EXPERT OPINION

Targeting IGF-1 signaling pathways in gynecologic malignancies

Ilan Bruchim & Haim Werner[†]

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

Introduction: The signaling pathways of the insulin-like growth factors (IGF) have been implicated in the etiology of a number of epithelial neoplasms including prostate, breast, colon and more recently, gynecologic cancers. The insulin-like growth factor-1 receptor (IGF-1R) is expressed in most transformed cells, where it displays potent anti-apoptotic, cell-survival and potentially, transforming activities. IGF-1R expression and activation are typical hallmarks associated with tumor initiation and progression. Multiple approaches have been used to abrogate IGF-1R signaling for targeted cancer therapy including antibodies and small molecule tyrosine kinase inhibitors. These novel IGF-1R targeting agents have produced significant experimental and clinical results in many cancers and generated considerable optimism in the field of cancer therapy.

Areas covered: The authors will review important research advances regarding the role of the IGF axis in cancer, particularly preclinical and clinical studies in cervical, uterine and ovarian cancers. The significance of tumor expression and circulating levels of the IGF pathway as well as targeting therapies of the IGF axis in the gynecologic cancers will be discussed.

Expert opinion: Accumulating data confirm that the IGF-1R pathway has an important role in gynecologic cancers and *in vivo* and *in vitro* studies have shown a significant impact of IGF-1R targeted therapies in these malignancies, mainly ovarian and endometrial cancers. Currently, ongoing preclinical and clinical trials are evaluating the efficacy of IGF-1R targeting. A better understanding of the complex mechanisms underlying the regulation of the IGF system will improve the ability to develop effective treatment modalities for these malignancies.

Keywords: cancer, cervix, gynecological cancer, IGF-1 receptor, insulin-like growth factor-1, ovary, targeted therapies, uterus

Expert Opin. Ther. Targets [Early Online]

1. Introduction

The insulin-like growth factor (IGF) system has an important role in regulating multiple cellular pathways, as well as in tumor initiation and progression. IGF-1 was first isolated in the mid-1950s and the 'insulin-like growth factors' were so named because their structure resembled that of proinsulin. The IGF system comprises ligands (IGF-1, IGF-2), cell-surface receptors (IGF-1 receptor (IGF-1R), IGF-2 receptor (IGF-2R)) and at least six binding proteins (IGFBPs) that control the normal growth and differentiation of most organs. The IGF axis regulates a wide array of physiological processes, including metabolic, nutritional, endocrine, growth and aging events. In addition to their normal actions, experimental, clinical and epidemiological evidence indicate that the IGF signaling pathways are

informa
healthcare

10.1517/14728222.2013.749863 © 2012 Informa UK, Ltd. ISSN 1472-8222, e-ISSN 1744-7631

Article highlights.

- The insulin-like growth factor (IGF) system has an important role in the development of gynecologic cancers.
- IGF-1R and IGF-2 are highly expressed in cervical, endometrial and ovarian cancers.
- IGF-1, IGF-2 and IGF-1R expression levels are associated with disease outcome in the gynecologic malignancies.
- Recent *in vitro* and *in vivo* studies showed significant growth inhibition with IGF-1R-targeted therapies in the gynecologic malignancies.
- IGF-1R *tumor expression and* IGF-1R expression on circulating tumor cells (CTCs) were suggested as possible biomarkers for the efficacy of IGF-1R-targeted therapies.
- Ongoing clinical trials are evaluating the efficacy of IGF-1R inhibitors in ovarian cancer in combination with chemotherapy or other biologic agents.

This box summarizes key points contained in the article.

important mediators in the biochemical and molecular chain of events that lead from a phenotypically normal cell to one harboring neoplastic traits.

The biological actions of the IGFs are mainly mediated by the IGF-1R, a transmembrane tyrosine kinase that is structurally related to the insulin receptor (INSR) [1-3]. The IGFs (IGF-1, IGF-2) are structurally and functionally related to insulin [4] and the IGF-1R displays a large similarity to the INSR. The structural and functional similarities between insulin and IGF-1 suggest that both molecules are derived from a common ancestral precursor that probably participated in food intake and nutritional regulation. Whereas the INSR (A and B isoforms) is mainly involved in metabolic types of action, the IGF-1R mediates primarily growth activities [5]. The downstream signaling the IGF axis is mediated by complex interactions between the IGF-1R, INSR and IGF-1R-INSR hybrid receptors (composed of an IGF-1R hemireceptor linked to an INSR hemireceptor). There is a certain degree of cross-talk between insulin, IGFs and their receptors. Specifically, insulin may interact with IGF-1R with low affinity, thus mediating growth activities, whereas IGFs may stimulate metabolic activities via interaction with the INSR [4]. Recent study showed co-overexpression of IGF-1R and INSR in cancer cells sensitive to anti-IGF-1R antibody (Ab) and demonstrated the presence of IGF-1R/INSR heterodimeric receptors in these cells [6]. The IGF-1R displays potent anti-apoptotic and, potentially, transforming activities and is considered a key factor in cancer development [7,8]. Early studies by Baserga and collaborators demonstrated that fibroblast cells lacking the IGF-1R were resistant to transformation by viral and cellular oncogenes [9,10].

In recent years, the IGF-1R has emerged as a promising therapeutic target and major efforts are being invested to translate experimental and preclinical data into solid medical protocols [11-14]. This review will evaluate the role of the IGF pathway in gynecologic malignancies.

Consistent with the proliferative role of IGF-1, large-scale epidemiological studies performed about 15 years ago suggested that elevated circulating IGF-1 levels were associated with increased risk for several cancers, including breast and prostate tumors [15,16]. In a prospective, nested control study (the Nurse's Health Study), the relative risk of breast cancer in premenopausal women was 4.6 in the upper tertile of IGF-1 values compared with women in the lower tertile. Furthermore, the relative risk increased to 7.3 when IGFBP3 concentrations were included in the analysis. Following these initial findings, numerous epidemiological studies were conducted worldwide, with diverse (and sometimes conflicting) outcomes [17-19]. A comprehensive meta-analysis of 21 studies by Renehan *et al.* concluded that despite certain controversies, circulating IGF-1 values are positively associated with cancer risk in prostate, premenopausal breast and colorectal tumors, although the relative risks were substantially lower than those reported in earlier studies [20]. Hence, although laboratory methods need to be standardized, these epidemiological observations could have major implications for risk assessment and cancer prevention.

