Blocking insulin-like growth factor-I receptor as a strategy for targeting cancer

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Clear links between cancer and cellular signaling triggered by the insulin-like growth factor-I (IGF-I) receptor (IGF-IR) and its cognate ligands (IGF-I and IGF-II) have been reported throughout the past two decades. Experimental results suggest that the pharmaceutical targeting of this signaling pathway could be beneficial for the treatment of cancer. Here, more recent advances towards potentially clinically viable strategies to interfere with the function of IGF-IR will be discussed.

The insulin-like growth factor-I (IGF-I) receptor (IGF-IR) is a member of the receptor tyrosine kinase family. Its molecular architecture comprises two α- and two β-subunits linked by disulfide bonds (Figure 1). Whereas the α-subunits are extracellular and responsible for ligand binding, the β-subunits span the plasma membrane and encompass an intracellular kinase domain devoted to the initiation of signal transduction cascades. The closest relative of IGF-IR is the insulin receptor (InsR), which has a high level of sequence identity, particularly within the intracellular kinase domain.

The peptides IGF-I and IGF-II are the cognate activating ligands of IGF-IR. The binding of IGF-I and IGF-II to the extracellular domain induces conformational changes that result in auto-transphosphorylation of each β-subunit at specific tyrosine residues, converting the receptor from the unphosphorylated to the activated form. Activation of the receptor triggers, through docking and phosphorylation of signal transduction molecules, the initiation of intracellular events [e.g. activation of the Ras–Raf–mitogen activated protein kinase (MAPK) and PI3K–protein kinase B (PKB) pathways] that primarily result in proliferation, transformation and inhibition of apoptosis (Figure 1) [1].

Several lines of epidemiological and mechanistic evidence link IGF-IR activation and signaling to tumor biology, implying that blocking its function in tumor cells could result in therapeutic benefits for cancer patients [1–4]. From an epidemiological perspective, upregulated levels of the receptor and its cognate ligands have been observed in a variety of solid human tumors. A broad range of experimental studies revealed that IGF-IR function is implicated in most of the hallmarks of cancer – self sufficiency in growth signals, evasion from apoptosis, tissue invasion and metastasis, as well as angiogenesis. A variety of approaches, including dominant-negative mutants, kinase defective mutants, antisense oligonucleotides, antisense expression plasmids, IGF-binding proteins, soluble forms of the receptor, antagonistic and/or neutralizing antibodies or small molecule kinase inhibitors have been used to study IGF-IR signaling by interfering with its function. These studies demonstrated that, in a variety of experimental settings, interference with IGF-IR function results in inhibition of cancer cell proliferation, survival, anchorage independent growth *in vitro*, inhibition of tumor growth and formation of metastasis *in vivo* and sensitization of cancer cells to various chemotherapeutic and radiation regimens. This

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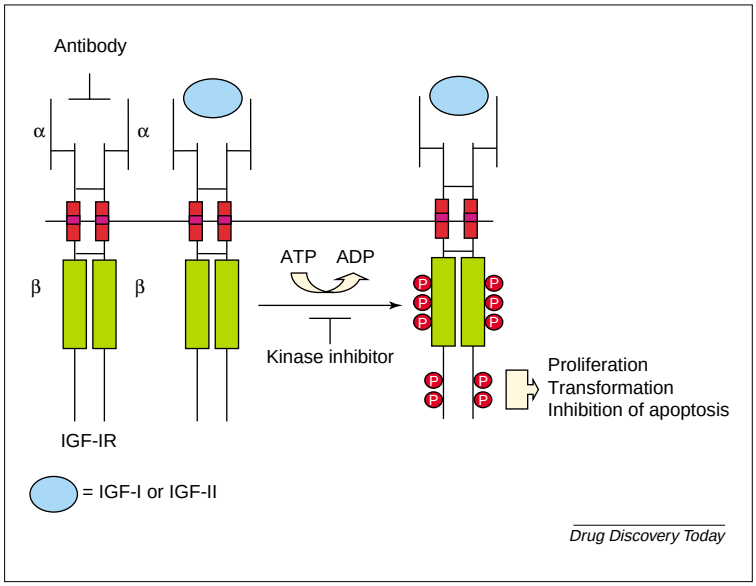

