REVIEW ARTICLE

Drugging IGF-1R in cancer: New insights and emerging opportunities

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Abstract   The insulin-like growth factor (IGF) axis plays important roles in cancer development and metastasis. The type 1 IGF receptor (IGF-1R) is a key member in the IGF axis and has long been recognized for its oncogenic role in multiple cancer lineages. Here we review the occurrence of IGF-1R aberrations and activation mechanisms in cancers, which justify the development of anti-IGF-1R therapies. We describe the therapeutic agents available for IGF-1R inhibition, with focuses on the recent or ongoing pre-clinical and clinical studies. These include antisense oligonucleotide, tyrosine kinase inhibitors and monoclonal antibodies which may be conjugated with cytotoxic drug. Remarkably, simultaneous targeting of IGF-1R and several other oncogenic vulnerabilities has shown early promise, highlighting the potential benefits of combination therapy. Further, we discuss the challenges in targeting IGF-1R so far and new concepts to improve therapeutic efficacy such as blockage of the nuclear translocation of IGF-1R.
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Introduction

Targeted cancer therapies are designed to harness the specific molecular abnormalities that drive tumorigenesis. Because of the specificity, targeted therapies are expected to achieve higher efficacy with lower toxicity compared with the traditional approaches such as chemotherapy. Next generation sequencing has facilitated the discovery of genomic abnormalities that underlie the development and progression of cancer.[1] These abnormalities represent potential therapeutic targets.

Gene amplification of *IGF1R*, which encodes insulin-like growth factor 1 receptor, is a frequent event across cancer types (Fig. 1A). IGF-1R is a key member of the insulin-like growth factor (IGF) axis, which involves in cancer development, metastasis and cancer therapeutic

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resistance.[2—4] The IGF axis consists of several ligands (insulin, IGF-1, IGF-2) and cell surface receptors, including IGF-1R, IGF-2 receptor (IGF-2R) and insulin receptor (InsR).[5] Additionally, the levels of circulating IGF ligands are regulated by seven soluble IGF binding proteins (IGFBP1—7) and IGFBP proteases.[5,6] IGFBPs act as the serum reservoirs of IGF-1 and IGF-2 by regulating their stability and their release at target tissues, thereby modulating the bioavailability of these two ligands to the receptors temporally and spatially.[7—9] Among the molecules of the IGF axis, IGF-1R is recognized as a promising target of cancer therapy because of (i) its frequent amplification and overexpression in cancers[2]; (ii) its established functions in transmitting tumorigenic signals[10,11]; and (iii) its role as the primary receptor of the IGFs.[5,8]

The mature IGF-1R is a heterotetramer comprised of two extracellular ligand binding α subunits and two transmembrane β subunits that contain the intracellular kinase domain.[12] Upon ligand binding, the tyrosine residues in the intracellular kinase domain of IGF-1R are autophosphorylated. This activation leads to the binding of the phosphorylated IGF-1R to docking proteins, including insulin receptor substrates 1 to 4 (IRS-1 to IRS-4) as well as Src homolog and collagen domain protein (Shc).[13] Recruitment of these docking proteins not only promotes the membrane retention of IGF-1R, but also mediates the activation of downstream signaling pathways of the IGF axis, which are primarily the phosphatidylinositol-3-kinase (PI3K)/Akt/mTOR and RAS/RAF/mitogen-activated protein kinase (MAPK) pathways.[14] In addition, IGF-1R is highly homologous to InsR (60% overall sequence homology and 80% homology in their kinase domains). IGF-1R and InsR (InsR-A or InsR-B) can heterodimerize to form IGF-1R/InsR hybrid,[15] potentially resulting in crosstalk between IGF-1R and insulin signaling which may have impacts on glucose uptake and other cellular functions.[16] Here, we review the recent pre-clinical and clinical evidence demonstrating the therapeutic potential of targeting IGF-1R in cancers. We also discuss the challenges and the emerging opportunities to inhibit IGF-1R in combinational therapies that can overcome therapeutic resistance and maximize the efficacy of cancer treatment.

Overexpression and activation of IGF-1R in human cancers

Mechanistically, activated IGF-1R promotes an array of cancer phenotypes such as growth and metastasis.[17,18] The activity of IGF-1R can be regulated through multiple mechanisms described below.

(i) IGF-1R overexpression caused by *IGF1R* gene aberrations

Overexpression of IGF-1R was reported in multiple human cancer lineages and was associated with poor prognosis of cancer patients.[19—21] In prostate cancer ($n = 136$), protein levels of IGF-1R were significantly higher in malignant epithelia compared with benign glands from the same biopsies.[19] In a head and neck squamous cell cancer cohort

($n = 346$), patients with high IGF-1R levels had significantly reduced overall survival (OS) and disease-specific survival.[20] At least 50% of breast cancer cases were found to express activated IGF-1R.[21] These studies showed strong IGF-1R staining in the membrane and/or cytoplasm and nucleus. Cytoplasmic localization of IGF-1R is thought to be an indicator of receptor activation which subsequently leads to receptor internalization, whereas nuclear IGF-1R has also been reported to transmit tumorigenic signal.[22] Interestingly, a breast cancer study ($n = 50$) revealed that estrogen receptor (ER)-positive breast cancer was significantly correlated with membranous and mixed IGF-1R expression whereas HER2-positive cases showed less membranous staining but mostly cytoplasmic and mixed patterns.[23] While these patterns have to be validated in larger independent cohorts, crosstalk between IGF-1R and ER or HER2 to stimulate oncogenic signal has been reported.[17,24,25]

Notably, alterations in *IGF1R* gene copy number have been found in 384 out of 10,953 (3.5%) pan-cancer patient samples from The Cancer Genome Atlas (TCGA). A positive correlation between *IGF1R* copy numbers and its mRNA levels could be observed (Fig. 1B). Single nucleotides mutations in *IGF1R* are also common. The majority of these mutations remain uncharacterized, therefore their functional significance and therapeutic implications are largely uncertain. Correlation analyses using cancer samples of the breast, colon, pancreas and skin have demonstrated significantly positive association between single nucleotide polymorphism (SNP) of *IGF1R* and an increased cancer risk.[26—29] Interestingly, there are also SNPs which were found to associate with protective effect on breast cancer or melanoma.[29,30] The molecular consequences driven by all these reported SNPs of *IGF1R* remain elusive. It would be interesting to examine the expression levels of IGF-1R in the presence of these *IGF1R* SNPs.