The typical features of the IGF-1R include potent anti-apoptotic and mitogenic capacities, important roles in invasion, metastasis and angiogenesis and involvement in oncogenic transformation [1,3,21,22]. **Figure 1** describes the IGF-1R signaling pathway leading to activation of the Ras-Raf-MAP kinase and PI3K-PDKI/PKB downstream pathways. Preclinical observations demonstrated that IGF-1R plays an important role in growth, angiogenesis and metastasis in different human malignancies. Increased local tumor expression of IGF-1, IGF-2 and IGF-1R have been documented in various malignancies, with positive correlations between IGF-1/IGF-2 and IGF-1R expression levels and tumor progression, tumor differentiation and disease stage [23-25]. Recent microarray analyses revealed expression 'signatures' specific for IGF-1 that correlate with poor breast cancer prognosis and with response to anti-IGF-1R inhibitors [26]. However, other studies reported conflicting results regarding the prognostic significance of IGF-1R expression [27]. Finally, regulation of IGF-1R gene expression is attained primarily at the transcriptional level. Evidence was provided that the IGF-1R gene transcription rate depends on a number of stimulatory nuclear proteins [5-8] and is modulated by negative transcriptional regulators, including p53/p63/p73 [28,29] and the breast cancer gene-1 (BRCA1) [30-32]. The level of expression of the IGF-1R gene is ultimately determined by complex interactions between stimulatory and inhibitory transcription factors.

1.1 IGF-1R pathway targeting

As mentioned above, the IGF axis is a very attractive oncological therapeutic target. More than 30 drugs in clinical and laboratory studies and over 60 Phase I – III clinical trials are currently being used to evaluate IGF-1R targeting for cancer

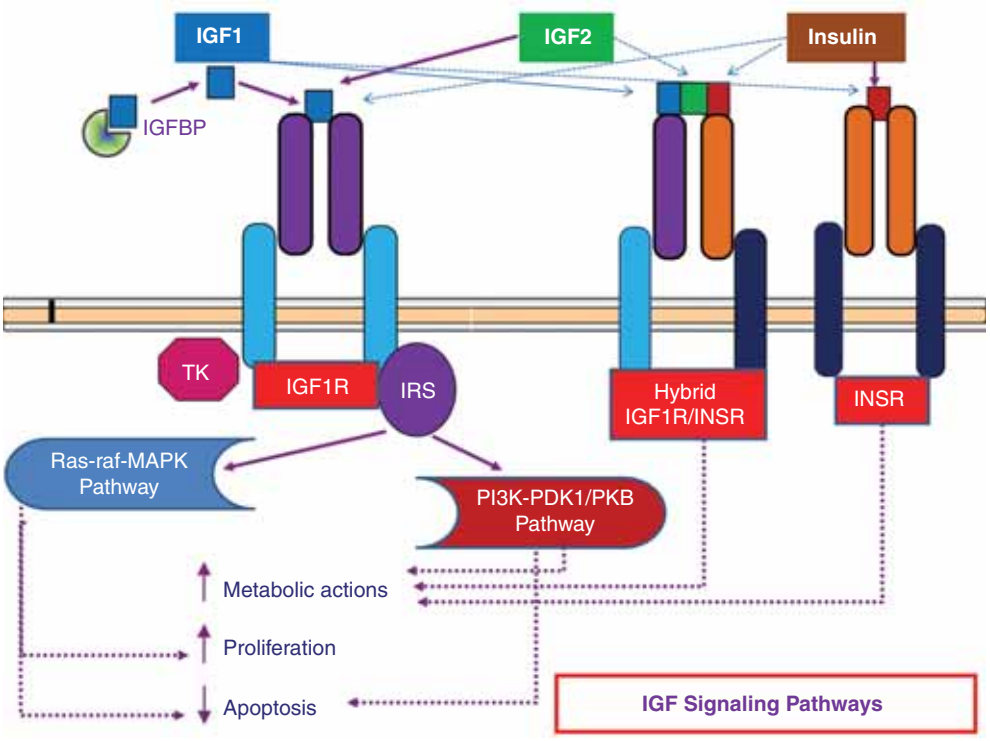

Figure 1. IGF signaling pathways. The biological actions of IGF-1 and IGF-2 are mainly mediated by the IGF-1R and modulated by a family of six IGFBPs. IGF-1/2 bind to the extracellular domain of the IGF-1R and induce autophosphorylation of its tyrosine kinase (TK) domain. After activation of the IGF-1R, the insulin receptor substrates (IRSs) become phosphorylated, leading to activation of two main cascades, the Ras-Raf-MAP kinase and the PI3K-PDK1-Akt/PKB pathways. There is a certain degree of cross-reactivity between ligands and receptors, and IGF-2 was shown to bind with relatively high affinity to the insulin receptor (INSR), A isoform. In addition, both ligands can activate hybrid receptors, composed of an IGF-1R hemireceptor linked to an INSR hemireceptor. 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.

therapy [33]. Three major classes of compounds being studied *in vitro* and in clinical trials are: i) Abs that target the IGF-1R; ii) small molecule tyrosine kinase inhibitors (TKI) that inhibit the IGF-1R kinase activity and iii) Abs that target the IGF ligands. Targeting the IGF-1R with a monoclonal Ab has been the most pursued method of blocking IGF signaling in clinical investigations to date. Most of these Abs induce IGF-1R internalization and degradation. Therefore, it has been suggested that IGF-1R Abs block signaling by two different mechanisms: i) abrogating ligand binding and ii) inducing receptor internalization and degradation [23]. *In vitro* and preclinical models showed that monoclonal Abs targeting the IGF-1R inhibit IGF-I/II-stimulated proliferation of many solid tumors and certain hematologic malignancies [34,35]. Furthermore, the anti-tumoral effect of IGF-1R Abs was enhanced by combined treatment with cytotoxic drugs [36]. *In vivo* studies using xenograft models showed that treatment with anti-IGF-1R markedly decreased tumor size and also augmented chemotherapeutic activity [37].

In recent years, several IGF-1R Abs have been developed, some of which were evaluated in Phase I and II clinical trials as monotherapy, as well as in combination with chemotherapy, radiotherapy and/or additional Abs. Currently, the greatest clinical impact of IGF system signaling inhibition for cancer treatment is prevention or reversal of resistance to anti-cancer therapies. Therapies directed against the IGF-1R were shown to enhance the cytotoxic effects of conventional treatments. Objective response to IGF-1R-targeting monotherapy is generally low [38]; therefore, these targeted therapies are usually expected to show their anti-tumor activity by augmenting the efficacy of cytotoxic and other biologic therapies. In a Phase I study of AMG-479 (ganitumab, Amgen Inc., Thousand Oaks, CA, USA, a monoclonal IGF-1R Ab) that enrolled 33 patients, three had an objective response and five had stable disease. The dose-limiting toxicity of this agent was thrombocytopenia; additional adverse effects included arthralgia, diarrhea and hyperglycemia [39]. Two Phase I trials evaluated the activity of an IgG2 monoclonal Ab targeting the IGF-1R (CP-751,871, figitumumab, Pfizer, New York, USA) in combination with docetaxel or carboplatin and paclitaxel in patients with advanced solid tumors and reported that this drug combination was well tolerated [40]. A Phase II study

recently validated the efficacy of AMG-479 in combination with gemcitabine in patients with pancreatic cancer and reported reasonable toxicity and a trend toward increased survival rates and longer progression intervals with combination treatment versus gemcitabine alone [41]. Phase I and II trials in small cell lung cancer, colorectal, pancreatic and ovarian cancers and in other solid tumors are currently underway. However, few of these IGF-1R Ab trials have progressed to or completed Phase III studies. Two Phase III trials testing figitumumab in conjunction with chemotherapy or combined with EGFR inhibitor (erlotinib) in advanced and in relapsed non-small cell lung cancer, were discontinued after data showed significant side effects [42].