FIGURE 1

Activation of IGF-IR and approaches for therapeutic intervention. The IGF-IR is a receptor tyrosine kinase formed by two α-and two β-subunits linked by disulfide bonds. The α-subunits are extracellular and responsible for ligand binding (IGF-I or IGF-II), which trigger the activation of the intracellular kinase domain located within the transmembrane β-subunits. Cross-phosphorylation of the β-subunits triggers downstream signaling cascades, which contribute to sustain proliferation, inhibit apoptosis and elicit transformation. Antagonistic antibodies that prevent ligand binding and specific small molecule kinase inhibitors represent two potential therapeutic approaches that target IGF-IR.

extensive experimental evidence has provided preclinical proof-of-concept to pursue IGF-IR as a target for therapeutic intervention in oncology.

Among the various strategies used to interfere with IGF-IR function at the preclinical level, the antagonistic antibodies and the small molecular mass kinase inhibitors (Figure 1) represent, at this point in time, the most probable clinically viable options. Other drug discovery approaches to target IGF-IR include antisense oligonucleotides and recombinant IGF-binding proteins [2,5].

Humanized monoclonal antibodies: blocking the physical interaction of IGF-IR with its growth factors

The feasibility of inhibiting IGF-IR function with a specific antibody was first demonstrated using a mouse monoclonal antibody (α-IR-3) directed against the α-subunit of IGF-IR [6]. This antibody inhibits the binding of IGF-I to its receptor, thereby preventing downstream signaling, tumor cell proliferation *in vitro* and tumor growth *in vivo* [7]. These seminal observations were then recapitulated in a variety of studies using a broad range of experimental systems [1].

With the aim of translating this concept into clinical practice, numerous groups have recently described the identification and characterization of antagonistic and/or neutralizing humanized antibodies targeting the extracellular domain of IGF-IR (Table 1). Although generated by applying different strategies, such potential biopharmaceuticals have been shown to bind specifically to IGF-IR, thereby preventing the activation of IGF-IR-mediated signaling.

EM164

The antagonistic antibody EM164 was originally generated by immunizing mice with murine cells overexpressing human IGF-IR [8]. The antibody specifically bound to IGF-IR, displaying a dissociation constant of 0.1 nM and inhibited IGF-I-induced IGF-IR phosphorylation, but not insulin-induced InsR activation. With regard to species specificity, EM164 recognized human and monkey, but not mouse, rat, Chinese hamster or goat IGF-IR. The IGF-I-mediated survival of several cancer cell lines in the absence of serum growth factors, as well as their serum-stimulated proliferation, were inhibited by the antibody; these effects were obtained at concentrations of EM164 (60–120 nM) that resulted in inhibition of downstream signaling, monitored at the level of phosphorylated insulin receptor substrate-1 (IRS-1), phosphorylated PKB or phosphorylated p70-S6 kinase. After intravenous administration, the *in vivo* antitumor efficacy of EM164 was demonstrated, as a single agent or in combination with the ribonucleotide reductase inhibitor Gemcitabine, by treating established, subcutaneously implanted BxPC-3 human pancreatic tumor xenografts. A humanized IgG version of EM164 that retains equivalent binding affinity and inhibitory activity has been obtained for potential clinical use and is being co-developed by ImmunoGen and Sanofi-Aventis (www.immunogen.com/prod_ovr.html).

IMC-A14

Following a different approach – screening a Fab phage-display library – a fully human antibody, IMC-A14, that binds to IGF-IR with an affinity of 0.04 nM has been generated [9]. In addition to binding to the receptor and preventing the interaction with its ligands, and consequently activation of the kinase and downstream signaling, the antibody induced receptor internalization and downregulation, thereby substantially reducing the number of available IGF-IRs on the cell surface. IGF-IR downregulation and inhibition of signaling was observed in BxPC-3 pancreatic tumor xenografts 6 h after a single dose of IMC-A14. When administered three times per week intraperitoneally to mice bearing subcutaneous MCF-7 human breast cancer xenografts, IMC-A14 inhibited tumor growth in a dose-dependent manner. The subsequent histological analyses of the tumors revealed a significant decrease in staining for the proliferation marker Ki67 and a strong increase in TUNEL-positive cells, indicating inhibition in proliferation and induction of apoptosis in tumors. Similarly, inhibition of tumor growth following administration of IMC-A14 as a single agent was observed in xenograft models based on pancreatic (BxPC-3), renal (Caki-I) and colorectal (Colo205 and HT-29) cancer cell lines [10], as well as androgen-dependent and androgen-independent prostate cancer xenografts (LuCaP35 and