(ii) Regulation of IGF-1R expression by modifier genes

Intriguingly, IGF-1R protein may act as an auto-activator by translocating to nucleus. Nuclear IGF-1R binds directly to its own promoter to upregulate its expression level.[31] In addition, increase of IGF-1R expression in cancer cells can be attributed to deregulation of modifier genes.[32—36] Examples of tumor suppressors that regulate *IGF1R* promoter are *VHL*, *TP53* and *BRCA1*,[32,37,38] whereas oncogenes that promotes *IGF1R* transcription include *MYC* and *EWS-WT1* gene fusion.[35,36] Lastly, the epidermal growth factor receptor (EGFR) may stabilize the IGF-1R protein by preventing IGF-1R proteasomal degradation through a mechanism that remains to be unraveled.[39]

(iii) Increased levels of ligands

Apart from upregulation of IGF-1R expression, increased levels of its ligands are expected to activate the signaling axis. Accordingly, high circulating IGF-1 level has been found to correlate with increased risk of developing cancers including that of breast, ovary and prostate.[40—42] Recently, an interesting study has demonstrated that IGF-2 secreted by cancer-associated fibroblasts in the stroma induced paracrine IGF1-R/InsR axis and downstream PI3K/Akt
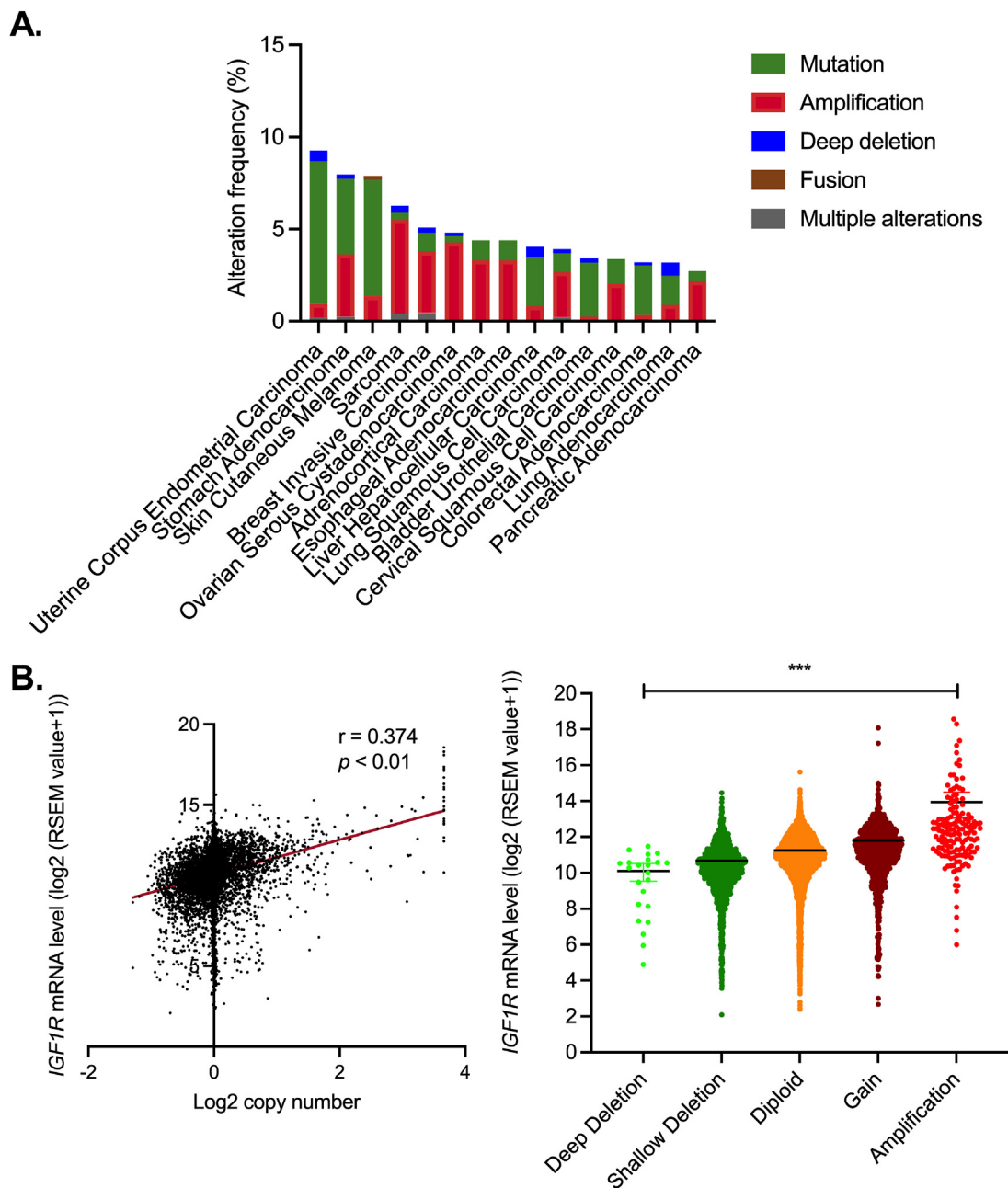
Figure 1 Amplification of *IGF1R* copy number is frequent in several common types of human cancer. **(A)** The top 15 most frequent cancer types that have been reported to have *IGF1R* mutations or copy number variations (TCGA; *n* = 10,953). **(B)** Correlation of *IGF1R* copy number and mRNA levels in TCGA cancer patients (*n* = 9,889).