Although most of these Abs do not bind to the INSR, some partially cross-react with the INSR leading to hyperglycemia in clinical studies. The potential effect of IGF-1R Abs on INSR signaling is of special concern, given that these Abs 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 could be potentially advantageous, because specific inhibition of the INSR in the tumor might increase the effective anti-tumoral activity [43]. Preliminary results from Phase I trials in patients with advanced cancer treated with CP-751,871 or IM Clone's A12 Abs showed only infrequent, mild, transient hyperglycemia with no dose-limiting toxicity [44,45].

In addition to IGF-1R Abs, a series of small-molecule IGF-1R TKIs have demonstrated tumor growth inhibitory properties in experimental studies. In general, these therapies indiscriminately inhibit both IGF-1R and INSR kinase domains, as these enzymatic domains are closely related [46]. However, several TKIs are more selective to the IGF-1R and have a 15- to 30-fold increased potency for IGF-1R kinase inhibition compared with INSR kinase inhibition [47]. NVP-AEW541 (Novartis International AG, Basel, Switzerland), an orally available, small molecule IGF-1R-specific TKI, inhibited IGF-1R signaling in tumor xenografts and significantly reduced the growth of IGF-1R-driven sarcomas [47]. In addition, another selective inhibitor of the IGF-1R TK, picropodophyllin (PPP), blocked IGF-1R activity and induced apoptosis and tumor regression in a xenograft mouse model [48]. Of importance, PPP was shown to inhibit the IGF-1R TK, but not the INSR [49]. INSM18 is a small molecule inhibitor of the IGF-1R that also acts against the HER2 receptor TK. In a clinical trial, this therapy appeared to be well tolerated among 15 patients with prostate cancer and showed response in 2 patients [46]. Only one IGF-targeting TKI, OSI-906 (linsitinib, OSI Pharmaceuticals, Melville, NY, USA), an orally available, small molecule has progressed to a Phase III trial and is being evaluated as a monotherapy in patients with advanced adrenocortical carcinoma [42].

Finally, several *in vivo* studies demonstrated that the inhibition of the IGF-1 and IGF-2 ligands using neutralizing Abs, resulted in potent antitumor activity and offers an effective approach to selectively target both the IGF-1R and INSR-A signaling pathways [50]. However, only MEDI-573, a fully human Ab that neutralizes IGF-1 and IGF-2 and inhibits IGF signaling through both the IGF-1R and INSR-A pathways, progressed into clinical studies (advanced solid tumors (NCT00816361) and metastatic breast cancer (NCT01446159)). To the best of authors' knowledge, this targeted strategy has never been evaluated in gynecologic malignancies.

1.2 Biomarkers for IGF-1R targeting

Growing clinical evidence suggests potential correlations between biomarkers related to the IGF-1R pathway and clinical benefits from IGF-1R-targeted therapies. High IGF-1R expression and elevated circulating IGF-1 levels were shown to be correlated with improved response to IGF-1R-targeted therapies in clinical trials [51,52]. Interestingly, IGF-1R expression on circulating tumor cells (CTCs) was associated with response to targeting IGF-1R (CP-751,871) in patients with advanced prostate cancer [53]. A recent study reported that increased nuclear localization of IGF-1R is associated with better overall survival for patients treated with IGF-1R Ab therapy [54]. Pretreatment levels of insulin receptor substrate-1 (IRS-1) were also reported to be correlated with response to IGF-1R targeting [55].

Circulating levels of IGFs or IGFBPs may be predictive for IGF-1R targeting, although it seems more likely that IGF tumor expression will have more significant predictive values of response. Tumor expression of IGF-1R/INSR/IRS-1 or BRCA1/2 and p53 may be correlated with IGF-1R targeting. Moreover, an association with IGF-1R in CTCs was suggested. Measuring tumor expression requires invasive methods, although isolation of CTCs still has technical limitations. Further studies of IGF expression in the tumors and in CTCs in patients treated with IGF-1R inhibitors will confirm their value as biomarkers for IGF-1R targeting.

2. Gynecologic cancers

Several studies have identified the IGF system as an important player in the development of gynecologic tumors. For example, in a study of 46 endometrial, 32 cervical and 20 ovarian cancers, Hirano *et al.* [56] reported that IGF-1R mRNA was highly expressed in 91, 100 and 87.5% of the gynecologic tumors, respectively. The authors suggested that IGF-1R signals might be involved in the growth of gynecologic tumors. The roles of the IGF-1R in cervical, endometrial and ovarian cancers are summarized below.

3. Cervical cancer

Early studies suggested a possible role for the IGF system in cervical tumorigenesis. Overexpression of the IGF-1R in primary cervical cancer cultures and cell lines compared with normal cervical cells was shown by Steller *et al.* [57]. Moreover, this group found elevated IGF-2 mRNA levels following EGF stimulation in the cervical cancer cell line HT-3, and suggested that autocrine production of IGF-2 and

overexpression of IGF-1R play an important role in mediating cervical cancer. The increase in IGF-2 levels was inhibited in a dose-dependent manner by IGFBP5, which binds to the IGF-2 and neutralizes its effect [58].

3.1 Circulating IGF levels

Mathur *et al.* [59] evaluated the serum levels of IGF-2 and IGFBP3 in patients with cervical cancer, cervical intraepithelial neoplasia (CIN) and in normal controls. Serum IGF-2 levels were elevated in patients with CIN and cervical cancers compared with controls. Of note, in patients successfully treated for CIN and cervical cancers, the IGF-2 levels returned to normal. Serum IGFBP3 showed a significant decrease in advanced stage disease and there was an inverse relationship between IGF-2 and IGFBP3 levels. The authors suggested that IGF-2 might be a marker for early diagnosis, while IGFBP3 might be a prognostic marker for patients with cervical cancer. Another study evaluated IGF-1 and IGFBP3 plasma levels in 44 patients with cervical cancer, 82 with CIN and 40 with a normal cervix [60]. They showed that the plasma levels of IGF-1 and the IGF-1:IGFBP3 ratio were significantly higher in patients with CIN compared with controls. There was, however, no significant association with cancer. The authors suggested that IGF-1 and the IGF-1:IGFBP3 ratio might be useful for early detection of cervical cancer. Finally, Serrano *et al.* reported that low levels of IGF-1 and low IGF-1:IGFBP3 ratio might be associated with cervical cancer [61]. Later, this group found that IGF-2 levels were significantly lower in cervical cancer patients versus controls and that IGFBP3 was significantly higher in high-grade squamous intraepithelial lesions (HGSIL) versus controls [62].