TABLE 1

Examples of antibodies that target the extracellular domain of IGF-1R

Antagonistic and/or neutralizing antibody	Company	Phase of development
CP-751,871	Pfizer	Phase I
EM164	ImmunoGen and Sanofi-Aventis	Preclinical
IMC-A14	ImClone	Preclinical
h7C10 (F50035)	Pierre Fabre and Merck	Preclinical
19D12	Schering-Plough	Preclinical

LuCaP35V) [11]. The potential synergistic effect of combining an antagonist IGF-IR antibody therapy with an approved treatment for colorectal cancer, such as the topoisomerase-I inhibitor Irinotecan, was demonstrated in an HT-29 xenograft model. More recently, further successful preclinical examples of the antitumor activity of IMC-A14 in combination with standards of care were reported in studies using an *in vivo* model of multiple myeloma (MM-1S), where, in addition to a direct effect on the viability of tumor cells, an antiangiogenic component was described [12]. In this multiple myeloma model, IMC-A14 demonstrated antitumor activity as a single agent or in combination with standard therapeutics, such as the alkylating agent Melphalan or the proteasome inhibitor Bortezomib; in all test cases, the antitumor activity translated into increased survival. These observations suggest that IMC-A14 can potentially be used as an efficacious combination partner of at least selected approved, standard chemotherapeutic agents.

CP-751,871

A fully human IgG2 anti-IGF-IR antibody, CP-751,871, was generated by application of the Xenomouse™ technology [13], in which the human immunoglobulin loci was introduced into the mouse genome, where the endogenous immunoglobulin locus was inactivated. This antibody specifically binds to IGF-IR with a dissociation constant of 1.5 nM, displaying species selectivity toward human and monkeys, as opposed to rat, dog, rabbit and marmoset. CP-751,871 inhibits IGF-I binding to cells in culture with an IC_{50} of 1.8 nM and IGF-I-induced receptor autophosphorylation with an IC_{50} of 0.42 nM. By binding to IGF-IR, it induces internalization of the receptor, resulting in downregulation of IGF-IR expression at the plasma membrane *in vitro* and *in vivo*. This feature is applicable as a measure of drug action (i.e. as pharmacodynamic read-out) in, for example, a surrogate tissue such as peripheral blood lymphocytes, where a dose-dependent downregulation of IGF-IR expression was observed after exposure to CP-751,871. *In vivo* antitumor efficacy of this antagonistic antibody was demonstrated as a single agent or in combination with Doxorubicin, 5-Fluorouracil or Tamoxifen in xenograft models derived from cells transformed by the overexpression of IGF-IR (3T3-IGF-IR), colorectal cancer cells (Colo205) or breast cancer cells (MCF-7). A Phase I clinical trial of CP-751,871 is in progress in patients with multiple myeloma (www.moffitt.usf.edu/about_moffitt/publications/clinical_trials_update/nbcmonths/2004s4.pd): to date, no data from this study have been reported.

h7C10 (F50035)