pathway of colon cancer cells.[43] The findings provide evidence that IGF-1R signaling can be regulated by extrinsic modulators in the tumor microenvironment.[43]

(iv) Transactivation by other membrane receptors

There is accumulating evidence that IGF-1R can be activated by other membrane receptors, in particular integrins and G-protein-coupled receptor (GPCR). Integrins, which are heterodimers formed by α and β chains, mediate

cell–cell and cell-extracellular matrix (ECM) interactions upon stimulation by ECM ligands or cell surface ligands.[44] Integrins αvβ3 and α6β4 have been shown to interact with IGF-1 and IGF-2 [45-47]. Integrin-binding defective IGF-1 or IGF-2 mutants could not activate IGF-1R.[45–47] This indicates that apart from binding to IGF-1R, direct binding of IGF-1/-2 to integrins is crucial to trigger IGF-1R signaling. Intriguingly, IGF-1 induces the formation of integrin/IGF-1/IGF-1R protein complex. All these observations together put forward a hypothetical model in which

integrins (at least αvβ3 and α6β4) are common co-receptors for IGF-1 and IGF-2 signaling.

GABA$_B$ receptor (a GPCR activated by the neurotransmitter γ-aminobutyric acid) has been demonstrated to activate IGF-1R.[48] Mechanistically, GABA$_B$ receptor and IGF-1R form a signaling complex mediated by focal adhesion kinase (FAK). The heterotrimeric G proteins are composed of three subunits: α (4 subfamilies: Gi/o, Gs, Gq/11, or G12/13), β, and γ (these two subunits bind to form Gβγ).[49] The involvement of the different subunits in IGF-1R activation has been investigated.[48,50,51] Inhibition of Gi/o by pertussis toxin prevented IGF-1R transactivation and recruitment of FAK to the complex, indicating the critical role of Gi/o.[48] While the Gi/o heterotrimer is constitutively associated with IGF-IR, ligand stimulation by IGF-I induces the release of Gβγ subunits from the IGF-IR complex.[50] Another study showed that the Gβγ subunit mediates the activation of MAPK by IGF-1 because the activation was impaired by Gβγ subunit antagonist.[52]

Therapeutic strategies for targeting IGF-1R

(1) Downregulation of IGF-1R expression by antisense oligonucleotide (AS-ODN) and small interfering RNA (siRNA)

Oligonucleotide therapy has been a subject of intensive research for novel cancer therapeutic modality. The primary effect of this strategy is depletion of the target gene which is theoretically an oncogene. Apart from this primary effect, much evaluations are directed to AS-ODN with immunostimulatory activity. Cancer immunotherapy has emerged as a promising cancer therapeutics. Autologous cell vaccine, which is a type of personalized immunotherapy, capitalizes on the patient's own tumor cells to induce cytotoxic T-cell response against endogenous tumor antigens and thereby tumor cell death. Multiple *in vitro* and *in vivo* studies using breast cancer and glioblastoma multiforme (GBM) models have provided evidence of the ability of AS-ODN targeting *IGF1R* to induce anti-tumor immune responses.[53,54] The immune stimulation effect of AS-ODN is attributed to the presence of unmethylated CpG motif within the sequence, which is recognized by Toll-like receptor 9 (TLR9) as pathogen-associated molecule. This reaction stimulates a cascade of innate and adaptive immune responses. In this regard, inhibition of IGF-1R signaling in breast cancer cells stimulated the secretion of pro-inflammatory cytokines (TNF-α and IFN-γ)[55] and was dependent on CD8$^+$ cell-mediated cytotoxicity via activation of the Fas/Fas ligand (FasL) death pathway.[53] In GBM, B cells and tumor-specific antibody production are thought to be the primary contribution to the immunity.[54]

Indeed, *IGF1R*/AS-ODN has a long history. Back in 2001, a pilot clinical study demonstrated encouraging therapeutic effects of *IGF1R*/AS-ODN in glioma patients (*n* = 12).[56] Sustained radiographic improvement was observed in 8 patients, who displayed tumor regression after treatment. In this study, an 18-mer phosphorothioate oligode-oxynucleotide (5′-GGACCCTCCTCCG-GAGCC-3′) which started 6 nucleotides downstream from the start codon was used. A recent study published in 2021

reported immune activation with enhanced overall survival of GBM patients treated with IGV-001, which is an autologous cell vaccine with autologous tumor cells integrated with an *IGF1R*/AS-ODN named IMV-001.[57] IMV-001 is an optimized phosphorothioate-linked AS-ODN complementary to the codons 2 to 7 of *IGF1R* mRNA (Fig. 2) with immunostimulatory properties to stimulate antigen presentation.[54] The IGV-001 was encapsulated in an bio-diffusion chamber which was then implanted to the abdomen of patients,[56] thereby inducing antigenicity of malignant cells and the innate immune response of patients to further promote immunogenic cell death.[54,58] In this phase Ib clinical trial (*n* = 33), the median progression-free survival (PFS) of patients receiving IGV-001 was significantly longer than that of patients receiving standard of care (radiation and temozolomide) (9.8 months *vs.* 6.5 months; *P* = 0.0003).[57]

Silencing *IGF1R* using siRNA is another molecular therapeutic strategy to inhibit gene expression. Short RNA duplexes of 20–25 base pairs bind to *IGF1R* mRNA specifically and induce mRNA degradation, thereby reducing the abundance of IGF-1R protein. To increase the stability of siRNA upon *in vivo* delivery, the siRNA was chemically modified by replacing the alternate 2′-hydroxy groups of the ribose sugar ring with 2′-O-Me.[59] Xenograft models of renal cancer cells showed induction of apoptosis and reduced cell survival upon *IGF1R* depletion.[59] Another *in vivo* study using murine breast cancer cell line supported the anit-tumor effect of *IGF1R* siRNA.[55] Interestingly, the suppression of tumor growth was accompanied with an induction of antitumor immune response and increased secretion of proinflammatory cytokines.[55] A new nanocarrier for *IGF1R* siRNA has recently been reported.[60] This lipid-polymer hybrid nanoparticles (LPHNP) using dimethyldioctadecylammonium bromide-methoxy poly(ethylene glycol)-poly (ε-caprolactone) (DDAB-mPEG-PCL) copolymer is suggested to be more stable, more biocompatible and less toxic with better cellular uptake. So far, there has been no siRNA clinical trials designed for *IGF1R*.