3.2 IGF tissue levels

Mathur *et al.* [63] measured IGF-2 levels in gynecological cancers (cervix, endometrium and ovary) using immunofluorescence and found significantly higher levels in cervical cancer biopsies compared with normal cervical biopsies. They also reported a significant correlation between IGF-2 levels in the tumors and pelvic lymph node metastases. In addition, small nests of malignant cells were identified in the lymph nodes by using IGF-2 as a marker.

3.3 IGF and human papilloma virus

Kuramoto *et al.* investigated the expression levels and activation status of IGF-1R in patients with CIN and cervical cancer [64]. IGF-1R expression was significantly elevated in CIN-III and invasive cancer specimens. Furthermore, IGF-1R phosphorylation was enhanced in all CIN and invasive cancer specimens and its intensity was related to tumor promotion. The authors suggested that human papilloma virus (HPV) infection contributes to the up-regulation of IGF-1R expression in cervical cancer and to tumor initiation and progression. Serrano *et al.* [65] showed that cervical cancer cell lines differ in the type of insulin and IGF-I receptors expressed according to the HPV status. Hence, HPV-positive cells express IGF-1R, INSR-A, INSR-B and INSR/IGF hybrid receptors, while HPV-negative cells express only INSR-A. Finally, Harris *et al.* assessed the correlation between total IGF-1 and IGFBP3 levels and the incidence of cervical oncogenic HPV and CIN in 137 women [66]. A high IGF-1:IGFBP3 ratio was associated with increased persistence of oncogenic HPV infection and women with high serum IGFBP3 levels had significantly lower rates of oncogenic HPV detection and HPV-positive CIN.

3.4 IGF-1R regulation in cervical cancer

The effect of IGF-1R signaling on cervical cancer formation was investigated in cell lines and *in vivo*. Shen *et al.* reported that the growth and invasiveness of cervical cancer cells stimulated by IGF-1 were dependent on IGF-1 dose [67]. Klopp *et al.* [68] investigated early gene expression changes after chemoradiation in patients with cervical cancer. They demonstrated that 262 genes were significantly changed and that the IGF-1R pathway was one of the important regulated signaling pathways. Moreover, IGF-2 gene expression was lower in patients with recurrent disease compared with those without recurrence. The clinical implications of the involvement of the IGF system in this malignancy were evaluated by Huang *et al.* [69]. They reported that the 5-year recurrence-free and overall survival rates were significantly lower among patients with high grade expression of IGF-1R and that this parameter was also an independent predictor of death and recurrence.

3.5 Targeted therapies

Treatment with IGF-1R blocking Ab significantly inhibited tumor growth and caused tumor regression in an SCID (severe combined immunodeficiency) mice model of cervical cancer. Moreover, IGF-1R phosphorylation and downstream Akt and Erk1/2 were remarkably decreased in animals treated with blocking IGF-1R Abs [67]. These data suggest that the IGF-1R pathway plays an important role in promoting the development and progression of cervical cancer.

4. Endometrial cancer

Endometrial cancer is the most widespread gynecologic cancer in Western countries, with a 2.5% lifetime risk of developing it [70]. Endometrial cancers are classified into two major groups, Type I and Type II. Type I tumors are estrogen-related and account for more than 80% of cases [71,72]. Type I tumors usually have an endometrioid, well-differentiated morphology and are associated with a relatively good prognosis as opposed to Type II tumors, which display a less differentiated phenotype and have a worse prognosis. Uterine serous papillary endometrial carcinoma (USPC) constitutes the predominant histological class among Type II tumors. It represents 10% of all endometrial carcinomas, is considered to be high grade and has a poorer prognosis than endometrioid tumors [73].

Diabetes mellitus type 2, a condition associated with chronic endogenous insulin excess, is a well-established risk factor for certain types of cancer, including endometrial tumors [74]. Moreover, at least 40% of endometrial cancers can be attributed to excess body weight. Recent epidemiological studies have demonstrated that the metabolic syndrome and its components (obesity, hyperlipidemia and high blood pressure) constitute risk factors not only for diabetes and cardiovascular diseases, but also for cancer [75]. Several studies have demonstrated that the insulin/IGF pathway is a major player in the chain of events linking the metabolic syndrome with cancer [1,2,76-78]. In addition, chronic hyperinsulinemia is a major link between obesity, lack of physical activity and development of ovarian androgen excess. Insulin resistance is an important potential risk factor for endometrial cancer. The high levels of circulating insulin, a consequence of the insulin resistance, exert both direct and indirect effects that possibly contribute to the development of endometrial cancer. Insulin directly promotes cell proliferation and survival through activation of the PI3K/Akt and Ras/MAPK pathways. Indirectly, insulin leads to changes in sex hormones, including increased estrogen levels [79]. In this context, recent studies showed a possible increased cancer risk in patients treated with human insulin and insulin analogs. Hemkens *et al.* [80] analyzed a data set that included 127,031 patients from Germany and showed a positive association between cancer incidence and insulin dose for all insulin types. Similar overall trends were reported in a Swedish study based on 114,841 diabetic patients and a Scottish study that included 49,197 patients [81,82].

IGF-1, which shares an extensive structural homology and downstream signaling pathways with insulin, has also gathered interest as a risk factor for endometrial carcinoma. Likewise, as mentioned above, the IGF-1R displays a large similarity to the INSR. In addition, several studies demonstrated a functional cross-talk between IGF-1 and estrogen signaling [83,84]. It was shown that IGF-1 induced activation of estrogen receptor α (ERα) in a ligand-independent manner and that activated IGF-1R interacts with ERα and stimulates cancer progression [85].

4.1 Circulating IGF levels

Cyclic changes in IGF-1 expression and signaling play an important role in regulating the transition of the premenopausal endometrium through proliferative, secretory and menstrual cycles. Several studies showed a significant role for IGF-1R action in endometrial cancer and emphasized the importance of altered IGF-1R gene expression in the development of a malignant phenotype [86-89]. Ayabe *et al.* [90] reported higher IGF-1 and lower IGFBP1 levels in postmenopausal patients with endometrial cancer compared with controls. Petridou *et al.* [91] reported that endometrial cancer was positively associated with IGF-2 serum levels and inversely associated with IGF-1. Another case-cohort study that included 250 incident endometrial cancer patients and 465 controls assessed the association between endometrial cancer risk and serum levels of IGF-1, IGFBP3, insulin and estradiol [92]. Low levels of free IGF-1 and high insulin levels were associated with endometrial cancer risk after adjustments for age, hormone therapy use and estradiol levels. Both associations were stronger among obese patients, especially the association between insulin and endometrioid adenocarcinoma. By contrast, other studies did not show any evidence of an overall association between endometrial cancer risk and serum levels of IGF-1, IGF-2, IGFBP1, IGFBP3 and insulin [93]. In summary, the data regarding the significance of circulating levels of the IGF components in endometrial cancer are inconsistent and more research is needed.

