

Expert Opinion

1. The IGF system of ligands, receptors and binding proteins
2. Similarities and differences between the IGF1 and insulin signaling pathways
3. The involvement of the IGF1 axis in cancer development
4. IGF1R overexpression in cancer: the classical dogma and some new concepts
5. Interactions between IGF1R and cancer genes
6. The interplay between tumor suppressors and the IGF1R gene
7. Epidemiological evidence of the involvement of the IGF1 axis in cancer
8. Selected examples of IGF1R involvement in human cancer
9. Approaches and methodological issues in IGF1R targeted therapy
10. Conclusions
11. Expert opinion

informa
healthcare

Targeting the IGF1 axis in cancer proliferation

Ilan Bruchim, Zohar Attias & Haim Werner[†]

[†]Tel Aviv University, Sackler School of Medicine, Department of Human Molecular Genetics and Biochemistry, Tel Aviv 69978, Israel

The IGF network of ligands, cell-surface receptors and IGF-binding proteins has important roles at multiple levels, including the cellular, organ and organism levels. The IGF system mediates growth, differentiation and developmental processes, and is also involved in various metabolic activities. Dysregulation of IGF system expression and action is linked to diverse pathologies, ranging from growth deficits to cancer development. Targeting of the IGF axis emerged in recent years as a promising therapeutic approach in conditions in which the IGF system is involved. Specific IGF1 receptor (IGF1R) targeting, in particular, produced the best experimental and clinical results so far, and generated significant optimism in the field. This review provides a basic analysis of the role of the IGF1R in cancer biology and explores the functional interactions between the IGF signaling pathways and various cancer genes (e.g., oncogenes, tumor suppressors). In addition, we review a number of specific malignancies in which the IGF system is involved and summarize recent data on preclinical and clinical studies employing IGF1R-targeted modalities.

Keywords: IGF-1 receptor (IGF1R), insulin-like growth factors (IGF), monoclonal antibodies, targeted therapy, tyrosine kinase inhibitors

Expert Opin. Ther. Targets (2009) 13(10):1-15

1. The IGF system of ligands, receptors and binding proteins

The IGFs are a system of secreted hormones, cell-surface receptors and binding proteins that control normal growth and differentiation of most organs [1,2]. The IGFs are active at most stages of the life cycle, including the fetal period, infancy and adulthood. In addition, the IGF status of the organism (i.e., circulating hormone levels, tissue receptor activation, interactions with other signaling pathways, etc) affects critical aspects of the aging process and, hence, seems to have a marked effect on longevity [3]. Unlike most other growth factor systems, the IGF axis displays biological activities at both the cell and organism levels. At the cellular level, IGFs function as cell progression factors, 'pushing' the cell through the various phases of the cell cycle. At the organism level, IGFs participate in the control of multiple systems, including neuronal activity, kidney function, reproduction, etc. The IGF network is also involved in a wide range of pathological processes, such as diabetes and cancer [4-7]. In the specific context of cancer, the IGF axis emerged in recent years as a promising candidate for targeted therapies [8-10]. The aim of this review is i) to provide a brief analysis of the role of the IGF system in the biology of cancer; and ii) to review the current status of ongoing efforts to target the IGF axis for therapeutic purposes.

The IGF system consists of two ligands (IGF1 and IGF2), two receptors [IGF1R and IGF2/mannose 6-phosphate receptor (M6P-R)], and at least six IGF-binding proteins (IGFBPs) [11]. The IGF network includes, in addition, a series of IGFBP-related proteins and IGFBP proteases. The existence of the IGFs was postulated in the late 1950's, following the seminal observation by Salmon

and Daughaday that growth hormone (GH) stimulated the incorporation of sulfate into cartilage in an indirect fashion, which involved activation of a specific serum factor [12]. The factor that was originally termed 'sulfation factor' and then 'somatomedin' is now accepted as IGF1. Circulating IGF1 levels are mostly dependent on liver output, a process that is tightly regulated by GH. Both IGF1 and IGF2 activate a common cell-surface receptor, the IGF1R, which signals mitogenic, antiapoptotic and transforming activities [13,14]. The IGF1R is coupled to several intracellular second messenger pathways, including the *ras-raf*-MAPK and PI3K-PKB/Akt signaling cascades (Figure 1). IGF1R is vital for cell survival, as illustrated by the lethal phenotype of mice in which the IGF1R gene was disrupted by homologous recombination. The IGF2/M6P-R, on the other hand, is apparently not involved in IGF signaling but is mainly responsible for targeting the highly mitogenic IGF2 for lysosomal degradation. In this respect, the IGF2/M6P-R functions as a tumor suppressor by protecting the organism from pervasive IGF2-induced IGF1R activation [15]. Importantly, significant portions of the proliferative activities of IGF2 have been shown to be mediated by a particular isoform of the insulin receptor (InsR), termed InsR-A.

Unlike insulin, which circulates in serum unbound, IGF1 and IGF2 are carried in serum and other body fluids (e.g., cerebrospinal fluid, saliva, etc) by a family of IGFbps [16,17]. In the serum, the majority of circulating IGFs are found in a ternary complex with IGFbp3 and an acid-labile subunit. This complex modulates IGF action by protecting the growth factors from proteolysis, thus prolonging their half-lives in the circulation. The release of the IGFs from the IGFbps involves IGFbps protease action. The ratio between free and bound IGFs in serum is of major importance in terms of proliferation and mitogenic potential. Finally, some IGFbps exert their biological effects in an IGF-independent manner, suggesting the existence of IGFbp receptors [18].

2. Similarities and differences between the IGF1 and insulin signaling pathways

The structural and functional similarities between insulin and IGF1 suggest that both molecules are derived from a common ancestral precursor that probably participated in food intake and nutritional regulation. A divergence of functions most probably occurred before the appearance of the first vertebrates, with insulin mostly active in the regulation of metabolism and IGF1 in growth processes. However, in view of their common evolutionary origins and conserved architecture there is a certain degree of cross-talk between insulin, IGFs and their receptors [19]. Specifically, insulin may interact with IGF1R with low affinity, thus mediating growth type of activities, whereas IGFs may stimulate metabolic activities via interaction with the InsR. The complexity of these interactions is further illustrated by the important role of InsR-A in mediating the mitogenic actions of IGF2.

The specificity of insulin and IGF1 physiological activities, despite marked structural similarities at both ligand and receptor levels, has been the topic of controversial research. In addition, the fact that the vast majority of the signaling modules downstream of IGF1R, InsR (A and B isoforms) and IGF1R-InsR hybrid receptors (composed of an IGF1R hemireceptor linked to an InsR hemireceptor) are shared by all of them raises the question as to how these receptors are able to engage in basically different biological activities. A number of potential mechanisms were postulated to explain this paradox, including a different tissue distribution of IGF1R and InsR [20], different internalization kinetics and subcellular distribution of the hormone-receptor complex [21], and different hormone-receptor affinities [22]. Furthermore, various substrates and signaling mediators that are preferentially activated by insulin or IGF1 have been identified. For example, the adapter protein Grb10 associates with InsR but not with IGF1R [23]. Differential activation of this and other substrates may partially explain the specificity of the receptors. Finally, and as mentioned above, the fact that IGF1, but not insulin, is carried in the circulation and extracellular fluids by IGFbps further contributes to the divergent actions of the ligands.

3. The involvement of the IGF1 axis in cancer development

A number of mechanisms have been proposed to rationalize the possible role/s of the IGF system in the initiation and/or progression of neoplasia [24,25]. Although IGF1 was shown to increase chromosomal fragility under experimental conditions, it is usually considered to be nongenotoxic [26]. A widely accepted model of IGF1 action in cancer cells postulates that, while unable to induce oncogenic transformation by itself, once a malignant transformation has already occurred, cell survival of transformed cells depends on IGF1 action. The disruption of internal checks and control mechanisms associated with the neoplastic phenotype is further emphasized by the finding that IGF1 action can override the cellular signals of apoptosis [13].

Unlike the IGF1 gene, overexpression of the IGF2 gene has been linked to the etiology of a number of overgrowth syndromes (e.g., Beckwith-Wiedemann Syndrome) and neoplasias (e.g., Wilms' tumor, rhabdomyosarcoma) [27]. The IGF2 gene is an imprinted one (i.e., it is only expressed from the paternal allele). Overexpression of IGF2 in cancer may be caused by a number of genetic events, including gene duplication, loss of heterozygosity, and loss of imprinting, leading to biallelic IGF2 expression [28]. Furthermore, and in agreement with its cell survival role, the initial proliferative switch in oncogene-induced transformation was correlated with focal activation of IGF2 [29]. Transfection with an antisense oligonucleotide to the IGF2 mRNA interfered with tumor cell proliferation *in vitro*, and transgenic mice homozygous for a disruption of the IGF2 gene developed tumors with reduced malignancy. Combined, these results suggest that IGF2 signaling is necessary to elicit hyperproliferation.