(2) Disrupting the binding between IGF-1R and its ligands

Inhibiting the binding of IGF ligands to the receptor can inactivate IGF-1R. This therapeutic strategy of IGF-1R inhibition has been reviewed previously.[61,62] We focus here on the monoclonal antibodies that entered late-stage clinical studies.

Monoclonal antibodies targeting IGF-1R

Anti-IGF-1R monoclonal antibody is among the first major therapeutic agents for IGF-1R inhibition. These antibodies inhibit the binding between IGF-1R and its ligands, thereby blocking the activation of IGF-1R homodimer and IGF-1R/InsR hybrid, but not the InsR homodimer.[63–65] A number of fully human monoclonal antibodies, which target the α subunit of IGF-1R, have demonstrated anti-tumor activities in pre-clinical studies. After completion of early phase clinical trials, three of the IGF-1R antibodies (figitumumab, ganitumab, and dalotuzumab) were tested in phase III clinical trials.[66–68]

5' – T*C*C*T*C*C*G*G*A*G*C*C*A*G*A*C*T*T – 3'

* **Phosphorothioate linkage**

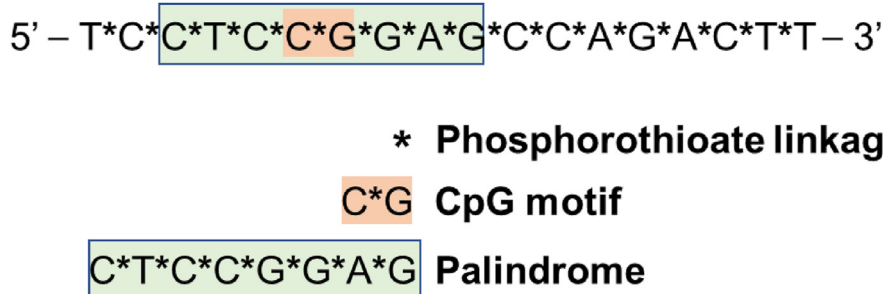C*G **CpG motif**

C*T*C*C*G*G*A*G **Palindrome**

Figure 2 The sequence and the corresponding immunostimulatory functions of motifs within IMV-001, which is complementary to *IGF1R*. The sequence contains a CpG motif and a palindromic sequence, which stimulate innate immune responses upon recognized by toll-like receptor 9. Phosphorothioate modification promotes nuclease resistance and binding of AS-ODN to plasma proteins, thereby decreasing renal loss and enhancing tissue delivery. The modification may also induce immune activities.

In a trial with advanced non-small-cell lung cancer (NSCLC) patients ($n = 671$), combination of figitumumab with chemotherapy did not improve PFS nor OS (4.7 months with chemotherapy plus figitumumab *vs.* 4.6 months with chemotherapy alone for median PFS; 8.6 months *vs.* 9.8 months for OS).[67] Adverse events including pneumonia, dehydration, asthenia and hyperglycemia were observed in a number of patients.[67] Similarly, the other antibody dalotuzumab did not show therapeutic benefit in a randomized trial involving *KRAS* wild-type metastatic colon cancer patients ($n = 344$).[68] The median PFS was 3.9 months and 5.4 months in patients provided with dalotuzumab weekly or once every two weeks respectively, whereas the PFS of placebo-treated patients was 5.6 months. Due to the lack of clear clinical benefit in these phase III trials, further clinical research on figitumumab and dalotuzumab is not being pursued.

The low efficacy of these IGF-1R antibodies might be explained by two reasons. First, although the antibodies are thought to prevent the ligand—receptor interaction and therefore IGF-1R kinase activity, it has been suggested that the IGF-1R-targeting antibody can act as "biased" IGF-1R agonist to promote IGF-1R/β-arrestin1 association.[69] This association results in ERK1/2 signaling pathway activation, despite the depletion of IGF-1R kinase activity. Whether this "biased" agonism also occurs via the IGF-1R/InsR hybrid receptor is unclear. In this regard, *in vitro* analysis has demonstrated that figitumumab can also recognize and downregulate the hybrid receptor.[70] Second, pro-tumorigenic activities of nuclear IGF-1R has been documented.[71] This nuclear pool of IGF-1R cannot be neutralized by the antibodies.

Although it appears that dalotuzumab might not be effective in these patients, exploratory biomarker analyses in the study revealed an intriguing observation that *IGF1* mRNA level could be a predictive marker of response. Among patients administered with weekly dalotuzumab, those tumors with high *IGF1* mRNA had better PFS (5.6 *vs.* 3.6 months, $HR = 0.59$, 95% $CI = 0.28$ to 1.23, $P = 0.16$) and OS (17.9 *vs.* 9.4 months, $HR = 0.67$, 95% $CI = 0.31$ to 1.45, $P = 0.31$) compared with those having high *IGF1* mRNA tumors in placebo group.[68] In contrast, high *IGF1* mRNA level predicted significantly lower response rate (17.6% *vs.* 37.3%, $P = 0.04$), shorter PFS (3.6 *vs.* 6.6 months, $HR = 2.15$, 95% $CI = 1.15$ to 4.02, $P = 0.02$), and shorter OS (9.4 *vs.* 15.5 months, $HR = 2.42$, 95% $CI = 1.21$ to 4.82, $P = 0.01$) than low *IGF1* mRNA level in placebo group.