4.2 IGF tissue levels

Consistent with a central role of IGF-1R in endometrial cancer, McCampbell *et al.* [94] reported a significant increase in IGF-1R expression in biopsies from hyperplastic endometrium and endometrial carcinoma compared with proliferative endometrium. Likewise, Hirano *et al.* [56] reported high expression of IGF-1R in all gynecological cancers, with mRNA expression in 91.3% of endometrial cancers. The correlation of IGF-1R and IGF-2 expression with endometrial cancer stage was investigated in a study that included specimens from 59 cases of endometrial adenocarcinoma, 10 of endometrial hyperplasia and 7 normal controls [95]. IGF-1R and IGF-2 expressions were much higher in advanced stage (stages III – IV) malignant tissue compared with early stages or endometrial hyperplasia. Finally, a recent study investigated the association between IGF-1R and VEGF-C expressions and lymphatic metastasis in 40 endometrial adenocarcinoma tumors and 14 normal endometrium samples using immunohistochemistry [96]. IGF-1R expression was associated with histological grade and with lymph node metastasis, but not with surgical stage. Moreover, IGF-1R and VEGF-C expressions were correlated in endometrial adenocarcinoma and lymphatic vessel density was closely related to both. The authors concluded that abnormal IGF-1R and VEGF-C expressions might be important markers in investigating lymph node metastasis of endometrial adenocarcinoma and might be potentially used to evaluate the prognosis. In summary, these studies suggest that IGF-1R and IGF-2 expressions are associated with poor outcome in endometrial cancer.

4.3 IGF regulation in endometrial cancer

Polymorphisms in genes associated with the IGF pathway might affect gene expression and protein function and influence cancer risk. A large case–control study investigated the associations between 44 polymorphisms within the IGF-1, IGF-2 and IGFBP3 genes and endometrial cancer risk, using 692 invasive endometrial cancer cases and 1923 matched controls [97]. A significant inverse association with two IGF-2 gene polymorphisms (rs3741211 and rs100446) and an IGFBP3 gene polymorphism (rs2453839) and endometrial cancer risk was observed. However, polymorphisms

in the IGF-1 and IGFBP1 genes were not associated with endometrial cancer risk.

Studies from the authors' group have shown that the IGF-1R system is regulated by the p53 and BRCA1/2 pathways in a number of malignancies, including endometrial cancer. p53 was shown to inhibit IGF-1R expression via a mechanism that involves repression of the IGF-1R promoter [88]. They also demonstrated that uterine serous carcinoma (USC) tumors overexpressing p53 are more likely to be affected by anti-IGF-1R therapies. In addition, they recently reported high protein expression of BRCA1 and IGF-1R in primary and metastatic USC tumors [89]. Moreover, they showed that BRCA1 suppresses IGF-1R gene expression and IGF-1R activity in USC cell lines. These findings suggest a possible biological link between the BRCA1 and the IGF-1 signaling pathways in USC.

4.4 Targeted therapy

In recent studies, the authors investigated the effect of IGF-1R targeting in Type I (endometrioid) and Type II (USPC) endometrial cancer cell lines. IGF-1R targeting with monoclonal Abs IMC-A12 (cixutumumab, Imclone Systems, Inc., New York, NY, USA), MK-0646 (dalotuzumab, Merck, Whitehouse Station, NJ, USA) and NVP-AEW-541 (a specific IGF-1R TKI, Novartis) inhibited IGF-induced proliferation in both types of endometrial carcinomas [98,99]. The results demonstrated that treatment with IGF-1R inhibitors blocked IGF-1-induced autophosphorylation of the IGF-1R. Moreover, it was shown that IGF-1R inhibitors abrogated IGF-1-stimulated proliferation and increased apoptosis. Mendivil *et al.* showed that a human monoclonal IGF-1R Ab, currently in Phase II study [100], inhibited endometrial cancer proliferation, predominantly through inhibition of the Akt and MAPK signaling pathways. Shu *et al.* demonstrated that RNA interference (RNAi)-mediated down-regulation of IGF-1R expression in endometrial carcinoma significantly decreased tumorigenesis and induced apoptosis [101]. Finally, to investigate the impact of IGF-1R activation on the development of chemotherapeutic resistance in endometrial cancer cells, a recent study evaluated the significance of lentivirus-mediated small or short hairpin RNA (shRNA) IGF-1R down-regulation on chemosensitivity [102]. Results obtained showed that cell proliferation was significantly inhibited in the transfected cells and that IGF-1R down-regulation resulted in sensitization to cisplatin. Taken together, these results suggest that IGF-1R inhibition is a promising therapeutic tool against endometrial cancer.

5. Ovarian cancer

Ovarian cancer is the second most common gynecological cancer and is the leading cause of death among all gynecological cancers in the Western word [70]. Ninety percent of ovarian malignancies are of epithelial origin. Most patients present with advanced disease and have a poor prognosis. The management of epithelial ovarian cancer (EOC) includes either primary or neo-adjuvant surgical cytoreduction and combined chemotherapy. Unfortunately, although primary treatment achieves high response rates, 75% of patients relapse with incurable disease [103]. Presently, there is no cure available for patients with recurrent ovarian cancer and new treatment approaches are needed to improve the outcomes of these patients.

Current research in advanced and recurrent ovarian cancer focuses on inhibition of signal transduction pathways and DNA repair mechanisms. The major advance in targeted therapy for EOC to date has been among agents that target angiogenesis. Bevacizumab (a VEGF-A monoclonal Ab, Avastin, Genentech, Inc., San Fransisco, CA, USA) has demonstrated significant activity in Phase II and III trials as first-line therapy (ICON-7, GOG-218) [104] and for recurrent disease (OCEANS trial) [105] with reasonable side effects. Other important research in ovarian cancer treatment is focused on targeting DNA repair with poly-ADP-ribose polymerase (PARP) inhibition, with several PARP inhibitors being evaluated in clinical trials.

IGFs and their receptors are known to play key roles in regulating the normal biology of ovarian surface epithelial cells and have been implicated in the transformed phenotype of ovarian carcinoma cells. A number of studies have shown expression of IGF-1, IGF-2, IGF-1R and IGFBPs in ovarian cancer cells [106,107]. In addition, it was reported that ovarian cancer cells display an autocrine growth loop mediated through the IGF-1R [107]. Ongoing clinical trials are currently evaluating the efficacy of IGF-1R inhibition in ovarian cancer patients.

5.1 Circulating IGF levels

Circulating IGF-1 and IGF-2 may play a potentially important role in the development of ovarian cancer. Two prospective studies reported a significant correlation between IGF-1 serum levels and ovarian cancer risk among women younger than 55 years at diagnosis [108]. Whereas, several retrospective studies found that serum IGF-1 levels in patients with malignant ovarian tumors were lower than in controls [109]. Serin *et al.* observed significantly decreased serum IGF-1 and IGFBP3 concentrations in 47 postmenopausal patients with ovarian tumors (23 malignant, 24 benign), compared with 31 controls [110]. Another large study of 222 cases and 599 controls, using data from three prospective control cohorts (the Nurses Health Study (NHS, NHSII) and the Women's Health Study (WHS)) did not find any significant association between IGF system proteins (IGF-1, IGFBP2, IGFBP3) and ovarian cancer risk [111].