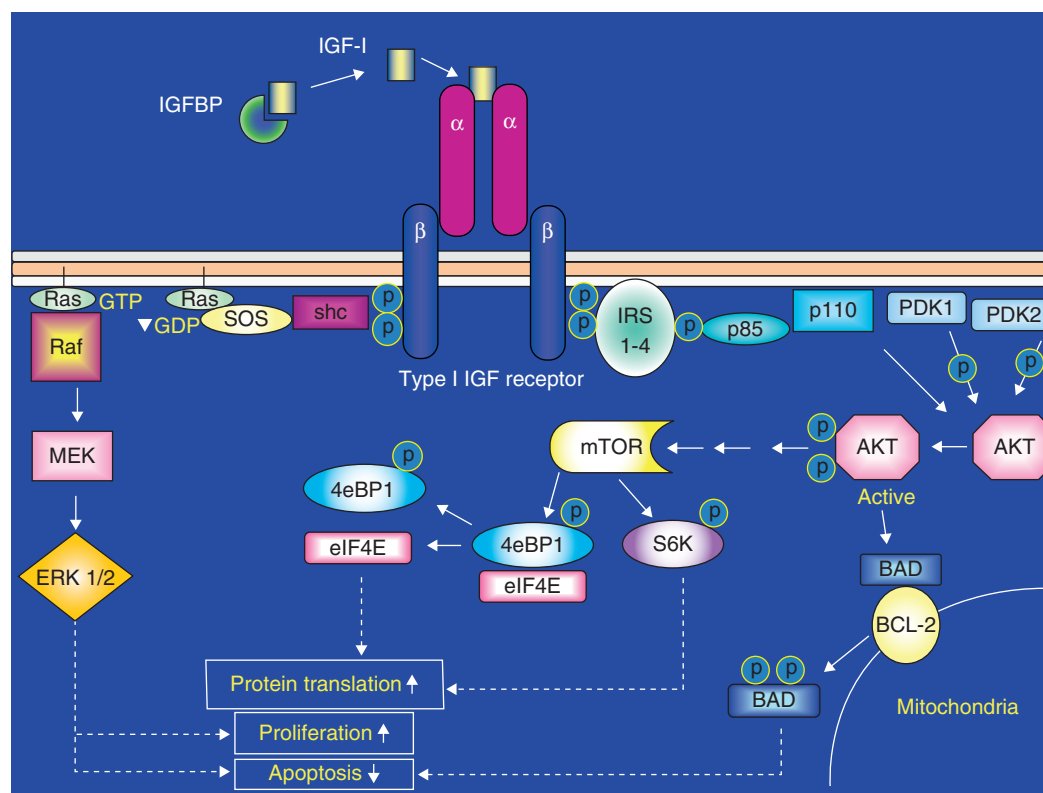


Figure 1. IGF1 signaling pathways. IGF1/2 binding to the extracellular domain of the IGF1R (or Type 1 IGF receptor) leads to autophosphorylation of its tyrosine kinase domain and subsequent activation of various cytoplasmic mediators. Particularly important pathways are the *Ras-Raf-MEK-ERK1/2* and the *PDK-AKT-mTOR* signaling systems. The net consequences of the concerted activation of these pathways are, among other biological effects, an increase in proliferation and a marked reduction in apoptosis.

Among all growth factor receptors described, the IGF1R displays one of the most potent antiapoptotic activities. This inherent feature of the receptor confers upon IGF1R-expressing cells the capacity not to die, a quintessential feature of cancer cells [30]. On the other hand, IGF1R-null cells (R⁻, derived from IGF1R knockout embryos) are unable to undergo transformation when exposed to different cellular and viral oncogenes [31]. The IGF1R displays, in addition, pivotal roles in invasion, metastasis and angiogenesis [5]. Combined, these data support the notion that IGF1R expression is a fundamental prerequisite for acquisition of a malignant phenotype. However, the presence of the IGF1R may not be an obligatory prerequisite, as demonstrated by the fact that certain oncogenes induce transformation via pathways that are IGF1R-independent. For example, transfection of R⁻ cells with the GTPase-deficient mutant α_{13} resulted in malignant transformation in spite of the absence of the IGF1R [32].

4. IGF1R overexpression in cancer: the classical dogma and some new concepts

Analysis of targeted disruption experiments, showing that disruption of the IGF1R gene is incompatible with life, along with ontogenetic studies, demonstrating an almost

universal pattern of distribution of the IGF1R during development, underscore its crucial role as a cell-survival and proliferation factor in most organs [33,34]. In accordance with this role, most primary tumors and malignant cells express increased IGF1R mRNA and protein levels and augmented IGF binding. These tumors include, breast, ovarian, prostate, colon, hematopoietic, rhabdomyosarcoma, renal, etc. Increased IGF1R expression in cancer reflects a reversal to a less differentiated, more primitive ontogenetic stage that, in most species and body organs, is characterized by very high IGF1R mRNA concentrations and IGF binding sites [35]. The molecular mechanisms responsible for the increased expression of the IGF1R gene in tumors, however, remain largely unidentified. Amplification of the IGF1R locus at band 15q26 has been reported in a small number of breast and melanoma cases [36].

The dogma that emerged from these comprehensive analyses postulated that IGF1R expression is a fundamental prerequisite for cellular transformation [31,37]. The strength of this paradigm resided in the fact that enhanced IGF1R levels and IGF1 signaling were considered key factors, indispensable for the cell, in order to adopt proliferative pathways. While correct in most aspects, some of the above notions are interpreted today as overgeneralizations and,

213 therefore, a more balanced examination is required [38]. Spe-
215 cifically, IGF1R overexpression is a common theme in most
pediatric tumors (that are, in many cases, associated with
recurring chromosomal translocations) and other solid can-
cers, including renal tumors [7,10]. The extent and biological
significance of IGF1R overexpression in adult epithelial
tumors is more complex. At the methodological level, the
220 large number of studies describing IGF1R overexpression in
breast, prostate and other tumors have been, for the most
part, based upon analyses of tissue homogenates or estab-
lished cancer cell lines for which appropriate normal con-
trols do not exist. The IGF1R content of homogenates, in
225 particular, can be affected by contamination with stroma,
which would dilute IGF1R content in normal epithelium or
small tumors.

More recent analyses of IGF1R expression in breast and
prostate cancer revealed that expression is tightly dependent
230 on tumor stage. Immunohistochemistry of primary breast
tumors and matched control samples revealed that IGF1R
and the downstream signaling molecule insulin receptor
substrate-1 (IRS-1) were expressed at high levels in control
tissues and in well and moderately differentiated carcinoma,
235 but at low levels in poorly differentiated cancers. InsR, on the
other hand, did not show a significant correlation with the
differentiation grade of the tumors [39]. Interestingly, a study
by Papa *et al.* [40] demonstrated that InsR content (as mea-
sured by radioimmunoassay) in a collection of 159 breast
240 cancer specimens was more than sixfold higher than in nor-
mal breast tissue. In addition, the receptor retained its capac-
ity to bind insulin and to undergo phosphorylation, suggesting
a role for the InsR in breast cancer.

In the prostate, IGF1R levels were significantly reduced
245 in prostate carcinoma, as compared with benign prostate
epithelium [41]. IGF1R expression was also diminished in a
majority of human prostate cancer bone marrow metastases,
although a recent study showed sustained upregulation of
IGF1R in metastases [42]. While the molecular and bio-
chemical nature of this reduction has not yet been estab-
lished, the results of studies showing that androgens and
estrogens control IGF1R levels can be interpreted to suggest
250 that an IGF1R decrease might be linked to the acquisition
of steroid hormone independence at advanced stages of the
disease [43,44]. Another potential explanation for the decrease
of IGF1R levels is the downregulation of the receptor by the
very high levels of *in situ*-produced IGF2 and stroma-produced
IGF1. Taken together, available data suggest that normal
prostate and breast epithelial cells respond to circulating
IGF1 to maintain an appropriate balance between prolifera-
260 tion and differentiation. Disruption of this balance (e.g., excess
of IGF secretion, IGF1R overexpression) leads to increased
cellular proliferation, and the probability that cells become
hyperplastic and premalignant. The transition to frank
malignancy, at least in the case of breast and prostate cancer,
265 seems to be associated with a reduction of IGF1R levels
and, therefore, IGF responsiveness. Kim *et al.* [45] have

shown that a constitutively active IGF1R causes transforma- 268
tion and xenograft growth of immortalized mammary epi- 270
thelial cells, accompanied by an epithelial to mesenchymal
transition. Interestingly, a recent study has shown that
tumors initiated by IGF1R have the ability to become inde-
pendent of this initiating oncogene, and IGF1R indepen-
dence was associated with an epithelial to mesenchymal
275 transition [46].

5. Interactions between IGF1R and cancer genes

Cellular and viral oncogenes can induce transformation by 280
'recruiting' and activating the IGF signaling pathway. For exam-
ple, transformation by pp60^{src}, the protein encoded by the *src*
oncogene of Rous sarcoma virus, results in the constitutive
tyrosine phosphorylation of the IGF1R tyrosine kinase
domain [47]. It has been estimated that ~ 10 – 50% of the 285
receptors are phosphorylated in the unstimulated *src*-transformed
cell, while addition of IGF1 synergistically increased the extent
of phosphorylation. These results raise the possibility that pp60^{src}
alters growth regulation by rendering the cells constitutively
290 subject to a mitogenic signal.

Additional oncogenes were shown to exhibit a direct
transactivating effect of the IGF1R gene. For instance, over-
expression of *c-myc* (the cellular equivalent of the viral
transforming oncogene *v-myb*) in Balb/c-3T3 cells induced
an increase in the levels of both the IGF1R and IGF1 tran- 295
scripts [48]. This event led to abrogation of the requirement
for IGF1 in the growth media, one of the distinctive hall-
marks of a malignantly transformed cell. An additional
oncoprotein shown to stimulate IGF1R gene transcription is
the hepatitis B virus X (HBx) gene product. In hepatocellular- 300
carcinoma-derived cell lines containing HBx protein, IGF1R
mRNA levels were approximately fivefold higher than in
cells that do not express HBx transcripts [49]. Furthermore,
expression of the HBx cDNA induced a large increase in
IGF1R promoter activity, mRNA and IGF binding. These 305
findings support the notion that the mechanism of action of
oncogene HBx in the specific context of hepatocellular car-
cinoma involves the transactivation of the IGF1R gene.