These together highlight the potential of the ligand expression level as stratifying marker to increase response rate. In addition to *IGF1* level, inspired by another study which showed a partial response (PR) to dalotuzumab of a patient with Ewing's sarcoma,[72] the *EWS/FLI1* fusion may represent another determinant of response. The *EWS/FLI1* fusion results from t(11; 22) (q24; q12) translocation and can be detected in ~90% of Ewing's sarcoma cases. The fusion protein is a transcription factor that drives the development of the disease. Because IGF-IR contributes to the transformation capacity of EWS/FLI1,[36,73] the anti-tumor effect of dalotuzumab in Ewing's sarcoma patient is most likely due to a direct functional interference of the fusion driver by blocking the interaction between IGF-1R and EWS/FLI1.

At present, ganitumab is the only IGF-1R monoclonal antibody with ongoing clinical trials. Ganitumab demonstrated notable clinical activity with acceptable toxicity in several phase I or II clinical trials of different cancer types.[74,75] Antitumor activity upon receipt of ganitumab was observed in patients with advanced solid malignancies or non-Hodgkin's lymphoma ($n = 53$). Two patients with Ewing/primitive neuroectodermal tumors (PNET) exhibited durable complete responses (CR) and unconfirmed PR respectively, whereas two patients with neuroendocrine tumors experienced PR or a minor response.[76] Notably, the patient with Ewing/PNET, who experienced a CR, remained disease free after 28 months of treatment.[76] In 2017, FDA granted an orphan drug designation to ganitumab for the treatment of patients with Ewing sarcoma.

There have been attempts to combine ganitumab with other therapies. A combination of ganitumab and a chemotherapeutic agent gemcitabine improved OS in patients with metastatic pancreatic cancer compared with that of placebo arm in a phase II study (8.7 months *vs.* 5.9 months; $n = 125$).[77] However, a subsequent phase III study demonstrated that such drug combination did not improve OS versus gemcitabine alone in an independent metastatic pancreatic cancer cohort ($n = 800$).[66] Apart from the difference in therapeutic outcomes, the results of biomarker assessment are different in these two studies. While analysis of the phase II trial data revealed that higher circulating levels of IGF-1, IGF-2, or IGFBP-3 associated with better response to the drug combination,[78] stratifying the patients in the phase III trial based on the levels of these IGFs did not result in a treatment effect on survival by

ganitumab.[66] It is possible that dichotomization simply by median level is not robust. Two rational clinical trials involving ganitumab are on-going. These include a phase II study on the combination of ganitumab and palbociclib (small molecular cyclin-dependent kinase) in patients with Ewing's sarcoma (NCT04129151). A selection criterion is the presence of gene translocation involving *EWSR1* or *FUS* with *ETS* family in the tumor. Another phase I/II trial (NCT03041701) will study the outcome of ganitumab and dasatinib (Src family kinase inhibitor) in patients with embryonal and alveolar rhabdomyosarcoma which has been suggested to be driven by Src.[79,80]

Bispecific antibodies can simultaneously bind two separate antigens and thus carry dual-specificity. A phase II study in which metastatic pancreatic cancer patients were treated with istiratumab (MM-141), which is a fully human tetravalent bispecific antibody that binds to and co-inhibits IGF-1R and HER3, has released its results in 2020.[81] Although istiratumab was shown to be effective in preclinical studies with ovarian cancer or pancreatic cancer cells,[82,83] the clinical trial demonstrated a lack of benefit of istiratumab (including patients with high levels of IGF-1 and the HER3 ligand heregulin).[81]

IGF neutralizing antibodies

Several other therapeutic agents were designed to disrupt the binding between the ligands and IGF-1R. These include IGF neutralizing antibodies, IGF ligand TRAPs which are engineered molecules that bind to the ligands at high affinity, recombinant IGFBPs which represent naturally occurring IGF inhibitors, and inhibitors of pregnancy-associated plasma protein-A (PAPP-A) which is a metalloprotease that enhances IGF bioactivity by proteolytic cleavage of IGFBPs. Among these strategies, IGF ligand TRAPs, recombinant IGFBPs and PAPP-A inhibitors were shown to be effective in inhibiting the IGF-1R axis in preclinical studies. While further investigations are warranted to examine the clinical effects of these agents, two IGF neutralizing antibodies (dusigitumab and xentuzumab) had entered clinical trials. In contrast to dusigitumab which did not show prominent activity on PFS in a phase Ib/II trial for patients with hormone receptor-positive breast cancer (NCT01446159), xentuzumab showed anti-tumor activity in a phase I study in which 2 patients with advanced solid cancer had partial responses.[84] Xentuzumab is an IgG1 monoclonal antibody that has high affinity to IGF-1 and IGF-2, thereby outcompeting the binding between these ligands and IGF-1R or InsR-A.[84,85] Because xentuzumab does not interfere with the metabolic effects of InsR-B or insulin, the reported incidence of hyperglycaemia with xentuzumab was low.

IGF-1R decoy receptor

Soluble IGF-1R truncated mutants that correspond to ligand-binding portion of the α subunits (aa 1−524; dnIGF1Rα) and the α subunit-interacting portion of the β subunits (aa 741−936; dnIGF1Rβ) have been developed.[86] These decoy receptors effectively blocked IGF-1R activity in a dominant-negative inhibition manner

and suppressed tumorigenicity of osteosarcoma cells *in vitro* and *in vivo*.[86] These mutants, particularly dnIGF1Rβ, may allow for more specific interactions with the IGF ligands and cause less interference with insulin signaling.

