IGF1R suppression by monoclonal antibodies or TKIs might be sufficient, it must be noted a significant number of women with breast cancer, have metabolic syndrome (Esposito et al. 2013, Bhandari et al. 2014) and it is possible their tumors are driven by high levels of insulin. Thus, a dual approach to IGF1R and INSR may be needed to completely suppress this pathway.

**Summary**

There was great appeal to target IGF1R signaling in breast cancer; yet, the clinical trials testing drugs designed to target this pathway were unsuccessful. Perhaps this could have been expected due to the design of the trials; no biomarkers were used to stratify patients, patients were enrolled with limited expression of the target, preclinical data outlining a potential role in multiple subtypes of breast cancer were not incorporated into clinical trial designs, and the reflex increase of serum insulin levels was not accounted for in trial design. Thus, there remains hope targeting of IGF1R could be a successful breast cancer therapy and ongoing clinical trials testing other strategies to block this pathway, primarily by neutralizing monoclonal antibodies, are ongoing. Certainly, attention to the factors outlined in this review will be needed to best optimize this strategy for women with breast cancer.
Declaration of interest
The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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