In summary, the fundamental requisite for a functional
IGF1R in order for a cell to undergo oncogenic transforma- 310
tion can be explained, at a molecular level, by the fact
that many oncogenes 'adopt' the IGF1R signaling path-
way as their mechanism of transformation. Certain onco-
genes (e.g., *c-myc*) are able to directly transactivate the
IGF1R promoter, thus drastically increasing receptor con- 315
centrations in the preneoplastic cell, while other onco-
genes (e.g., pp60^{src}) induce a large increase in IGF1R β
subunit phosphorylation [47,48]. Regardless of the mecha-
nism of action of the oncogene in the specific context of
IGF1R control (i.e., regulation at the transcriptional or 320
post-transcriptional levels), the transformed cells display
essentially identical phenotypes. 322

6. The interplay between tumor suppressors and the IGF1R gene

Molecular characterization of the IGF1R gene regulatory region identified a number of *cis*-acting elements and *trans*-acting factors that are directly involved in control of IGF1R gene expression [50]. Some of these transcription factors were identified as tumor suppressor gene products, and they include p53, p63, p73, BRCA1, the von Hippel-Lindau protein (VHL), the Wilms' tumor protein (WT1) and the Kruppel-like factor-6 (KLF6) [51-58]. As described below, most of these tumor suppressors negatively regulate IGF1R gene transcription. Accordingly, it has been postulated that inhibitory control of the IGF1R gene by tumor suppressors constitutes a mechanism that prevents from the cell from engaging in mitogenic activities.

A typical example of a tumor suppressor involved in regulation of the IGF1R gene is p53, a nuclear protein that, in its hyperphosphorylated state, blocks progression of cells through the cell cycle. The p53 protein functions as a transcription factor that binds specifically to DNA sequences in various promoters and stimulates their transcriptional activity. Mutations of the p53 gene are the most frequent event in human cancers. In addition, p53 can also function as a transcriptional repressor of many growth-regulated genes. Thus, transient expression of wild type p53 in osteosarcoma and rhabdomyosarcoma cell lines suppressed the activity of a cotransfected IGF1R promoter construct by ~ 90% [51]. On the other hand, cotransfection of tumor-derived, mutant p53 stimulated promoter activity by several-fold. In addition, wild type p53 decreased the IGF1-induced tyrosine phosphorylation of IGF1R and IRS-1, whereas mutant p53 stimulated phosphorylation [59,60]. These results support the view that, at least part of, the effects of wild type p53 on apoptosis and cell cycle arrest are mediated via suppression of the IGF1R promoter. Lack of inhibition, or even stimulation, by mutant p53 may accelerate tumor growth and inhibit apoptosis, thus providing an increased survival capacity to malignant cells.

Inactivation of the VHL gene is a frequent event in the etiology of clear cell renal cell cancer (CC-RCC) [61]. The potential regulation of the IGF1R gene by VHL was recently examined [58]. IGF1R mRNA levels were significantly higher in CC-RCC biopsies than in benign kidney. IGF1R protein levels were unaffected by hypoxia, but were higher in CC-RCC cells harboring a mutant inactive VHL than in isogenic cells expressing a wild type VHL. Furthermore, IGF1R mRNA and promoter activities were lower in CC-RCC cells expressing a wild type VHL, consistent with a transcriptional effect. In terms of the mechanism of action of VHL, it was shown that the negative effect of VHL involves interaction with and, potentially, sequestration of, zinc finger protein Sp1, a potent transactivator of the IGF1R gene. In addition, VHL was shown to control IGF1R mRNA stability. Hence, this study identified a new, hypoxia-independent role for VHL in

suppressing IGF1R transcription and mRNA stability. VHL inactivation leads to IGF1R upregulation, contributing to renal tumorigenesis and, potentially, also to chemoresistance.

BRCA1 has been identified as a tumor suppressor gene that, when mutated, increases the risk of breast and ovarian cancer. BRCA1 participates in multiple biological pathways, including DNA damage repair, cell growth and apoptosis, and gene transcription. The BRCA1 gene product has been shown to inhibit IGF1R transcription in breast cancer cell lines, suggesting that a potential mechanism of action of BRCA1 involves suppression of IGF1R gene expression [56]. In contrast, mutant BRCA1 proteins lacking transcriptional activity are impaired in their ability to suppress the IGF1R promoter, with resulting increments in IGF1R mRNA and IGF binding in mammary tumors [55,56]. Similar to the mechanism of action of VHL, BRCA1 was shown to specifically bind Sp1, thus preventing from the zinc finger from binding to, and transactivating, the IGF1R gene [56]. Finally, and consistent with the postulate that mutant BRCA1 may lead to dysregulated IGF1R expression, a recent immunohistochemical analysis revealed a significant elevation of IGF1R levels in primary breast tumors derived from BRCA1 mutation carriers compared to non-carriers [62].

7. Epidemiological evidence of the involvement of the IGF1 axis in cancer

The importance of IGF action in cancer biology is supported by epidemiological studies showing a correlation between circulating IGF1 values and cancer incidence. Seminal studies from the group of Michael Pollack published in 1998 identified IGF1 as a risk factor in breast and prostate cancers [63,64]. Specifically, in a prospective nested control study (the Nurse's Health Study) the relative risk (RR) of breast cancer in premenopausal women was 4.6 in the upper tertile of IGF1 values, in comparison to women in the lower tertile. The RR increased to 7.3 when the concentrations of IGFBP3 were included in the analysis. Likewise, the RR of prostate cancer in men (evaluated in the Physician's Health Study) in the upper quartile of IGF1 values was 2.4 (4.3 when normalized for IGFBP3). Noteworthy is that IGF1 levels were measured an average of seven years before the diagnosis of the disease. A meta-analysis of 14 studies confirmed the association between IGF1 levels and prostate cancer [65]. In addition to hormone-dependent prostate and breast carcinomas, the importance of IGF1 as a risk factor was evaluated in various non-hormone-dependent types of cancers. Analysis of colon cancer risk in the Nurse's and Physician's Health Studies showed an increased cancer risk in individuals with the highest IGF1 values [66,67]. A recent meta-regression analysis by Renehan and coworkers identified 21 eligible studies, which included 3609 cases and 7137 controls [68]. The study concluded that high concentrations of IGF1 were associated with an increased risk of prostate cancer (odds ratio comparing 75th with 25th percentile

1.49, 95% CI 1.14 – 1.95) and premenopausal breast cancer (1.65, 1.26 – 2.08) and high concentrations of IGFBP3 were associated with increased risk of premenopausal breast cancer (1.51, 1.01 – 2.27). Associations were larger in assessments of plasma samples than in serum samples, and in standard case-control studies compared with nested studies. In summary, serum levels of IGF1 and IGFBP3 are associated with increased risk of common cancers, but associations seem to be smaller than those reported in earlier studies. Furthermore, the correlations vary between sites. Although laboratory methods need to be standardized, these epidemiological observations could have major implications for assessment of risk and prevention of cancer. Finally, the importance of circulating IGF1 levels in cancer is further underscored by animal studies showing reduced susceptibility to skin and other types of cancer in mice with low serum IGF1 values [69]. Of interest, and in spite of its potent mitogenic potential, no epidemiological link has been reported between serum IGF2 and cancer risk.

8. Selected examples of IGF1R involvement in human cancer

8.1 Breast cancer

Epidemiological, clinical, and *in vitro* and *in vivo* experimental evidence support a key role for the IGF axis in breast cancer development. The expression of IGF ligands, receptors, and IGFBPs is changed in breast cancer tissue compared with normal breast tissue. Moreover, many components of the IGF axis are altered in the circulation of breast cancer patients. Recent epidemiological studies indicate that evaluation of IGF1 levels in breast cancer patients might be important both in terms of prognosis and diagnosis. However, the results of some of these studies are controversial. A number of systematic reviews and meta-analyses of prospective and case-control studies indicated a relationship between IGF1 and IGFBP3 regarding breast cancer risk in premenopausal but not postmenopausal women [68,70-72]. However, a recent case-cohort study, which included 423 breast cancer cases and 1901 controls, reported that IGF1 and IGFBP3 were positively associated with breast cancer risk in patients older than 50, but not in younger women [73]. Likewise, the European Prospective Investigation into Cancer and Nutrition (EPIC) study analyzed data from 1081 patients and 2098 controls, and reported that high levels of IGF1 or IGFBP3 are associated with a 40% increased risk for breast cancer in women older than 50 years of age, but not in younger women [74]. On the other hand, the Nurses' Health Study II found no association between circulating IGF1, IGFBP1, and IGFBP3 levels and breast cancer risk in a large cohort of premenopausal women [75]. Moreover, a recent study including 835 incident breast cancer patients and 816 controls from the Women's Health Initiative Observational Study (WHI-OS) found no association between total and free IGF1 levels and breast cancer risk. Notably, a modest positive

association was reported between free IGF1 and risk of breast cancer among nonusers of hormone replacement therapy [76]. Renehan *et al.* [77] suggested that these controversial findings may be a result of lack of standardization of assays, variation in study design, and/or variability in IGFBP3 proteolysis in serum samples.

Regarding IGF1R expression in primary tumors, previous studies have shown that the IGF1R gene is highly expressed (39 – 93%) in breast carcinomas [78] yet conflicting data exists regarding its biological significance. Data have suggested lower IGF1R levels in benign lesions and normal breast tissue, compared with their malignant counterpart [79,80]. Shimizu *et al.* [81] demonstrated IGF1R overexpression in 48% of primary breast tumors, although expression levels did not correlate with tumor size, nodal status, hormone receptor status, histological grade, or prognosis. Finally, an additional study demonstrated that IGF1R expression in breast cancer was correlated with lower grade and hormone receptor positivity [82].