Anti-idiotypic antibody

Anti-idiotypic antibody may represent an alternative immunotherapeutic treatment strategy. Anti-idiotypic antibody directed against another anti-IGF-1 antibody functionally mimic the 3D structure of the original IGF-1 antigen. An IGF-1 anti-idiotypic monoclonal antibody (named B003-2A) that can specifically bind to IGF-1R has been identified.[87] B003-2A mimics an epitope on IGF-1 and competes with IGF-1 for receptor binding. IGF-1R-mediated signaling was inhibited by B003-2A, accompanied by downregulation of IGF-1R protein and ovarian tumor growth inhibition in xenograft.[87]

(3) Tyrosine Kinase Inhibitors (TKIs)

Several small molecule TKIs have been developed for inactivating IGF-1R. These TKIs are categorized according to their ATP or non-ATP competitive activities. IGF-1R and InsR are 80% identical in the kinase domains. The ATP-binding domains in the two receptors are 100% identical. Given the roles of InsR in glucose metabolism, the majority of the ATP-competitive IGF-1R TKIs have been reported to cause hyperinsulinemia and hyperglycemia due to the simultaneous inhibition of InsR.[88,89] Therefore, it is reasonable to deduce that non-ATP competitive IGF-1R TKIs may result in less toxicities associated with disrupting glucose homeostasis.

Linsitinib (OSI-906) is an ATP-competitive TKI against both IGF-1R and InsR. Preclinical study has reported the synergistic anti-tumor effect of linsitinib in combination with two validated approaches to treat multiple myeloma (proteasome inhibitors bortezomib and dexamethasone).[90] Building on the preclinical data, a phase I trial was initiated to assess the effect of this drug combination in patients with multiple myeloma ($n = 19$).[91] Because of the predicted disturbance of glucose metabolism by this dual IGF-1R/InsR TKI, patients were required to have good glycemic control during the course of the study. This study showed that the drug combination was safe and well tolerated. Hyperglycemia was detected in only 11% (2/19) patients, at grades 1 and 3 severity respectively. The drug combination did not significantly improve overall responses when compared with historical controls of bortezomib and dexamethasone alone. However, 4 of 5 bortezomib-refractory patients showed PR or stable disease, suggesting that a subset of patients may be responsive to such combinatorial regimen.

AXL-1717 is a non-ATP competitive IGF-1R TKI. It demonstrated promising anti-tumor efficacy as a single agent and acceptable safety profile in two phase I trials, including 15 heavily pretreated NSCLC patients[92] and patients with recurrent or progressive malignant astrocytomas ($n = 9$).[93] AXL-1717 has been granted orphan drug designation for the treatment of patients with relapsed malignant astrocytomas. The optimal dose, tolerability and

efficacy of AXL-1717 in treating recurrent malignant astrocytomas will be further examined in a phase II clinical trial (NCT01721577).

NT157 is a non-ATP competitive IGF1R-IRS1/2 inhibitor that has been shown to effectively target chronic myeloid leukemia (CML) cells and to overcome BCR-ABL1 TKI resistance. Imatinib is a frontline TKI in CML treatment. Common mechanism of imatinib resistance includes T315I mutation in the kinase domain of BCR-ABL1. NT157 allosterically binds to IRS1/2 which are the common cytoplasmic effectors of IGF-1R and BCR-ABL1, thereby inhibiting these two signaling axes.[94] In preclinical models of CML, NT157 caused apoptosis independent of the mutation status of *BCR-ABL1*, indicating the potential of NT157 in overcoming TKI resistance driven by T315I mutation.[94] Of note, the combination of NT157 and imatinib produced an additive anti-tumor effect.[94]

(4) Cytotoxic drug conjugate

Antibody—drug conjugate (ADC) represents another major focus in oncology therapeutics. About 80 ADCs are currently being tested in clinical trials and 4 ADCs have been approved by the European Medicine Agency and FDA for the treatment of metastatic cancers.[95,96] ADC has been exploited to treat IGF-1R overexpressing tumors. W0101, which tethers a monoclonal antibody (hz208F2-4) to a cytotoxic derivative of auristatin via a chemical linker (noncleavable maleimidocaproyl linker), is another new ADC. W0101 demonstrates high specific binding to IGF-1R but not InsR. Upon binding of W0101 to the membranous IGF-1R, the receptor readily undergoes internalization and auristatin can then be released in the tumor cells. Auristatin blocks cell division by repressing the polymerization of tubulin in the cytoplasm.[97] The effective delivery of this ADC to the intracellular compartment is acquired by catabolism of ADC in the lysosomes, which release the free cytotoxic drug to cytoplasm.[97] In IGF-1R-overexpressing cancer cell line models MCF-7 (breast) and NCI—H2122 (lung), W0101 induced a potent cytotoxic activity.[97] A phase I/II clinical trial is currently recruiting to assess the clinical safety and efficacy of W0101 in advanced or metastatic solid tumors (NCT03316638).

Apart from antibody—drug conjugate, peptide—drug conjugate that targets IGF-1R-overexpressing tumors has been explored. IGF-1 is the primary ligand of the IGF-1R. However, when IGF-1 is bound to IGFBPs, IGF-1 cannot bind to IGF-1R.[7,9] To enhance the chance of binding to IGF-1R, an IGF-1 variant (765IGF) which has reduced affinity for IGFBPs has been engineered. IGF-methotrexate (IGF-MTX) is a conjugate of 765IGF and an antifolate drug methotrexate (MTX; a chemotherapeutic and immunosuppressant agent). The conjugate can selectively enter cancer cells through binding to IGF-1R.[98] The high binding affinity of IGF-MTX to IGF-1R contributes to its promising anti-tumor activities in LNCaP and MCF7 xenograft compared with free MTX.[98,99] It has been suggested that low doses of IGF-MTX are sufficient to induce anti-tumor effects and cause less side effects as well as systemic toxicity.[99] The smaller size of peptide—drug conjugate compared with antibody—drug conjugate may also allow for efficient cellular uptake. Two phase I clinical trials in patients with advance solid and hematologic malignancy (NCT02045368) or high-grade myelodysplastic syndromes ($n = 2$, NCT03175978) have suggested promising efficacy of IGF-MTX at tolerable dose and desirable toxicity profile.[98,100] Stable disease could be observed in 20% of patients ($n = 3$ out of 15) with refractory solid or hematologic malignancies[98] and in patients ($n = 2$ out of 2) with myelodysplastic syndromes.[100]