A recombinant humanized IgG1 anti-IGF-IR antibody, known as h7C10 or F50035, has been identified and characterized [14]. This antibody inhibited IGF-I and IGF-II binding to the receptor with IC_{50} values of 4.2 nM and 3.1 nM, respectively. Consistent with this observation, the antibody inhibited IGF-I-mediated receptor phosphorylation, downstream signaling and cellular proliferation. Moreover, exposing cells to h7C10 caused a rapid internalization and degradation of IGF-IR, thereby decreasing the number of available binding sites for the cognate activating ligands on the cell surface. This antagonistic antibody demonstrated *in vivo* antitumor efficacy as a single agent against established breast (MCF-7) and non-small cell lung cancer (A549) xenografts when administered intraperitoneally. Antitumor efficacy in the A549 model was enhanced by combining h7C10 with the tubulin depolymerizing agent Vinorelbine, resulting in a significant prolongation of survival. In the same model, improved efficacy and survival were observed following combination of h7C10 with an antibody (225Mab) targeting the epidermal growth factor receptor (EGFR). Part of the *in vivo* antitumor efficacy observed with h7C10 could potentially be ascribed to an immune response – antibody-dependent cell-mediated cytotoxicity (ADCC) – that is triggered by antibodies coating target cells, which are then rendered vulnerable to attack by immune cells. Indeed, h7C10 was found *in vitro* to target MCF-7 or A549 cancer cells to natural killer cells. h7C10 is being co-developed by Pierre Fabre and Merck (www.forbes.com/2004/08/05/0805automarketscan02_print.html).

19D12

Another fully humanized neutralizing anti-IGF-IR antibody, 19D12, is specific for IGF-IR, blocks IGF-IR autophosphorylation, as well as downstream signaling, and induces a downregulation of IGF-IR expression *in vitro* and *in vivo* [15–17]. In addition to inhibiting the *in vitro* proliferation of several cancer cell lines, this antibody proved efficacious in a variety of xenograft models, ranging from ovarian (A2780) and non-small cell lung cancer (H322) to breast (MCF-7) and colon (HT-29) cancer.

scFv-Fc-IGF-IR

Based on a previously characterized mouse monoclonal antibody (1H7) that blocks the binding of IGF-I and IGF-II to their receptor [18], a chimeric single-chain antibody, termed scFv-Fc-IGF-IR, was generated by fusing the Fc domain of human IgG1 with the Fv region of 1H7 [19]. After exposure of cells to this antibody, an immediate and

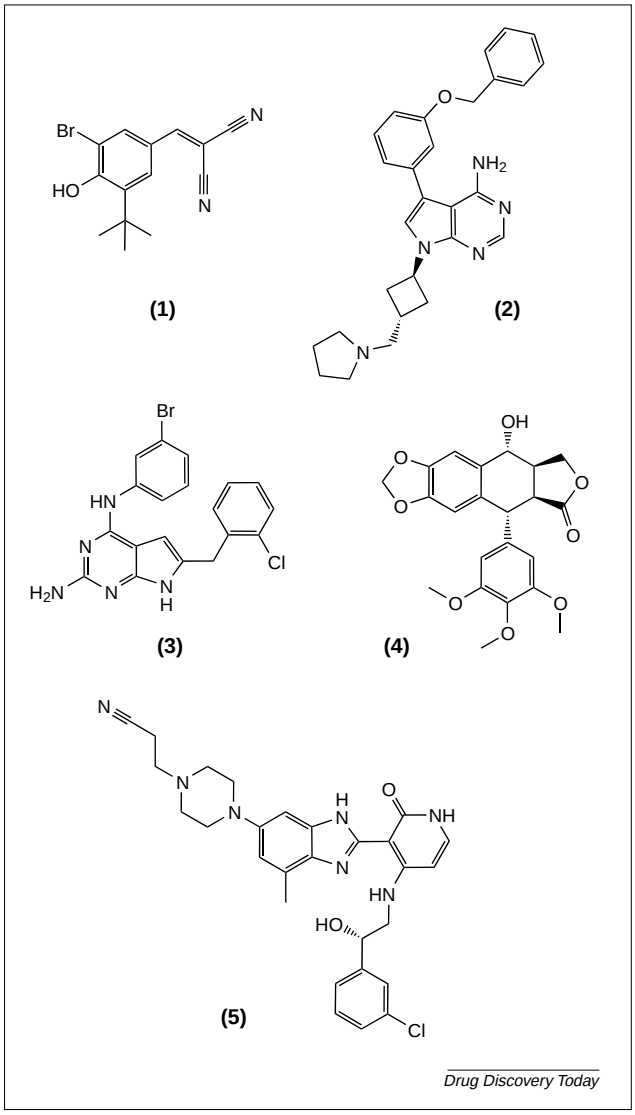

FIGURE 2

Examples of IGF-IR kinase inhibitors. (1) tyrphostin derivative; **(2)** NVP-ADW742; **(3)** 7H-pyrrolo[2,3-d]pyrimidin-2,4-diamine derivative; **(4)** cyclolignan derivative, PPP; and **(5)** BMS-554417.