Besides its role in cancer progression, the IGF1R has also a role in mediating resistance to various targeted and non-targeted cancer therapies [83]. Compared with HER2⁺ breast cancers, which represent 20 – 25% of all breast cancers, the high-level-IGF1R-expression breast cancers represent a much broader potential group of patients that may be candidates for targeted therapy [84]. The IGF1R has been demonstrated to mediate resistance to radiotherapy in breast cancer, *in vitro* and *in vivo* [85]. Moreover, treatment of breast cancer cells with IGF1R antibody can sensitize these cells to the effect of doxorubicin [86]. Conversely, other *in vitro* studies showed that increased IGF1 signaling in breast cancer cell lines is correlated with increased response to various chemotherapies [87].

8.2 Prostate cancer

Growing evidence has accumulated suggesting that the IGF1 axis plays an important role in normal prostate gland growth and development as well as in prostate cancer etiology. As indicated above, multiple epidemiological studies have addressed the potential correlation between serum IGF1 levels and prostate cancer incidence. In terms of IGF1R expression in prostate cancer, Hellawel *et al.* [42] showed that IGF1R mRNA and protein were significantly upregulated, compared with benign prostatic epithelium, in a study including 54 primary prostate specimens. In addition, in a study using frozen tissue sections, widespread IGF1R expression was found in normal prostate, prostate cancer, and metastases, with more intense staining in the stromal tissue surrounding the tumor [88]. Upregulation of IGF axis components, including IGF1, IGF2, IGF1R and IRS-1, in prostatic intraepithelial neoplasia in a collection of 56 tissue specimens, has been shown to be correlated with tumor grade [89]. As discussed above, other studies reported a marked reduction of IGF1R levels during progression of prostate cancer from a benign to a metastatic stage [40].

IGF1R expression in prostate cancer seems to be dependent on androgen receptor (AR) status. A study by Pandini *et al.* [44] has shown that androgens upregulate IGF1R levels in cultured prostate cancer cells and sensitize the cells to the biological effects of IGF1. In addition, IGF1R levels are also correlated with BRCA1 status, a tumor suppressor whose involvement in prostate cancer is still a controversial issue. Significantly elevated BRCA1 levels were seen in prostate cancer in comparison to normal prostate tissue. In addition, an inverse correlation between BRCA1 and IGF1R levels was observed in the AR-negative P69 and M12 prostate cancer-derived cell lines. Coexpression experiments in M12 cells revealed that BRCA1 was able to suppress IGF1R promoter activity and endogenous IGF1R levels. On the other hand, BRCA1 enhanced IGF1R levels in LnCaP C4-2 cells expressing an endogenous AR [90]. These findings are of relevance because they demonstrate a new mechanism for IGF and AR stimulation of prostate cancer and further support the relevance of targeting AR and IGF1R in prostate cancer with BRCA1 expression as a marker for defining the target activity.

8.3 Cervical cancer

In vitro and *in vivo* data suggest a possible role for the IGF system in cervical tumorigenesis. Early studies by Steller *et al.* [91] found an elevation of IGF2, but not IGF1, mRNA levels following EGF stimulation in the cervical cancer cell line HT-3, and suggested that IGF2 plays a central role in mediating cervical cancer. In a follow-up report, the same group reported overexpression of the IGF1R gene in primary cervical cancer cell cultures and cell lines, compared with normal cervical cells [92].

A recent study evaluated IGF1R expression levels and activation status in patients with cervical intraepithelial neoplasia (CIN) and cervical cancer [93]. IGF1R expression was elevated in CIN-stage III and invasive cancer. Furthermore, IGF1R phosphorylation was promoted in all CIN and invasive cancers, and its intensity was related to tumor promotion. The authors suggested that human papilloma virus (HPV) infection contributes to upregulation of IGF1R expression in cervical cancer and to the initiation and progression of the tumors. Another study of 137 women assessed the correlation between serum IGF1 and IGFBP3 and the incidence of cervical oncogenic HPV and CIN [94]. Having a high IGF1:IGFBP3 ratio was associated with increased persistence of oncogenic HPV infection and women with high serum IGFBP3 had lower rates of oncogenic HPV detection and HPV-positive CIN. Finally, a recent study of 72 patients with early cervical cancer investigated the clinical implication of the IGF system in this malignancy [95]. Notably, the 5-year recurrence free and overall survival rates were significantly lower among patients with high grade IGF1R expression [95]. Moreover, IGF1R expression was an independent predictor of death and recurrence. Treatment with an IGF1R-blocking antibody decreased IGF1R phosphorylation and inhibited tumor growth in SCID mice [95].

8.4 Ovarian cancer

IGFs and their receptors play key roles in regulating the normal biology of ovarian epithelial cells and have been implicated in the transformed phenotype of ovarian carcinoma cells [96]. IGF1, IGF2 and the IGF1R have been shown to be produced *in vitro* by ovarian cancer cell lines, displaying autocrine growth loops mediated through the IGF1R. In a recent study, Brokaw *et al.* [97] analyzed the IGF1 mRNA expression and protein levels in 215 epithelial ovarian cancer (EOC) patients and reported that high IGF1 mRNA values are associated with increased risk of disease progression. Another study investigated the expression of IGF axis genes in relation to EOC outcome using microarray profiles from 64 patients with advanced disease [98]. In this study, IGFBP4 and IGF2/M6PR gene expression were inversely associated with survival. Moreover, the expression patterns of several gene subsets of the IGF family were also associated with prognosis of EOC.

An important role of IGF1R signaling in resistance to chemotherapy in ovarian cancer was recently reported [99]. Specifically, the authors reported that IGF1R expression levels were correlated with cisplatin resistance and IGF1-induced cisplatin resistance. Finally, Gotlieb *et al.* [100] observed a growth inhibition of ovarian cancer cell lines treated with NVP-AEW541, a small molecular weight IGF1R inhibitor (see below), associated with decreased activity of the downstream IGF1R signaling pathway and enhanced apoptosis.

8.5 Endometrial cancer

The elevated estrogen levels in obese women were suggested to explain the relationship between obesity and endometrial cancer. Recent hypotheses regarding this link, however, have focused on hyperinsulinemia, as insulin is a known mitogen. In the uterus, cyclic changes in IGF1 expression and signaling play an important role in regulating the transition of the premenopausal endometrium through proliferative, secretory and menstrual cycles. In addition, a number of studies showed a correlation between components of the IGF system and endometrial cancer risk. For example, Ayabe *et al.* [101] reported higher IGF1 and lower IGFBP1 levels in postmenopausal endometrial cancer patients compared with controls. Petridou *et al.* [102], in a study of 84 endometrial cancer patients and 84 control women, reported that endometrial cancer was positively associated with IGF2 blood levels and inversely associated with IGF1. A recent study including 250 incident endometrial cancer patients and 465 controls from the WHI-OS assessed the association between endometrial cancer risk and serum levels of IGF1, IGFBP3, insulin and estradiol [103]. Low levels of free IGF1 and high levels of insulin were associated with endometrial cancer risk. Both associations were stronger among obese patients.

Consistent with the important role of the IGF axis in endometrial cancer, McCampbell *et al.* [104] reported a significant increase in IGF1R expression in biopsies from hyperplastic endometrium and endometrial carcinoma compared with

proliferative endometrium. Finally, the correlation between IGF1R and IGF2 expression and endometrial cancer stage was investigated in a study that included 59 endometrial adenocarcinomas, 10 endometrial hyperplasias and 7 normal tissues [105]. The expression of IGF1R and IGF2 was much higher in malignant tissue at advanced stages (stages III-IV) compared with early stages or endometrial hyperplasia.

9. Approaches and methodological issues in IGF1R targeted therapy

IGF1R targeting emerged in recent years as a very active area in cancer therapeutics. IGF1R targeting is expected to result in: i) inhibition of IGF1R expression; ii) blockade of ligand–receptor interaction; and/or iii) impairment of receptor activation. Targeting methods are evaluated for their ability to: i) inhibit cancer cell proliferation, survival, and anchorage independent growth *in vitro*; ii) reverse tumor growth and metastases formation *in vivo*; and iii) sensitize cancer cells to chemotherapy, radiotherapy, hormonal and biological therapies. Various experimental methods are currently being employed to downregulate IGF1R expression and signaling (Figure 2). These approaches include, among others, IGF1R antibodies and IGF1R-specific low-molecular-weight tyrosine kinase inhibitors. A number of differences exist between the mechanisms of action of antibodies and kinase inhibitors, leading to different outcomes and, potentially, distinct side effects. Thus, humanized IGF1R antibodies are designed to prevent IGF1 binding, with ensuing receptor degradation whereas tyrosine kinase inhibitors, on the other hand, are designed to inhibit IGF1R's kinase activity without affecting IGF1R expression. A number of selected examples of recent attempts to target the IGF1R are described below.

9.1 IGF1R blocking antibodies

Targeting of the IGF1R with specific monoclonal antibodies has been the most pursued method of blocking IGF signaling employed in clinical investigations to date. The feasibility of this targeting approach was first demonstrated using a mouse monoclonal antibody directed against the IGF1R α -subunit (α -IR3) [106]. It has been suggested that IGF1R antibodies block signaling by two mechanisms: i) abrogation of ligand binding; and ii) induction of receptor internalization and degradation [107]. Several IGF1R antibodies have been developed in recent years and some of them are currently in Phase I and II clinical trials (Table 1) [108]. For example, monoclonal antibody EM164 (Sanofi) has been shown to inhibit IGF1/2-stimulated proliferation and survival of diverse cancer cell lines. Furthermore, its antitumoral effect was enhanced by combined treatment with a cytotoxic agent [109]. Similarly, Wu *et al.* [110], in a study using a human prostate cancer xenograft model, showed that treatment with IM Clone's A12 antibody markedly decreased tumor size and also augmented the inhibitory effect of docetaxel. Another extensively studied IGF1R monoclonal

antibody is CP-751,871 (Pfizer). A recent dose-escalation clinical trial including 47 patients with multiple myeloma revealed no significant response to single agent CP-751,871 therapy. However, 28 patients had stable disease and 9 had objective response when dexamethasone was added to the antibody [111]. In a Phase I study of AMG-479 (Amgen) that enrolled 33 patients, three objective responses and five incidences of stable disease were observed [112]. The dose-limiting toxicity of this agent was thrombocytopenia while additional adverse effects included arthralgia, diarrhea and hyperglycemia.