Genetic variations in the IGF-1, IGFBP1 and IGFBP3 genes were evaluated in relation to ovarian cancer risk by genotyping 29 haplotype-tagged single nucleotide polymorphisms (SNP) in 1173 cases and 1201 controls from the New England Case–Control study and NHS [112]. Interestingly, the results indicated that some haplotypes and

SNPs in IGF pathway genes might be associated with ovarian cancer risk. Of particular interest was the IGFBP3 SNP rs2270628, which was associated both with increased IGF-1 plasma levels and with increased risk of ovarian carcinoma. Another large, three-center study, which included 1135 patients with ovarian cancer, 321 women with borderline epithelial tumors and 1880 controls, assessed the association between tag SNPs of the IGF signaling axis and the risk of ovarian cancer [113]. Five tag SNPs in the IGF-2 gene were associated with risk of ovarian cancer, while there were no associations with variations in the IGF-1, IGFBP1 and IGFBP3 genes. Finally, a recent study showed that IGF-2 gene methylation was associated with low mRNA expression in a promoter-specific manner and that promoter 3 methylation and expression appeared to be critical parameters in ovarian cancer [114]. The authors suggested that DNA methylation regulates IGF-2 promoter-specific expression in ovarian cancer and this regulation may play a role in disease progression.

5.2 IGF tissue levels

Several studies observed overexpression of IGF ligands and IGF-1R in ovarian cancer tissues [115-118]. Thus, IGF-1 and IGF-1R transcripts were identified in 100% of freshly isolated ovarian cancer specimens [119]. IGF-1 levels were found to be higher in cystic fluid from invasive malignant ovarian neoplasms compared with that from benign neoplasms [120]. Using quantitative reverse transcription polymerase chain reaction (qRT-PCR), Sayer *et al.* found significantly higher IGF-2 mRNA levels in 109 EOCs compared with eight normal ovaries [121]. Moreover, high IGF-2 gene expression was associated with high-grade advanced stage disease and poor survival. The expression of IGF-1 and IGF-1R was also evaluated in low-grade serous ovarian cancer cells [122]. mRNA analysis and immunostaining revealed significantly higher IGF-1 expression in low-grade serous ovarian cancer compared with serous borderline ovarian tumors or high-grade ovarian cancer cells. In addition, low-grade ovarian cancer cells were more sensitive to IGF-1 stimulation and IGF-1R inhibition than were high-grade ovarian cancer cells. In summary, these studies suggest that high IGF-2 and IGF-1 mRNA expression are associated with poor survival.

5.3 Outcome in relation to IGF axis

Sayer *et al.* reported that IGF-2 gene expression was associated with high-grade advanced stage disease and poor survival [121]. Later, Brokaw *et al.* analyzed the IGF-1 mRNA expression and protein levels in 215 patients with EOC and reported that high IGF-1 mRNA and protein levels were associated with increased risk of disease progression [123]. Another study investigated the expression of IGF axis genes in relation to outcome using microarray profiles from 64 patients with advanced EOC [124]. In this study, IGFBP4 and IGF-2 receptor gene expression were inversely associated with survival. Eckstein *et al.* recently reported on an important role of the IGF-1R signaling pathway in chemotherapy resistance in ovarian cancer [125]. Specifically, they demonstrated that IGF-1R expression levels were correlated with cisplatin resistance. Treatment with small molecule inhibitors showed that IGF-1R and PI3K were essential for cisplatin resistance. Finally, Huang *et al.* demonstrated that taxol-resistant cells have higher IGF-2 expression and that IGF pathway inhibition sensitizes drug-resistant ovarian carcinoma cells to taxol [126]. This study also reported that high IGF-2 tumor expression is significantly correlated with tumor grade, advanced stage disease and shorter survival. Such novel findings suggest that the IGF-1R pathway represents a therapeutic target in ovarian cancer, particularly in regard to chemotherapy resistance.

5.4 Targeted therapies

Clinical trial data are accumulating for anti-angiogenic therapy, including VEGF inhibitors as well as PARP inhibitors. Other targeted therapies, including the IGF-1R inhibitors are in earlier phases of development for ovarian cancer. In an attempt to find improvements in ovarian cancer therapy, Gest *et al.* evaluated the effectiveness of 10 biological agents in decreasing ovarian cancer cell aggressiveness, using models based on the capacity for invasion and vasculogenic mimicry [127]. Interestingly, among the agents tested, inhibitors of IGF-1R, Stat3 and Rho GTPase were found to be the most promising. NVP-AEW541, a small molecule IGF-1R inhibitor, demonstrated anti-proliferative activity in ovarian cancer cells [107] in association with decreased activity of the IGF-1R downstream signaling pathway. The same group showed that BMS-536924, an IGF-1R inhibitor, sensitizes ovarian cancer cells to the PARP inhibitor, 3-aminobezamide [128]. The authors suggested that the combination of IGF-1R inhibitor with a PARP inhibitor might be an effective strategy to decrease resistance to treatment in clinical settings. A recent study observed up-regulation of IGF-1 in tumor and stromal cells in a xenograft model treated with a VEGF inhibitor (bevacizumab) [129]. Dual anti-VEGF and IGF blockade with bevacizumab and cixutumumab resulted in increased inhibition of tumor growth and increased tumor cell apoptosis.

Currently, several clinical studies are evaluating the activity of IGF-1R inhibitors in ovarian cancer. **Table 1** summarizes the current ongoing targeted IGF-1R therapy clinical trials that include patients with ovarian cancer. Two Phase II studies are currently evaluating the efficacy and safety of AMG-479 as a second-line therapy in patients with recurrent platinum-sensitive ovarian cancer (NCT00719212) and in combination with standard chemotherapy agents as first-line therapy (NCT00718523). Another Phase I/II trial is studying intermittent and continuous therapy with OSI-906, a dual TKI of IGF-1R and INSR, in combination with weekly paclitaxel in patients with recurrent EOC (NCT00889382). Combination therapy of IGF-1R inhibitor MK-0646 with either mTOR (mammalian target of rapamycin) inhibitor or Akt inhibitor is being evaluated in a Phase I

Table 1. Clinical trials with selected IGF-1R monoclonal Abs and small molecule TKIs, enrolling patients with EOC.