transient activation of IGF-IR phosphorylation and downstream signaling was observed; despite this short-term agonistic activity, abrogation of response to IGF-I and inhibition of cellular proliferation resulted from a subsequent strong and durable downregulation of the receptor. *In vivo* antitumor efficacy was demonstrated when scFv-Fc-IGF-IR was used as a single agent against MCF-7 breast cancer xenografts and in combination with Tamoxifen using the T61 human breast cancer model [20].

Di-diabody

A novel approach using a single biopharmaceutical for targeted combination therapy was explored by attempting to generate bispecific antibodies. Experimental evidence indicates that IGF-IR and EGFR have a significant role in a variety of human cancers, thereby suggesting that a combined therapy targeting both receptors could be advantageous in treating such tumors [21]. To this end, a fully human recombinant bispecific antibody, BsAb-IGF-IR-EGFR, was constructed to combine two previously identified neutralizing antibodies targeting IGF-IR and EGFR in a single antibody [22]. This bispecific antibody bound to both targets simultaneously and blocked IGF and EGF binding to their respective receptors, resulting in inhibition of downstream signaling and cellular proliferation. The *in vivo* antitumor efficacy of a similar bispecific antibody, now referred to as Di-diabody, has been recently reported [23], indicating an intriguing new twist in exploiting the features of engineered antibodies.

Modulators of IGF-IR kinase activity: a major challenge for medicinal chemistry

Parallel to the efforts directed at blocking the physical interaction between IGF-IR and its growth factors, drug discovery activities have also been aimed at modulating IGF-IR tyrosine kinase activity by targeting its intracellular kinase domain. The identification of specific low-molecular mass kinase inhibitors of IGF-IR kinase activity has proven to be a major challenge for medicinal chemistry because of the high sequence identity at the kinase domains of IGF-IR and InsR (~84%) and, in particular, at the ATP-binding pocket (100%) [24]. Notably, the amino acids of these two kinases that line the ATP-binding cleft are strictly conserved, and only two residues (Ala85 and His87 in InsR versus Thr and Arg at the respective positions in IGF-IR), which do not have a direct interaction with ATP but are in close proximity to the binding site, are different. The root-mean square deviation for the Cα atoms of the kinase domains of IGF-IR and InsR in their X-ray crystal structures, which is only 2.5 Å, exemplifies the high structural similarity of these two proteins [25]. On the basis of these structural data, it would be reasonable to predict that the identification and development of selective IGF-IR kinase inhibitors is beyond attainment, but this assumption was proved to be wrong.

ATP antagonists

Initial attempts to inhibit IGF-IR enzymatic activity resulted in the identification of several tyrphostin-type compounds [e.g. **1** (Figure 2)] that showed weak activity in blocking IGF-IR autophosphorylation (IC_{50} of ≈7–13 μM), but some selectivity over InsR (fourfold to eightfold) [26]. Improved IGF-IR inhibitory activity and cellular selectivity over InsR have been reported recently for a new series of pyrrolo[2,3-*d*]pyrimidine derivatives. As expected from the available structural information and amino acid sequence identity, these compounds are equipotent against IGF-IR and InsR in biochemical assays (IC_{50} <200 nM), which are based on the recombinant truncated versions of the receptors encompassing the respective kinase domains, but they show selectivity for IGF-IR over InsR (10–50-fold) in cellular autophosphorylation assays (i.e. when the assay is performed with the native forms of the receptors) [27,28]. For example, NVP-ADW742 (**2**; Figure 2) inhibits

IGF-IR autophosphorylation with an IC_{50} value of 170 nM, and, under similar experimental conditions, shows 16-fold selectivity over the native InsR and only weak activity (IC_{50} >5 μM) against other receptor and cytosolic tyrosine kinases [28].