Several *in vivo* studies evaluated the anti-tumor activity of anti-IGF1R as monotherapy as well as in combination with chemotherapy, radiotherapy or additional antibodies. As mentioned above, the potential effect of IGF1R antibodies on InsR signaling is of special concern given that these antibodies can co-target or alter InsR function, leading to insulin resistance and adverse effects on glucose and carbohydrate metabolism. On the other hand, InsR targeting (and, in particular, isoform A) could become an advantage because specific inhibition of the InsR in the tumor could add to the effective antitumoral activity [113]. Preliminary results from Phase I trials in patients with advanced cancer treated with CP-751,871 or A12 antibodies showed only infrequent mild transient hyperglycemia with no dose-limiting toxicity [114-116].

9.2 Small molecule IGF1R kinase inhibitors

In addition to IGF1R antibodies, a series of small-molecule IGF1R kinase inhibitors have been used in experimental studies demonstrating tumor growth inhibitory properties (Table 1). However, a potential pitfall of these therapies is the fact that they might indiscriminately inhibit the kinase domains of all IGF/insulin receptors, as they share high homology at these domains [84]. Remarkable exceptions are the NVP-ADW742 and NVP-AEW541 pyrrolo[2,3-D]pyrimidine derivatives (Novartis), which display a 15 – 30-fold increased potency for IGF1R kinase inhibition compared with InsR kinase [117,118].

Mitsiades *et al.* [118] reported *in vitro* and *in vivo* antitumoral activity of NVP-ADW742 in multiple myeloma. Similarly, Garcia-Echeverria *et al.* [117] reported *in vivo* antitumoral activity of the NVP-AEW541 compound in the MCF-7 and NWT-21 cell lines. This orally bioavailable compound also inhibited IGF1R signaling in tumor xenografts and significantly reduced the growth of IGF1R-driven fibrosarcomas. In addition, Scotlandi *et al.* [119] reported that NVP-AEW541 effectively inhibited the *in vitro* tumor growth of Ewing sarcoma and displayed a synergistic effect with combined chemotherapy. Hopfner *et al.* [120] showed *in vitro* growth inhibition of gastrointestinal neuroendocrine tumors and hepatocellular carcinoma cells by NVP-AEW541, both alone and in combination with chemotherapy. Finally, Gotlieb *et al.* [100] showed an inhibitory effect of NVP-AEW541 in ovarian cancer cell lines and this activity was associated with decreased Akt phosphorylation and increased PARP cleavage.

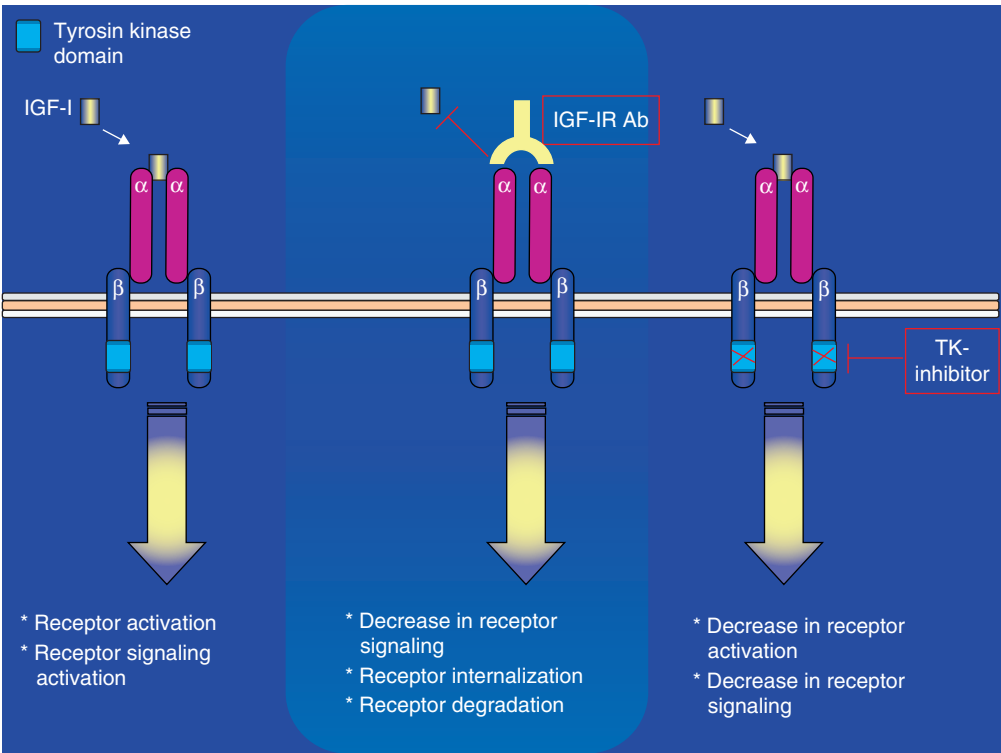


Figure 2. IGF1R targeted therapies. This scheme depicts two of the most commonly used approaches for IGF1R targeting: anti-IGF1R monoclonal antibodies (centre) and small molecular weight IGF1R trosine kinase inhibitors (right). IGF1R blockade by specific antibodies (usually against the extracellular domain) leads to a decrease in ligand binding and IGF1R activation, followed by enhanced receptor internalization and degradation. Low-molecular-weight tyrosine kinase inhibitors prevent IGF1R activation and signaling, without major effect on IGF1R expression.

Table 1. Selected IGF1R monoclonal antibodies and small molecule tyrosine kinase inhibitors.

Compound	Company	Status	Ref.
<i>Monoclonal antibodies</i>			
IMC-A12	IMClone	Phase I/II	[110,115]
CP-751,871	Pfizer	Phase II	[111,114,116]
EM164	Sanofi	Preclinical	[109]
AMG479	Amgen	Phase I	[112]
MK-0646/h7C10	Merck	Preclinical/Phase I	[108]
<i>Tyrosine kinase inhibitors</i>			
NVP-AEW541-ADW742	Novartis	Preclinical	[117-120]
BMS-554417	Bristol-Myers Squibb	Preclinical	[121]
Picropodophyllin (PPP)	Karolinska	Preclinical	[122,123]

Recently, Haluska *et al.* [121] reported the *in vitro* inhibition of colon, ovarian, and breast cancer cells, as well as of the *in vivo* growth of a mouse xenograft, using a dual IGF1R/InsR kinase inhibitor, BMS-554417. This small-molecule compound inhibited both InsR and IGF1R with similar potency. However, at the most effective dose tested, transient hyperglycemia and supraphysiologic elevation of secreted insulin was observed.

Another selective inhibitor of the IGF1R tyrosine kinase is picropodophyllin (PPP). PPP efficiently blocked IGF1R activity and induced apoptosis and tumor regression in a xenograft mouse model [122]. PPP was shown to inhibit the IGF1R, but not the InsR, tyrosine kinase [123]. Finally, INSM18, a small-molecule IGF1R inhibitor that has also activity against HER2, has been shown in clinical trials to be well tolerated

among 15 patients with prostate cancer and showed response in 2 patients [84].

9.3 Molecular approaches to IGF1R targeting

A number of nucleic acid-based strategies, including antisense oligonucleotides and siRNAs, have been employed to target the IGF1R, mainly in experimental systems. For example, antisense oligomers against IGF1R mRNA led to reduced receptor levels and inhibition of IGF signaling in various types of cancer, including lung, breast, prostate and melanoma [9,124]. The clinical use of these approaches, however, has been so far very limited. A pilot study published a few years ago investigated the effect of *ex vivo* treatment of autologous malignant astrocytoma with antisense oligomers against IGF1R mRNA. Results of this study showed that this approach induced apoptosis and a host response *in vivo*, associated with radiographic and clinical improvements, without major side effects [125]. Likewise, injection of antisense oligomers into human psoriasis lesions grafted onto nude mice led to reversal of epidermal hyperproliferation [126].

10. Conclusions

The IGF network has a central role in normal and pathological growth. We have presented evidence suggesting that the mechanism of action of certain oncogenic agents are strongly linked to the IGF signaling pathways. The interplay between cancer genes and the IGF axis may involve oncogenic transactivation of the IGF1R promoter (with ensuing increases in IGF1R mRNA), constitutive activation of the IGF1R kinase domain and downstream mediators by oncogenic agents, transcriptional dysregulation of the IGF1R promoter by mutated tumor suppressors and other mechanisms. As a corollary to the involvement of the IGF axis in cancer biology, targeted therapy of the IGF1R emerged as a biologically plausible approach. Most experimental, preclinical and clinical data generated in the last few years corroborate the hypothesis that the IGF axis and, in particular, the IGF1R are promising targets in cancer therapy.

11. Expert opinion

The huge amount of information accumulated in the IGF field since the early 1980s led to the recognition that the IGF signaling network fulfills a pivotal role in cancer cells. Consequently, this paradigm led to the logical prediction that targeting of the IGF axis may constitute an important strategy in cancer therapy. While early studies attempted to assess the clinical value of various candidate targets along the GH-IGF axis (GH, GHR, IGF ligands, etc), most recent work focused on the IGF1R as a clinically relevant therapeutic target. As discussed in this review, the central role of this receptor as an important mediator of the proliferative and cell survival actions of IGF1 and IGF2 has been amply validated.