To further improve the therapeutic efficacy of ADC exploiting IGF-1R, approach to advance the conjugated drug by chemical modification has been explored.[101] For example, maytansine (DM1) kills cancer cells by inhibiting microtubule assembly. Anti-IGF-1R antibody cixutumumab conjugated to PEGylated DM1 may gain potency because modifying DM1 through PEGylation renders DM1 the ability to evade from drug efflux pumps.[101]

Combinational therapy involving IGF-1R inhibition

Overexpression of IGF-1R may confer resistance towards radiotherapy, chemotherapy and targeted therapy in multiple cancer types.[102] The therapeutic resistance is mainly mediated through two mechanisms: (i) crosstalk with other signaling pathways resulting in network rewiring and compensatory survival signal; and (ii) promotion of homologous and non-homologous end-joining recombination to repair DNA double-strand breaks induced by radio- and chemotherapeutic agents.[103—105] Intriguingly, recent pre-clinical studies have also highlighted the role of IGF-1R in the nucleus.[71,106] In non-small cell lung and cervical cancer cell lines, nuclear IGF-1R might upregulate the promoter activities of target genes involved in the WNT pathway, for examples *CCND1* (encoding cyclin D1) and *AXIN2* (encoding Axin 2).[107] As a result, proliferation of cancer stem cells could be maintained via the sustained activation of WNT signaling. This potential role of nuclear IGF-1R in maintaining cancer stemness may present another mechanism of drug resistance.

Activation of IGF-1R was found in prostate cancer cells resistant to an EGFR inhibitor gefitinib.[108] Along this line, co-treatment of gefitinib and an IGF-1R inhibitor AG1024 resulted in reduced cell proliferation and invasion capability compared with single treatment in these cells.[108] Treatment of *EGFR*-mutated NSCLC with osimertinib (a third-generation EGFR-TKI selectively inhibiting EGFR with exon 19 deletion, L858R, and T790M) combined with IGF-1R TKI linsitinib (OSI-906) led to tumor eradication in xenograft and PDX models.[109] Although a phase I study ($n = 95$) has demonstrated the potential efficacy of combined EGFR and IGF-1R inhibition (by erlotinib and linsitinib, respectively) in multiple advanced solid tumor types,[110] such combination did not lead to better therapeutic outcome compared with erlotinib alone in a phase II clinical trial involving NSCLC patients ($n = 88$).[111]

HER2 and ER signaling are major drivers of breast cancer. Circulating IGF-2 levels in HER2-positive breast cancer patients with poor response to trastuzumab (Herceptin; anti-HER2 monoclonal antibody) were significantly higher than that of the patients with better response.[112] Enhanced anti-
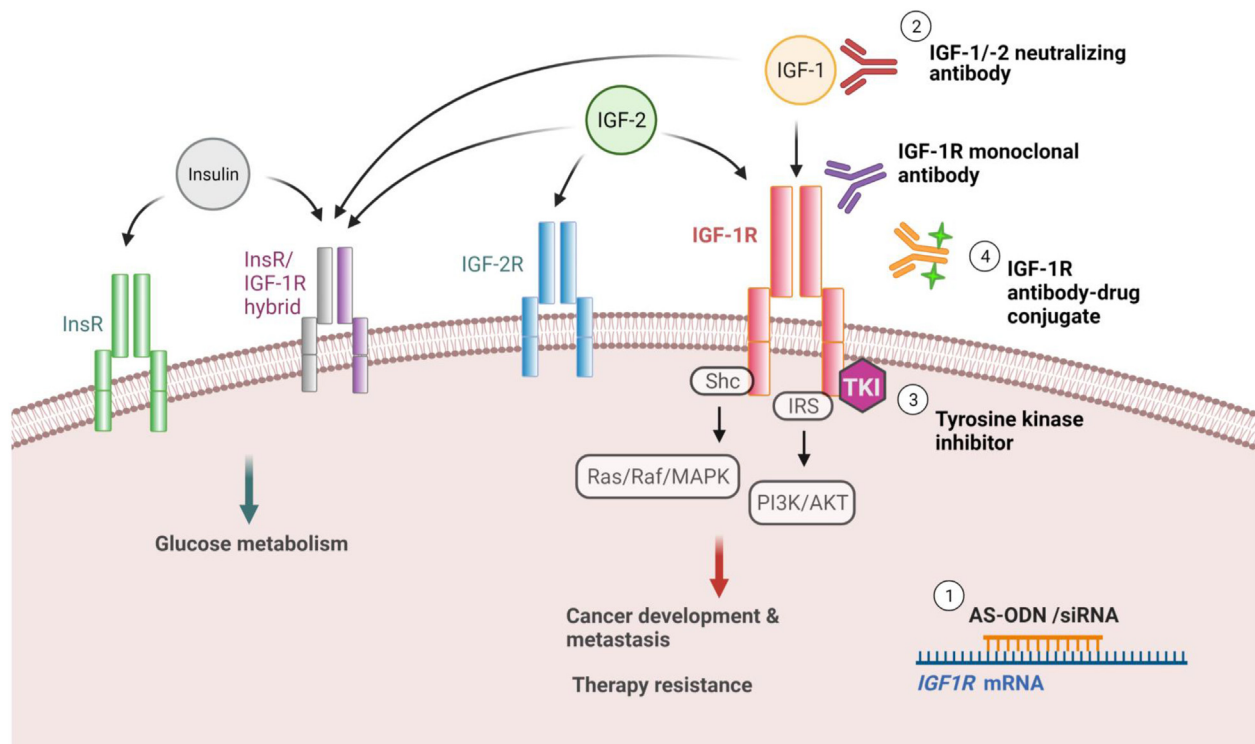
Figure 3 Overview of the therapeutic strategies developed to inhibit IGF-1R signaling. The schematic shows the key components of the IGF axis and the four major approaches for IGF-1R inhibition: (1) by suppressing IGF-1R expression using antisense oligo-nucleotide (AS-ODN) or siRNA; (2) by blocking the binding of IGF-1R with ligands (IGF-1R monoclonal antibodies or IGF-1/-2 neutralizing antibodies); (3) by repressing IGF-1R kinase activity using tyrosine kinase inhibitor (TKI); and (4) by conjugating cytotoxic drug to IGF-1R antibody.