Compound	Population	Phase	Combination	Company	Clinicaltrials.gov Identifier
Monoclonal Abs					
MK-0646	Resistant metastatic or recurrent EOC	I	mTOR or Akt inhibitors	Merck	NCT01243762
AMG-479	First-line therapy EOC	II	Carboplatin and taxol	Amgen	NCT00718523
AMG-479	Recurrent platinum-sensitive EOC	II	None	Amgen	NCT00719212
TKIs					
BMS-754807	Advanced solid tumors	I	Carboplatin and taxol	Bristol-Myers Squibb	NCT00793897
OSI-906	Recurrent/relapsed EOC and other solid tumors	II	Taxol	OSI	NCT00889382

Ab: Antibody; EOC: Epithelial ovarian cancer; mTOR: Mammalian target of rapamycin; TKI: Tyrosine kinase inhibitor.

study in patients with advanced cancers, with preliminary anti-tumor subgroup assessment in patients with resistant metastatic or recurrent ovarian cancer (NCT01243762). It was recently reported that these targeted combinations of MK-0646 with MK-2206 or with MK-0752 are both tolerable (Brana *et al.*, Abstract no. 3027, ASCO 2012). Combination of epidermal growth factor (HER) inhibitor with IGF-1R inhibitor (CP-751,87, figitumumab) was feasible and has revealed potential clinical benefit in patients with solid tumors (Calvo *et al.*, Abstract no. 3026, ASCO 2010). Combined treatment of another small molecule IGF-1R inhibitor (BMS-754807, Bristol-Myers Squibb, New York, USA), with paclitaxel and carboplatin in patients with advanced or metastatic solid tumors, including ovarian cancer is also being evaluated in a Phase I study (NCT00793897). Finally, another Phase I trial is studying the side effects of a DNA plasmid based on advanced ovarian cancer (NCT01322802) vaccine encoding amino acids 1 – 163 of IGFBP2 in patients.

6. Expert opinion

It is currently well accepted that the IGF network plays a crucial role in normal cell development, as well as in establishing and maintaining malignant phenotypes. The interplay between the IGF axis and cancer genes may involve oncogenic transactivation of the IGF-1R promoter, activation of the IGF-1R kinase domain and downstream mediators by oncogenic agents, as well as deregulation of the IGF-1R promoter by mutated tumor suppressors and other mechanisms. As a consequence of the involvement of the IGF axis in cancer initiation and progression, targeted IGF-1R therapy has emerged as a biologically plausible approach. In this review, the authors presented evidence demonstrating that the etiology of gynecologic malignancies, including cervical, uterine and ovarian cancers, is closely linked to the IGF system. Specifically, accumulating data confirm an important role of the IGF-1R pathway in gynecologic cancers and ongoing preclinical and clinical trials are evaluating the efficacy of IGF-1R targeting.

Overexpression of the IGF-1R was demonstrated in all gynecologic cancer cell lines and in tumor tissues. In endometrial cancer, IGF-1R expression was associated with advanced stage, histologic grade and lymph node metastasis. Correlation between IGF-1R expression and survival was also demonstrated in cervical cancer. High IGF-2 protein and mRNA expression levels were reported in cervical, endometrial and ovarian cancer tissues. Increased IGF-2 expression was associated with disease progression and survival in endometrial and ovarian cancer. These studies also demonstrated a correlation between IGF-1R expression and chemosensitivity. Data regarding IGF serum levels in gynecologic malignancies are inconsistent. Several studies reported significant correlation of serum IGF levels, while others found no correlation.

The expression and action of the IGF regulatory network depend on multiple factors, ranging from environmental causes, hormonal interactions, ligand availability and genetic characteristics. In cervical cancer, for example, it was suggested that oncogenic HPV up-regulated the IGF-1R pathway [64]. In endometrial cancer, tumor suppressors p53 and BRCA were demonstrated to have an important role in IGF-1R pathway regulation [79,80]. In this context, loss-of-function mutation of BRCA1 in ovarian cancer was shown to abolish its tumor protective action, leading to constitutive activation of the IGF-1R signaling pathway.

Various technologies are currently being employed to down-regulate IGF-1R expression and signaling. These approaches include, among others, anti-IGF-1R Abs and IGF-1R-specific small molecule TKIs. Both *in vitro* and *in vivo* studies have shown a significant impact of IGF-1R targeted therapies in gynecologic cancers, mainly ovarian and endometrial cancers. Moreover, it was demonstrated that IGF-1R inhibition enhanced sensitivity to chemotherapy, suggesting that combination therapies with IGF-1R may provide significant advantages over monotherapy.

Until recently, only a few clinical trials assessed the activity of IGF-1R targeted therapies in gynecologic malignancies.

Ongoing Phase I – II studies are evaluating the activity of IGF inhibitors as a single agent or in combination with chemotherapy or other biologic agents in patients with primary or recurrent ovarian cancer. Moreover, several Phase I – III clinical studies that evaluated the safety profile and response rates to IGF-1R inhibitors in patients with solid tumors also enrolled patients with gynecologic cancers, most with advanced or recurrent disease. While some of these clinical trials were disappointing in that they resulted in minimal response and had a number of side effects, others were encouraging, demonstrating effectiveness mainly in combined therapies. Therefore, identification of the subset of patients with tumor histology and specific biomarker profiles that can predict responsiveness to IGF-1R-targeted therapy will significantly improve the rate of success of these therapies. In summary, the IGF axis has emerged as a promising therapeutic target both in gynecologic cancers and in cancer therapy, in general. Further basic and clinical research is needed to achieve better therapeutic outcomes.

Acknowledgements

The authors would like to thank F Schreiber MS, Meir Medical Center, Kfar Saba, Israel for assistance with English language editing.

Declaration of interest

Work in the laboratory of H Werner and I Bruchim is supported by grants from the US–Israel Binational Science Foundation, Israel Science Foundation, Israel Cancer Association, Insulin-Dependent Diabetes Trust (IDDT, UK) and Israel Cancer Research Fund (ICRF, Montreal, Canada). The authors declare no other conflict of interest.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer 2008;8:915-28
2. LeRoith D, Yakar S. Mechanisms of disease: metabolic effects of growth hormone and insulin-like growth factor-1. Nat Clin Pract Endocrinol Metab 2007;3:302-10
3. Werner H, Bruchim I. The insulin-like growth factor-I receptor as an oncogene. Arch Physiol Biochem 2009;115:58-71
4. 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
5. LeRoith D, Werner H, Beitner-Johnson D, Roberts CT Jr. Molecular and cellular aspects of the insulin-like growth factor I receptor. Endocr Rev 1995;16:143-63
6. Kim JG, Kang MJ, Yoon YK, et al. Heterodimerization of glycosylated insulin-like growth factor-1 receptors and insulin receptors in cancer cells sensitive to anti-IGF1R antibody. PLoS One 2012;7:e33322
7. Chitnis MM, Yuen JSP, Protheroe AS, et al. The type I insulin-like growth factor-I receptor pathway. Clin Cancer Res 2008;14:6364-70
8. Werner H. The pathophysiological significance of IGF-I receptor overexpression: new insights. Ped Endocrinol Rev 2009;7:2-5
9. 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
10. Sell C, Dumenil G, Deveaud C, et al. Effect of a null mutation of the insulin-like growth factor I receptor gene on growth and transformation of mouse embryo fibroblasts. Mol Cell Biol 1994;14:3604-12
11. Scotlandi K, Picci P. Targeting insulin-like growth factor 1 receptor in sarcomas. Curr Opin Oncol 2008;20:419-27
12. Yuen JS, Macaulay VM. Targeting the type 1 insulin-like growth factor receptor as a treatment for cancer. Exp Opin Ther Targets 2008;12:589-603
13. Bruchim I, Attias Z, Werner H. Targeting the IGF1 axis in cancer proliferation. Exp Opin Ther Targets 2009;13:1179-92
14. Gualberto A, Pollak M. Emerging role of insulin-like growth factor receptor inhibitors in oncology: early clinical trial results and future directions. Oncogene 2009;28:3009-21
15. 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
16. 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
17. 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
18. 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
19. 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
20. 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
21. Baserga R. The IGF-I receptor in cancer research. Exp Cell Res 1999;253:1-6
22. Piecewicz SM, Pandey A, Roy B, et al. Insulin-like growth factors promote vasculogenesis in embryonic stem cells. PLoS One 2012;7(2):e32191
23. Samani AA, Yakar S, LeRoith D, Brodt P. The role of the IGF system in cancer growth and metastasis: overview and recent insights. Endocr Rev 2007;28:20-47