Various technologies are currently being employed to downregulate IGF1R expression and signaling. These approaches include, among others, anti-IGF1R antibodies and IGF1R-specific low-molecular-weight tyrosine kinase inhibitors. However, a number of fundamental differences exist between the mechanisms of action of monoclonal antibodies and kinase inhibitors, leading to different outcomes and, potentially, complications. Thus, humanized IGF1R antibodies (e.g., Pfizer's CP-751871, Sanofi's EM164, ImClone's A12, etc) are designed to prevent IGF1 binding, with ensuing receptor degradation. On the other hand, tyrosine kinase inhibitors (e.g., tyrphostins, Novartis' pyrrolo [2,3-D]pyrimidine derivatives, Biovitrum's picropodophyllin, etc) are designed to inhibit IGF1R's kinase activity without affecting IGF1R expression.

While most published preclinical data highlights the potential of IGF1R-targeted therapies, a number of obstacles must be resolved. These difficulties are primarily due to the large similarity between the mature forms of IGF1R and InsR, reaching 84% homology in the kinase domain. In addition, and as indicated above, the downstream pathways elicited by IGF1R and InsR are almost identical. Thus, the potential effect of IGF1R targeting on insulin signaling, leading to potential complications such as the development of insulin resistance, is of special concern. On the other hand, experts in the field advise that combined targeting of both IGF1R and InsR (and hybrid receptors) may have an added value in specific types of cancer.

Anti-IGF1R in combination therapy may also provide a significant advantage over anti-IGF1R monotherapy. Combination therapies may include, in addition to anti-IGF1R, the use of a supplemental biological targeting reagent (e.g., epidermal growth factor receptor antibodies) or conventional chemotherapy and/or radiotherapy. A number of studies have already demonstrated the benefit of the combined approach. It is expected that post genomic technological developments, including analyses of molecular signatures of tumors and identification of biomarkers linked to cancer response, will allow in the near future delivery of targeted therapies in a more rational and personalized fashion.

In summary, the combined efforts of basic scientists and clinicians in the fields of IGF and cancer research, pharmacology, endocrinology, bioinformatics and others, for many years, are paving the way for important translational developments that will undoubtedly impinge on our ability to move on 'from the bench to the bedside'.

Declaration of interest

The authors' work is supported by grants from the Insulin-Dependent Diabetes Trust (IDDT, UK), the US-Israel Binational Science Foundation (BSF, Grant 2003341), and the Israel Cancer Research Fund (ICRF, New York). I Bruchim wishes to thank the Israel Cancer Research Fund (Montreal, Canada) for their generous support.

Bibliography

- Kaplan SA, Cohen P. The somatomedin hypothesis 2007: 50 years later. *J Clin Endocrinol Metab* 2007;92:4529-35
- Werner H, Leroith D. New concepts in regulation and function of the insulin-like growth factors: implications for understanding normal growth and neoplasia. *Cell Mol Life Sci* 2000;57:932-42
- Bartke A. The somatotrophic axis and aging: Mechanisms and persistent questions about practical implications. *Exp Gerontol* 2009;44:372-4
- Klammt J, Pfaffle R, Werner H, et al. IGF signaling defects as causes of growth failure and IUGR. *Trends Endocrinol Metab* 2008;19:197-205
- Samani AA, Yakar S, LeRoith D, et al. The role of the IGF system in cancer growth and metastasis: overview and recent insights. *Endocrine Rev* 2007;28:20-47
- Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. *Nat Rev Cancer* 2008;8:915-28
- Werner H, LeRoith D. The role of the insulin-like growth factor system in human cancer. *Adv Cancer Res* 1996;68:183-223
- Baserga R. The insulin-like growth factor-I receptor as a target for cancer therapy. *Expert Opin Ther Targets* 2005;9:753-68
- Yuen JSP, Macaulay VM. Targeting the type 1 insulin-like growth factor receptor as a treatment for cancer. *Exp Opin Ther Targets* 2008;12:589-603
- LeRoith D, Helman LJ. The new kid on the block(ade) of the IGF-1 receptor. *Cancer Cell* 2004;5:201-2. Erratum in 403
- Cohen P. Overview of the IGF-I system. *Horm Res* 2006;65(Suppl 1):3-8
- Salmon WD, Daughaday WH. A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. *J Lab Clin Med* 1957;49:825-36
- Baserga R, Peruzzi F, Reiss K. The IGF-1 receptor in cancer biology. *Int J Cancer* 2003;107:873-7
- Werner H, Bruchim I. The insulin-like growth factor-I receptor as an oncogene. *Arch Physiol Biochem* 2009;115:58-71
- Lemamy GJ, Sahla ME, Berthe ML, et al. Is the mannose-6-phosphate/IGF-2 receptor coded by a breast cancer suppressor gene? *Adv Exp Med Biol* 2008;617:305-10
- Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. *Endocr Rev* 2002;23:824-54
- Holly J, Perks C. The role of insulin-like growth factor binding proteins. *Neuroendocrinology* 2006;83:154-60
- Jogie-Brahim S, Min HK, Oh Y. Potential of proteomics towards the investigation of the IGF-independent actions of IGFBP3. *Expert Rev Proteomics* 2005;2:71-86
- Werner H, Weinstein D, Bentov I. Similarities and differences between insulin and IGF-I: structures, receptors, and signaling pathways. *Arch Physiol Biochem* 2008;114:17-22
- Werner H, Woloschak M, Stannard B, et al. Insulin-like growth factor receptor: molecular biology, heterogeneity, and regulation. In: LeRoith D, editor, *Insulin-like growth factors: molecular and cellular aspects*. Boca Raton, FL: CRC Press; 1991. p. 17-47
- Zapf A, Hsu D, Olefsky JM. Comparison of the intracellular itineraries of insulin-like growth factor-I and insulin and their receptors in Rat-1 fibroblasts. *Endocrinology* 1994;134:2445-52
- Mastick CC, Brady MJ, Printen JA, et al. Spatial determinants of specificity in insulin action. *Mol Cell Biochem* 1998;182:65-71
- Laviola L, Giorgino F, Chow JC, et al. The adapter protein Grb10 associates preferentially with the insulin receptor as compared with the IGF-I receptor in mouse fibroblasts. *J Biol Chem* 1997;99:830-7
- Holly JMP, Gunnell DJ, Davey Smith G. Growth hormone, IGF-I and cancer. Less intervention to avoid cancer? More intervention to prevent cancer? *J Endocrinol* 1999;162:321-30
- Pollak MN. Insulin-like growth factors and neoplasia. *Novartis Found Symp* 2004;262:84-98
- Cianfarani S, Tedeschi B, Germani D, et al. In vitro effects of growth hormone (GH) and insulin-like growth factor I and II (IGF-I and -II) on chromosome fragility and p53 protein expression in human lymphocytes. *Eur J Clin Invest* 1998;28:41-7
- Brown KW, Villar AJ, Bickmore W, et al. Imprinting mutation in the Beckwith-Wiedemann syndrome leads to biallelic IGF-2 expression through an H19-independent pathway. *Hum Mol Genet* 1996;5:2027-32
- Steenman MJ, Rainier S, Dobry CJ, et al. Loss of imprinting of IGF2 is linked to reduced expression and abnormal methylation of H19 in Wilms' tumor. *Nat Genet* 1994;7:433-9
- Christofori G, Naik P, Hanahan D. A second signal supplied by insulin-like growth factor II in oncogene-induced tumorigenesis. *Nat* 1994;369:414-8
- Resnicoff M, Burgaud J-L, Rotman HL, et al. Correlation between apoptosis, tumorigenesis, and levels of insulin-like growth factor I receptors. *Cancer Res* 1995;55:3739-41
- Sell C, Rubini M, Rubin R, et al. Simian virus 40 large tumor antigen is unable to transform mouse embryonic fibroblasts lacking type 1 insulin-like growth factor receptor. *Proc Natl Acad Sci USA* 1993;90:11217-21
- Liu J-L, Blakesley VA, Gutkind JS, et al. The constitutively active mutant Gα13 transforms mouse fibroblast cells deficient in insulin-like growth factor-I receptor. *J Biol Chem* 1997;272:29438-42
- Bondy CA, Werner H, Roberts CT Jr, et al. Cellular pattern of insulin-like growth factor I (IGF-I) and type I IGF receptor gene expression in early organogenesis: comparison with IGF-II gene expression. *Mol Endocrinol* 1990;4:1386-98
- Bondy CA, Werner H, Roberts CT Jr, et al. Cellular pattern of Type I insulin-like growth factor receptor gene expression during maturation of the rat brain: comparison with insulin-like growth factors I and II. *Neuroscience* 1992;46:909-23
- Werner H, Roberts CT Jr. The IGF-I receptor gene: a molecular target for disrupted transcription factors. *Genes Chromosomes Cancer* 2003;36:113-20
- Almeida A, Muleris M, Dutrillaux B, et al. The insulin-like growth factor I receptor gene is the target for the 15q26 amplicon in breast cancer. *Genes Chromosomes Cancer* 1994;11:63-5
- Baserga R. The contradictions of the insulin-like growth factor 1 receptor. *Oncogene* 2000;19:5574-81
- Werner H, Maor S. The insulin-like growth factor-I receptor gene: a downstream target for oncogene and tumor suppressor action. *Trends Endocrinol Metab* 2006;17:236-42
- Schnarr B, Strunz K, Ohsam J, et al. Down-regulation of insulin-like growth factor-I receptor and insulin receptor