The selectivity achieved at the cellular level by these pyrrolo[2,3-*d*]pyrimidine derivatives suggests conformational differences between the native forms of IGF-IR and InsR – from the inactive to the active form – that can effectively be exploited for drug discovery. The conversion of inactive IGF-IR and InsR to their active kinase forms must require complex conformational rearrangements that cannot be fully recapitulated in biochemical assays using monomeric recombinant and fully activated kinase domains. Although the resolution of the structures of the full-length IGF-IR and InsR proteins has yet to be accomplished, the available structural information – in particular, the kinase domains of IGF-IR and InsR [25,29–32] – has provided some insight into the activation dynamics of these enzymes. Thus, the transition from the inactive form to the fully activated form requires conformational changes with a swing-out of the activation loop to open the ATP-binding cleft [30,33]. Without doubt, a better understanding of the molecular dynamics of the activation of IGF-IR and InsR could provide new avenues in the identification of inhibitors able to differentiate between these two structurally similar enzymes. In this context, it is of interest to note that the possibility to target the unphosphorylated form of IGF-IR has been explored using a continuous coupled spectrophotometric assay [34]. In this biochemical assay, production of ADP is coupled to the oxidation of NADH, and the decline of NADH absorbance at 340 nm enables the real-time monitoring of the rate of steady state ATP hydrolysis. This approach led to the identification of a group of 6–5 ring-fused compounds (e.g. **3**; Figure 2) that, as in the case of tyrphostins, showed some selectivity over InsR (>threefold) [34].

Similar to the activity profile observed with monoclonal antibodies, NVP-ADW742 and derivatives can inhibit the signal-transduction pathways that mediate the IGF-I effects *in vitro* and *in vivo* [28]. Notably, IGF-IR kinase inhibition by NVP-ADW742 results in a plethora of proapoptotic molecular events (e.g. decreased levels of caspase inhibitors) that could account for its effectiveness as a single agent and in enhancing the antitumor activity of a broad spectrum of chemotherapeutic agents [28], in the presence of which IGF-I signaling has a protective antiapoptotic effect [1].

Proof-of-concept of the potential therapeutic benefit of blocking IGF-IR kinase activity in tumor cells has been obtained in a multiple myeloma orthotopic model [28]. In this mouse model, multiple myeloma lesions are established after intravenous injection of luciferase-expressing human MM-1S myeloma cells: tumor burden and its response to therapy are quantified by whole-body bioluminescence imaging. The observed anatomic distribution of bone injuries (e.g. spine, skull and lower extremities) is consistent with the presentation of disease in human multiple myeloma patients. When used alone or in combination with cytotoxic agents (e.g. Melphalan), NVP-ADW742 suppresses tumor growth and prolongs survival of mice without significant toxicity. This preclinical finding and additional studies [35,36] support the potential application of IGF-IR kinase inhibitors in combination with established antitumor modalities for the treatment of IGF-responsive neoplasias or other IGF-I-dependent diseases (e.g. retinopathy of prematurity) [37–41].

More recently, two small molecules, BMS-536924 and BMS-554417 (**5**; Figure 2), almost equipotently inhibit IGF-IR, InsR and fokal adhesion kinase [42,43]. Both compounds showed antitumor activity *in vivo* in, for example, an IGF-IR-driven mechanistic model (salivary gland xenograft); however, at efficacious doses, and in keeping with its lack of selectivity for InsR, a tenfold increase in insulin level was observed during an oral glucose tolerance test.