proliferative effects of trastuzumab in combination with IGF-1R TKIs BMS-536924 or NVP-AEW541 were observed in HER2-positive breast cancer cell lines.[113] Indeed, mechanistically, formation of IGF-1R/HER-2 heterodimer was observed in trastuzumab-resistant breast cancer cells.[25] Moreover, trastuzumab resistance in HER2-overexpressing breast cancer cells is attributed to IGF-1R-activated FAK-Src signalling and can be reversed by an IGF-1R monoclonal antibody (α-IR3).[17] It has been shown that IGF-1R could induce the phosphorylation and activation of ER through the PI3K/AKT and Ras/Raf/MAPK signaling pathways.[114] As a result, combinatory inhibition of IGF-IR (by a monoclonal antibody αIGF-1R scFv-Fc) and ER (by tamoxifen) resulted in enhanced therapeutic effect in breast cancer cell line.[115] Co-targeting IGF-1R (by TKIs NVP-AEW541 or BMS-536924 or AG1024) and ER (by tamoxifen or fulvestrant) significantly enhanced cell cycle arrest at G1 phase or apoptosis in the breast cancer cell lines.[116,117]

Another combination strategy shown to be effective *in vitro* and *in vivo* involves combined inhibition of IGF-1R (by linsitinib or figitumumab) and MEK 1/2 (by U0126 or selumetinib). These combinations caused synergistic anti-proliferative effects in a panel of colorectal cancer cell lines and xenograft[118] as well as in small cell lung cancer cell line.[119] Interestingly, building on the facts that IGF-1R inhibitor may induce hyperglycemia and that metformin has both hypo-glycemic and anti-cancer effects, the effects of combining metformin and an anti-IGF-1R monoclonal antibody figitumumab or BMS-754807 (an IGF-1R inhibitor) were

examined in SCLC or breast cancer cell line respectively.[119,120] The combination resulted in superior therapeutic effects in both studies. However, a study suggested that BMS-754807 might inhibit additional protein kinase targets apart from IGF-1R and its anti-tumorigenic activity may be independent of IGF-1R inhibition.[121]

The regulation of the IGFR-1R signaling by the tumor microenvironment might be exploited in the design of combination therapy. Upon treatment with an IGF-1R monoclonal antibody cixutumumab, STAT3 was activated to upregulate the transcription of IGF-2 which then acts on IGF-2R on the stromal cells.[122] The infiltrated stromal cells produce pro-angiogenic cytokines, thereby recruiting vascular endothelial cells and enhancing tumor angiogenesis.[122] In light of these data, co-targeting IGF-1R and STAT3 may inhibit tumor metastasis and overcome STAT3-mediated resistance to anti-IGF-1R therapy.

Conclusions and future prospective

IGF-1R remains an attractive druggable target of cancer. Multiple therapeutic strategies revolving around this receptor have been developed and investigated (Fig. 3). Although the outcomes of clinical trials so far are conflicting, these studies have generated new and important insights into strategies that target IGF-1R. First, the lack of patient stratification could be a leading cause of the conflicting treatment outcomes. Investigation on the molecular profiles of responsive

and non-responsive patients will shed light on potential bio-markers of responses. Second, rational combinational targeted approaches that inhibit IGF-1R signaling and the linked networks have demonstrated additive anti-tumor activities. Elucidating the signaling network and drug resistant mechanism associated with IGF-1R will uncover more potential drug combination to improve therapeutic outcomes. Third, the hyperglycemic side effect associated with simultaneous inhibition of InsR might be circumvented by several approaches. These include the development of new inhibitors that spare InsR. Interfering with the IRS-1 and IRS-2 proteins, which are the signal adaptors of IGF-1R, may be effective in patients with high expressions of IGF-1R. Inhibiting IRS-1/2 may suppress tumor growth without affecting the normal glucose homeostasis mediated by the other adapter proteins in insulin target organs. Alternatively, the incorporation of metformin, which induces hypoglycemic and anti-cancer effects, with IGF-1R inhibition may reduce the occurrence of hyperglycaemia. Study that examines the structural different between InsR and IGF-1R may also help reveal novel class of specific allosteric inhibitor.[123]

Further, insights from biological research will continue to foster the development of effective anti-IGF-1R therapy. Accumulating evidence has highlighted the functional significance of nuclear IGF-1R in therapeutic resistance.[71] It has been found in colorectal and prostate cancers that high abundance of nuclear IGF-1R promoted tumorigenesis and hindered the effectiveness of chemotherapy and targeted therapies (inhibition of IGF-1R by ganitumab or NVP-AEW541; inhibition of Src by dasatinib).[124,125] A poor OS has been reported in colorectal cancer patients with high levels of nuclear phosphorylated IGF-1R compared with low/negative levels (median 16.7 months *vs.* 24.6 months; $P < 0.01$).[124] Building on these findings, inhibiting the nuclear sequestration event of IGF-1R may re-sensitize cancer cells to anti-IGF-1R therapy. Strikingly, a preclinical study using prostate cancer cells has revealed a novel function of IGF-1R TKI (AZ12253801) in the downregulation of the total and phosphorylated IGF-1R protein in the nucleus, shedding light on the possibility to reposition IGF-1R inhibitors in future combinational drug studies.[106] Nuclear translocation of IGF-1R is mediated by clathrin-dependent endocytosis and SUMOlyation.[106,126,127] Thorough understanding of the nuclear translocation process may lead to viable strategy that abrogates the translocation.

Conflict of interests

The authors declare no conflict of interests.

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