- substrate-1 expression in advanced human breast cancer. *Int J Cancer* 2000;89:506-13
40. Papa V, Pezzino V, Costantino A, et al. Elevated insulin receptor content in breast cancer. *J Clin Invest* 1990;86:1503-10
41. Plymate S, Bae V, Madison L, et al. Re-expression of the type I insulin-like growth factor receptor inhibits the malignant phenotype of simian virus 40 T antigen immortalized human prostate epithelial cells. *Endocrinology* 1997;138:1728-35
42. Hellawell GO, Turner GD, Davies DR, et al. Expression of the type 1 insulin-like growth factor receptor is up-regulated in primary prostate cancer and commonly persists in metastatic disease. *Cancer Res* 2002;62:2942-50
43. Maor S, Mayer D, Yarden RI, et al. Estrogen receptor regulates insulin-like growth factor-I receptor gene expression in breast tumor cells: involvement of transcription factor Sp1. *J Endocrinol* 2006;191:605-12
44. Pandini G, Mineo R, Frasca F, et al. Androgens up-regulate the insulin-like growth factor-I receptor in prostate cancer cells. *Cancer Res* 2005;65:1849-57
45. Kim HJ, Litzemberger BC, Cui X, et al. Constitutively active type I insulin-like growth factor receptor causes transformation and xenograft growth of immortalized mammary epithelial cells and is accompanied by an epithelial-to-mesenchymal transition mediated by NF- κ B and snail. *Mol Cell Biol* 2007;27:3165-75
46. Jones RA, Campbell CI, Wood GA, et al. Reversibility and recurrence of IGF-IR-induced mammary tumors. *Oncogene* 2009; [Epub ahead of print, 20 April]
47. Peterson JE, Jelinek T, Kaleko M, et al. C phosphorylation and activation of the IGF-I receptor in src-transformed cells. *J Biol Chem* 1994;269:27315-21
48. Reiss K, Ferber A, Travalì S, et al. The protooncogene c-myc increases the expression of insulin-like growth factor I and insulin-like growth factor I receptor messenger RNAs by a transcriptional mechanism. *Cancer Res* 1991;51:5997-6000
49. Kim SO, Park JG, Lee YI. Increased expression of the insulin-like growth factor I (IGF-I) receptor gene in hepatocellular carcinoma cell lines: implications of IGF-I receptor gene activation by hepatitis B virus X gene product. *Cancer Res* 1996;56:3831-6
50. Werner H, Bach MA, Stannard B, et al. Structural and functional analysis of the insulin-like growth factor I receptor gene promoter. *Mol Endocrinol* 1992;6:1545-58
51. Werner H, Karnieli E, Rauscher FJ, et al. Wild type and mutant p53 differentially regulate transcription of the insulin-like growth factor I receptor gene. *Proc Natl Acad Sci USA* 1996;93:8318-23
52. Nahor I, Abramovitch S, Engeland K, et al. The p53-family members p63 and p73 inhibit insulin-like growth factor-I receptor gene expression in colon cancer cells. *Growth Horm IGF Res* 2005;15:388-96
53. Idelman G, Glaser T, Roberts CT, et al. WT1-p53 interactions in insulin like growth factor-I receptor gene regulation. *J Biol Chem* 2003;278:3474-82
54. Werner H, Re GG, Drummond IA, et al. Increased expression of the insulin-like growth factor-I receptor gene, IGFIR, in Wilms' tumor is correlated with modulation of IGFIR promoter activity by the WT1 Wilms' tumor gene product. *Proc Natl Acad Sci USA* 1993;90:5828-32
55. Maor SB, Abramovitch S, Erdos MR, et al. BRCA1 suppresses insulin-like growth factor-I receptor promoter activity: potential interaction between BRCA1 and Sp1. *Mol Genet Metab* 2000;69:130-6
56. Abramovitch S, Glaser T, Ouchi T, et al. BRCA1-Sp1 interactions in transcriptional regulation of the IGF-IR gene. *FEBS Lett* 2003;541:149-54
57. Rubinstein M, Idelman G, Plymate SR, et al. Transcriptional activation of the IGF-I receptor gene by the Kruppel-like factor-6 (KLF6) tumor suppressor protein: potential interactions between KLF6 and p53. *Endocrinology* 2004;145:3769-77
58. Yuen JSP, Cockman ME, Sullivan M, et al. The VHL tumor suppressor inhibits expression of the IGF1R and its loss induces IGF1R upregulation in human clear cell renal carcinoma. *Oncogene* 2007;26:6499-508
59. Ohlsson C, Kley N, Werner H, et al. p53 regulates IGF-I receptor expression and IGF-I induced tyrosine phosphorylation in an osteosarcoma cell line: Interaction between p53 and Sp1. *Endocrinology* 1998;139:1101-7
60. Prisco M, Hongo A, Rizzo MG, et al. The insulin-like growth factor I receptor as a physiologically relevant target of p53 in apoptosis caused by interleukin-3 withdrawal. *Mol Cell Biol* 1997;17:1084-92
61. Gnarr JR, Tory K, Weng Y, et al. Mutations of the VHL tumor suppressor gene in renal carcinoma. *Nat Genet* 1994;7:85-90
62. Maor S, Yosepovich A, Papa MZ, et al. Elevated insulin-like growth factor-I receptor (IGF-IR) levels in primary breast tumors associated with BRCA1 mutations. *Cancer Lett* 2007;257:236-43
63. Hankinson SE, Willett WC, Colditz GA, et al. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. *Lancet* 1998;351:1393-6
64. Chan JM, Stampfer MJ, Giovannucci E, et al. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. *Science* 1998;279:563-6
65. Shi R, Berkel HJ, Yu H. Insulin-like growth factor-I and prostate cancer: a meta-analysis. *Br J Cancer* 2001;85:991-6
66. Giovannucci E, Pollak MN, Platz EA, et al. A prospective study of plasma insulin-like growth factor-1 and binding protein-3 and risk of colorectal neoplasia in women. *Cancer Epidemiol Biomarkers Prev* 2000;9:345-9
67. Ma J, Pollack MN, Giovannucci E, et al. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. *J Natl Cancer Inst* 1999;91:620-5
68. Renehan AG, Zwahlen M, Minder C, et al. Insulin-like growth factor-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. *Lancet* 2004;363:1346-53
69. Moore T, Carbajal S, Beltran L, et al. Reduced susceptibility to two-stage skin carcinogenesis in mice with low circulating insulin-like growth factor-I levels. *Cancer Res* 2008;68:3680-8
70. Fletcher O, Gibson L, Johnson N, et al. Polymorphisms and circulating levels in the insulin-like growth factor system and risk of breast cancer: a systematic review. *Cancer Epidemiol Biomarkers Prev* 2005;14:2-19
71. Shi R, Yu H, McLarty J, et al. IGF-I and breast cancer: a meta analysis. *Int J Cancer* 2004;111:418-23
72. Sugumar A, Liu YC, Xia Q, et al. Insulin-like growth factor (IGF)-I and IGF-binding protein 3 and the risk of