Non-ATP antagonists

Another intriguing and selective inhibitor of IGF-IR kinase activity is a cyclolignan derivative picropodophyllin [PPP (**4**; Figure 2)], which was originally identified at the Karolinska Cancer Institute (Sweden) and is currently being developed by Biovitrum. PPP potently inhibited IGF-IR autophosphorylation (IC_{50} of 0.04 μM) in intact cells and was selective against a panel of other receptor tyrosine kinases, including InsR [44]. ATP-kinetic studies indicated that the compound did not interfere with IGF-IR tyrosine kinase activity by binding to the ATP-binding site (i.e. as an ATP competitive inhibitor), suggesting an alternative mechanism of action. Additional efforts to elucidate its kinase inhibitory mechanism showed that PPP interferes with the phosphorylation of Tyr1136 in the activation loop of the kinase, while sparing the other two tyrosines (Tyr1131 and Tyr1135) [45]. As has been shown by X-ray crystallography, P-Tyr1136 stabilizes the conformation of the activation loop, whereas P-Tyr1131 and P-Tyr1135 destabilize the autoinhibitory conformation of the activation loop. Without structural information it is difficult to understand how PPP preferentially blocks Tyr1136 phosphorylation, but this effect has an interesting cellular outcome – PPP treatment of IGF-IR overexpressing cells results in the preferential inhibition of the PI3K/PKB pathway, as opposed to the MAPK pathway [45]. The consequences of the unusual mechanism of PPP on its antitumor activity and/or toxicity have yet to be elucidated, but, independently of this, the identification of PPP and the characterization of its mechanism of action confirm that other therapeutic approaches besides ATP mimetics can be exploited for this challenging kinase.

INSM18 (structure and biological activity not disclosed) was reported by Insmed and appears to be the first IGF-IR kinase inhibitor to have entered Phase I clinical trials. In preclinical studies, this compound, which is also active

TABLE 2

Examples of small molecule inhibitors that target IGF-1R kinase

Small molecule kinase inhibitor	Company	Phase of development
INSM18	Insmed	Phase I
PPP	Karolinska Cancer Institute and Biovitrum	Preclinical
NVP-ADW742	Novartis Pharma	Preclinical
NVP-AEW541	Novartis Pharma	Preclinical
BMS-536924	Bristol-Myers Squibb	Preclinical
BMS-554417	Bristol-Myers Squibb	Preclinical

against the human HER2 receptor, has demonstrated antitumor activity in breast, lung, pancreatic and prostate cancers. In late 2004, Insmed announced that it will initiate a dose-escalating clinical study designed to define the maximum tolerated dose of INSM18 in patients with relapsed prostate cancer (www.drugresearcher.com/news/news-ng.asp?n=55546-insmed-trials-encourage): at this time, no data from this study have been disclosed.

In addition to the preceding IGF-IR kinase inhibitors (Table 2), several patent specifications have been published describing receptor tyrosine kinase inhibitors, including compounds that are claimed to be active against IGF-IR. Although, in general, no kinase selectivity data are provided in these patents, the diverse chemical scaffolds covered in these specifications could represent promising new chemotypes for the future generation of IGF-IR kinase-selective inhibitors.

Conclusions and outlook

In the past few years, drug discovery activities have successfully identified biopharmaceuticals and low-molecular mass kinase inhibitors able to modulate IGF-IR activity in ways not previously possible. These IGF-IR modulators have provided proof-of-concept in preclinical cellular and animal settings. As these agents progress through the clinic, the therapeutic effectiveness and relative advantages and disadvantages of the different strategies will become apparent. The two approaches bear differences in terms of route of administration (intravenous for antibodies versus oral for most small molecule kinase inhibitors) and mode of action (blocking access to the activating ligand and downregulating receptor expression at the plasma membrane for most antibodies versus inhibiting the receptor kinase activity for small molecules). These peculiar features could result in different exposures, kinetics and duration of action, which, in turn, might positively or negatively influence their efficacy and toxicity profiles. Of special concern for these anti-IGF-IR therapies is their potential effect on InsR signaling. Anti-IGF-IR agents might co-target or alter InsR function and cause insulin resistance, but this could potentially transpire to be an advantage because specific inhibition of InsR or hybrid receptors in tumor or host cells [46–48] could be required for effective antitumor therapy. Another major issue in the development of these targeted agents will be the design of their clinical trials. Although a substantial number of epidemiological and experimental studies support an important role for IGF-IR in the biology of human cancers, the generic complexity and heterogeneity of tumors might limit the probability of positive responses to an anti-IGF-IR agent when used alone. Certainly, patient stratification and the selection of combination regimes will be major challenges for the clinical evaluation of anti-IGF-IR agents. Hopefully, these hurdles and challenges will be successfully overcome in the near future.

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