- premenopausal breast cancer: a meta-analysis of literature. *Int J Cancer* 2004;111:293-7
73. Baglietto L, English DR, Hopper JL, et al. Circulating insulin-like growth factor-I and binding protein-3 levels and the risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* 2007;16:763-8
 74. Rinaldi S, Peeters PH, Berrino F, et al. IGF-I, IGFBP-3 and breast cancer risk in women: The European Prospective Investigation into Cancer and Nutrition (EPIC). *Endocr Relat Cancer* 2006;13:593-605
 75. Schernhammer ES, Holly JM, Hunter DJ, et al. Insulin-like growth factor-I, its binding proteins (IGFBP-1 and IGFBP-3), and growth hormone and breast cancer risk in The Nurses Health Study II. *Endocr Relat Cancer* 2006;13:583-92
 76. Gunter MJ, Hoover DR, Yu H, et al. Insulin, insulin-like growth factor-I, and risk of breast cancer in postmenopausal women. *J Natl Cancer Inst* 2009;101:48-60
 77. Renehan AG, Harvie M, Howell A. Insulin-like growth factor-I, IGFBP3, and breast cancer risk: eight years on. *Endocr Relat Cancer* 2006;13:273-8
 78. Happerfield LC, Miles DW, Barnes DM, et al. The localization of the insulin-like growth factor receptor 1 (IGFR-1) in benign and malignant breast tissue. *J Pathol* 1997;183:412-7
 79. Lee AV, Hilsenbeck SG, Yee D. IGF system components as prognostic markers in breast cancer. *Breast Cancer Res Treat* 1998;47:295-302
 80. Peyrat J-P, Bonnetterre J, Beuscart R, et al. Insulin-like growth factor I receptors in human breast cancer and their relation to estradiol and progesterone receptors. *Cancer Res* 1988;48:6429-36
 81. Shimizu C, Hasegawa T, Tani Y, et al. Expression of insulin-like growth factor 1 receptor in primary breast cancer: immunohistochemical analysis. *Hum Pathol* 2004;35:1537-42
 82. Ueda S, Tsuda H, Sato K, et al. Alternative tyrosine phosphorylation of signaling kinases according to hormone receptor status in breast cancer overexpressing the IGF receptor type 1. *Cancer Sci* 2006;97:597-604
 83. Belfiore A, Frasca F. IGF and insulin receptor signaling in breast cancer. *J Mammary Gland Biol Neoplasia* 2008;13:381-406
 84. Weroha SJ, Haluska P. IGF1 receptor inhibitors in clinical trials-early lessons. *J Mammary Gland Biol Neoplasia* 2008;13:471-83
 85. Casa AJ, Dearth RK, Litzenburger BC, et al. The type 1 insulin-like growth factor receptor pathway: a key player in cancer therapeutic resistance. *Front Biosci* 2008;13:3273-87
 86. Beech DJ, Parekh N, Pang Y. Insulin-like growth factor-I receptor antagonism results in increased cytotoxicity of breast cancer cells to doxorubicin and taxol. *Oncol Rep* 2001;8:325-9
 87. Gooch JL, Van Den Berg CL, Yee D. IGF1 rescues breast cancer cells from chemotherapy-induced cell death-proliferative and antiapoptotic effect. *Breast Cancer Res Treat* 1999;56:1-10
 88. Ryan CJ, Haqq CM, Simko J, et al. Expression of insulin-like growth factor-I receptor in local and metastatic prostate cancer. *Urol Oncol* 2007;25:134-40
 89. Liao Y, Abel U, Grobholz R, et al. Up-regulation of insulin-like growth factor axis components in human primary prostate cancer correlates with tumor grade. *Hum Pathol* 2005;36:1186-96
 90. Schayek H, Haugk K, Sun S, et al. Tumor suppressor BRCA1 is expressed in prostate cancer and control IGF1-R gene transcription in an androgen receptor-dependent manner. *Clin Cancer Res* 2009;15:1558-65
 91. Steller MA, Delgado CH, Zou Z. Insulin-like growth factor II mediates epidermal growth factor-induced mitogenesis in cervical cancer cells. *Proc Natl Acad Sci USA* 1995;92:11970-4
 92. Steller MA, Zou Z, Schiller JT, et al. Transformation by human papillomavirus 16 E6 and E7: Role of the insulin-like growth factor 1 receptor. *Cancer Res* 1996;56:5087-91
 93. Kuramoto H, Hongo A, Liu YX, et al. Immunohistochemical evaluation of insulin-like growth factor I receptor status in cervical cancer specimens. *Acta Med Okayama* 2008;62:251-9
 94. Harris TG, Burk RD, Yu H, et al. Insulin-like growth factor axis and oncogenic human papillomavirus natural history. *Cancer Epidemiol Biomarkers Prev* 2008;17:245-8
 95. Huang YF, Shen MR, Hsu KF, et al. Clinical implications of insulin-like growth factor 1 system in early-stage cervical cancer. *Br J Cancer* 2008;99:1096-102
 96. Kalli KR, Conover CA. The insulin-like growth factor/insulin system in epithelial ovarian cancer. *Front Biosci* 2003;8:714-22
 97. Brokaw J, Katsaros D, Wiley A, et al. IGF-I in epithelial ovarian cancer and its role in disease progression. *Growth Factors* 2007;25:346-54
 98. Spentzos D, Cannistra SA, Grall F, et al. IGF axis gene expression patterns are prognostic of survival in epithelial ovarian cancer. *Endocr Relat Cancer* 2007;14:781-90
 99. Eckstein N, Servan K, Hildebrandt B, et al. Hyperactivation of the insulin-like growth factor receptor I signaling pathway is an essential event for cisplatin resistance of ovarian cancer cells. *Cancer Res* 2009;69:2996-3003
 100. Gotlieb WH, Bruchim I, Gu J, et al. Insulin-like growth factor receptor I targeting in epithelial ovarian cancer. *Gynecol Oncol* 2006;100:389-96
 101. Ayabe T, Tsutsumi O, Sakai H, et al. Increased circulating levels of IGF-I and decreased circulating levels of IGFBP1 in postmenopausal women with endometrial cancer. *Endocr J* 1997;44:419-24
 102. Petridou E, Koukoulomatis P, Alexe DM, et al. Endometrial cancer and the IGF system: a case-control study in Greece. *Oncology* 2003;64:341-5
 103. Gunter MJ, Hoover DR, Yu H, et al. A prospective evaluation of insulin and insulin-like growth factor-I as risk factors for endometrial cancer. *Cancer Epidemiol Biomarkers Prev* 2008;17:921-9
 104. McCampbell AS, Broaddus RR, Loose DS, et al. Overexpression of the insulin-like growth factor-I receptor and activation of the AKT pathway in hyperplastic endometrium. *Clin Cancer Res* 2006;12:6363-78
 105. Pavelic J, Radakovic B, Pavelic K. Insulin-like growth factor 2 and its receptors (IGF 1R and IGF 2R/mannose 6-phosphate) in endometrial adenocarcinoma. *Gynecol Oncol* 2007;105:727-35
 106. Kull FC, Jacobs S, Su Y-F, et al. Monoclonal antibodies to receptors for insulin and somatomedin-c. *J Biol Chem* 1983;258:6561-6

107. Gennigens C, Menetrier-Caux C, Droz JP. Insulin-like growth factor (IGF) family and prostate cancer. *Crit Rev Oncol Hematol* 2006;58:124-45
108. Hartog H, Wesseling J, Boezen HM, et al. The insulin-like growth factor 1 receptor in cancer: old focus, new future. *Eur J Cancer* 2007;43:1895-904
109. Maloney EK, McLaughlin JL, Dagdigian NE, et al. An anti-insulin-like growth factor-I receptor antibody that is a potent inhibitor of cancer cell proliferation. *Cancer Res* 2003;63:5073-5-83
110. Wu JD, Odman A, Higgins LM, et al. In vivo effects of the human type I insulin-like growth factor receptor antibody A12 on androgen-dependent and androgen-independent xenograft human prostate tumors. *Clin Cancer Res* 2005;11:3065-74
111. Lacy MQ, Alsina M, Fonseca R, et al. Phase I, pharmacokinetic and pharmacodynamic study of the anti-insulinlike growth factor type 1 receptor monoclonal antibody CP-751,871 in patients with multiple myeloma. *J Clin Oncol* 2008;26:3196-203
112. Beltran PJ, Mitchell P, Chung YA, et al. AMG 479, a fully human anti-insulin-like growth factor receptor type I monoclonal antibody, inhibits the growth and survival of pancreatic carcinoma cells. *Mol Cancer Ther* 2009;8:1095-105
113. Hofmann F, Garcia-Echeverria C. Blocking the insulin-like growth factor-I receptor as a strategy for targeting cancer. *Drug Discov Today* 2005;10:1041-7
114. Fong PC, Molife R, Spicer J, et al. Phase I trial involving the pharmacodynamic study of circulating tumour cells, of CP-751,871, a monoclonal antibody against the insulin-like growth factor-I receptor, with docetaxel in patients with advanced cancer. *Eur J Cancer Suppl* 2006;4(12):61-2
115. Higano C, Gordon M, Lorusso P, et al. A Phase I dose escalation study of weekly IMC-A12, a fully human insulin-like growth factor-1 receptor IgG1 monoclonal antibody in patients with advanced cancer. *Eur J Cancer Suppl* 2006;4:195
116. Haluska P, Shaw HM, Batzel GN, et al. Phase I dose escalation study of the anti insulin-like growth factor-I receptor monoclonal antibody CP-751,871 in patients with refractory solid tumors. *Clin Cancer Res* 2007;13:5834-40
117. Garcia-Echeverria C, Pearson MA, Marti A, et al. In vivo antitumor activity of NVP-AEW541-A novel, potent, and selective inhibitor of the IGF-IR kinase. *Cancer Cell* 2004;5:231-9
118. Mitsiades CS, Mitsiades NS, McMullan CJ, et al. Inhibition of the insulin-like growth factor receptor-1 tyrosine kinase activity as a therapeutic strategy for multiple myeloma, other hematologic malignancies, and solid tumors. *Cancer Cell* 2004;5:221-30
119. Scotlandi K, Manara MC, Nicoletti G, et al. Antitumor activity of the insulin-like growth factor-I receptor kinase inhibitor NVP-AEW541 in musculoskeletal tumors. *Cancer Res* 2005;65:3868-76
120. Hopfner M, Huether A, Sutter AP, et al. Blockade of IGF-1 receptor tyrosine kinase has antineoplastic effects in hepatocellular carcinoma cells. *Biochem Pharmacol* 2006;71:1435-48
121. Haluska P, Carboni JM, Loegering DA, et al. In vitro and in vivo antitumor effects of the dual insulin-like growth factor-I/ insulin receptor inhibitor, BMS-554417. *Cancer Res* 2006;66:362-71
122. Girnita A, Girnita L, del Prete F, et al. Cyclolignans as inhibitors of the insulin-like growth factor-1 receptor and malignant cell growth. *Cancer Res* 2004;64:236-42
123. Vasilcanu R, Vasilcanu D, Rosengren L, et al. Picropodophyllin induces downregulation of the insulin-like growth factor 1 receptor: potential mechanistic involvement of Mdm2 and β -arrestin1. *Oncogene* 2008;27:1629-38
124. Hellawell GO, Ferguson DJ, Brewster SF, et al. Chemosensitization of human prostate cancer using antisense agents targeting the type 1 insulin-like growth factor receptor. *BJU Int* 2003;91:271-7
125. Andrews DW, Resnicoff M, Flanders AE, et al. Results of a pilot study involving the use of an antisense oligodeoxynucleotide directed against the insulin-like growth factor type I receptor in malignant astrocytomas. *J Clin Oncol* 2001;19:2189-200
126. Wraight CJ, White PJ, McKean SC, et al. Reversal of epidermal hyperproliferation in psoriasis by insulin-like growth factor I receptor antisense oligonucleotides. *Nat Biotechnol* 2000;18:521-6

Affiliation

Ilan Bruchim^{1,2} MD, Zohar Attias¹ MSc & Haim Werner^{†1} PhD

[†]Author for correspondence

¹Tel Aviv University, Sackler School of Medicine, Department of Human Molecular Genetics and Biochemistry, Tel Aviv 69978, Israel
Tel: +972 3 640 8542; Fax: +972 3 640 6087; E-mail: hwerner@post.tau.ac.il

²Meir Medical Center, Gynecologic Oncology Unit, Department of Obstetrics and Gynecology, Kfar Saba 44281, Israel