24. Werner H, LeRoith D. The role of the insulin-like growth factor system in human cancer. Adv Cancer Res 1996;68:183-223

25. Kuklinski A, Kamocki Z, Koda M, et al. IGF-IR in patients with advanced colorectal cancer in correlation with certain clinico-morphological factors: initial report. Oncol Lett 2011;2:1155-9

26. Boone DN, Lee AV. Targeting the insulin-like growth factor receptor: developing biomarkers from gene expression profiling. Crit Rev Oncog 2012;17:161-73

27. 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

28. Werner H, Karnieli E, Rauscher FJ III, LeRoith D. 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

29. Nahor I, Abramovitch S, Engeland K, Werner H. 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

30. 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 Gen Metab 2000;69:130-6

31. Abramovitch S, Glaser T, Ouchi T, Werner H. BRCA1-Sp1 interactions in transcriptional regulation of the IGF-IR gene. FEBS Lett 2003;541:149-54

32. Abramovitch S, Werner H. Functional and physical interactions between BRCA1 and p53 in transcriptional regulation of the IGF-IR gene. Horm Metab Res 2003;35:758-62

33. King ER, Wong KK. Insulin-like growth factor: current concepts and new developments in cancer therapy. Recent Pat Anticancer Drug Discov 2012;7:14-30

34. Gualberto A, Pollak M. Clinical development of inhibitors of the insulin-like growth factor receptor in oncology. Curr Drug Targets 2009;10:923-36

35. Bruchim I, Attias Z, Werner H. Targeting the IGF1 axis in cancer proliferation. Expert Opin Ther Targets 2009;13:1179-92

36. 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;63:5073-83

37. 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

38. Reidy-Lagunes DL, Vakiani E, Segal MF, et al. A phase 2 study of the insulin-like growth factor-1 receptor inhibitor MK-0646 in patients with metastatic, well-differentiated neuroendocrine tumors. Cancer 2012; [Epub ahead of print]

39. 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

40. Molife LR, Fong PC, Paccagnella L, et al. The insulin-like growth factor-I receptor inhibitor figitumumab (CP-751,871) in combination with docetaxel in patients with advanced solid tumours: results of a phase Ib dose-escalation, open-label study. Br J Cancer 2010;103:332-9

41. Kindler HL, Richards DA, Garbo LE, et al. A randomized, placebo-controlled phase 2 study of ganitumab (AMG 479; or conatumumab (AMG 655;in combination with gemcitabine in patients with metastatic pancreatic cancer. Ann Oncol 2012; [Epub ahead of print]

42. Gao J, Chang YS, Jallal B, Viner J. Targeting the insulin-like growth factor axis for the development of novel therapeutics in oncology. Cancer Res 2012;721:3-12

43. Hofmann F, Garcia-Echeverria C. Blocking the insulin-like growth factor-I receptor as a strategy for targeting cancer. Drugs Discov Today 2005;10:1041-7

44. Fong PC, Molife R, Spicer J, et al. Phase I trial involving the pharmacodynamic study of circulating tumour cells, of CP-751,871 (C), a monoclonal antibody against the insulin-like growth factor-I receptor, with docetaxel in patients with advanced cancer. Eur J Cancer Suppl 2006;4:61

45. Higano C, Gordon M, LoRusso P, et al. A phase 1 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

46. Weroha SJ, Haluska P. IGF-1 receptor inhibitors in clinical trials-early lessons. J Mammary Gland Biol Neoplasia 2008;13:471-83

47. 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

48. 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

49. 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 beta-arrestin1. Oncogene 2008;27:1629-38

50. Gao J, Chesebrough JW, Cartlidge SA, et al. Dual IGF-I/II-neutralizing antibody MEDI-573 potently inhibits IGF signaling and tumor growth. Cancer Res 2011;71:1029-40

51. Olmos D, Martins AS, Jones RL, et al. Targeting the insulin-like growth factor 1 receptor in Ewing's sarcoma: reality and expectations. Sarcoma 2011;2011:402508

52. Gualberto A, Hixon ML, Karp DD, et al. Pre-treatment levels of circulating free IGF-1 identify NSCLC patients who derive clinical benefit from figitumumab. Br J Cancer 2011;104:68-74

53. de Bono JS, Attard G, Adjei A, et al. Potential applications for circulating tumor cells expressing the insulin-like growth factor-I receptor. Clin Cancer Res 2007;13:3611-16

54. Asmane I, Watkin E, Alberti L, et al. Insulin-like growth factor type 1 receptor (IGF-1R) exclusive nuclear staining: a predictive biomarker for IGF-1R

monoclonal antibody (Ab) therapy in sarcomas. Eur J Cancer 2012;48(16):3027-35

55. Baserga R. The insulin receptor substrate-1: a biomarker for cancer? Exp Cell Res 2009;315:727-32

56. Hirano S, Ito N, Takahashi S, Tamaya T. Clinical implications of insulin-like growth factors through the presence of their binding proteins and receptors expressed in gynecological cancers. Eur J Gynaecol Oncol 2004;25:187-91

•• **Reported overexpression of IGF-1R in gynecological cancers (cervical, uterine, ovarian).**

57. Steller MA, Delgado CH, Bartels CJ, et al. Overexpression of the insulin-like growth factor-1 receptor and autocrine stimulation in human cervical cancer cells. Cancer Res 1996;56:1761-5

• **The first to report overexpression of IGF-1R in cervical cancer.**

58. 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

59. Mathur SP, Mathur RS, Underwood PB, et al. Circulating levels of insulin-like growth factor-II and IGF-binding protein 3 in cervical cancer. Gynecol Oncol 2003;91:486-93

60. Lee SW, Lee SY, Lee SR, et al. Plasma levels of insulin-like growth factor-1 and insulin-like growth factor binding protein-3 in women with cervical neoplasia. Gynecol Oncol 2010;21:174-80

61. Serrano ML, Romero A, Cendales R, et al. Serum levels of insulin-like growth factor-I and -II and insulin-like growth factor binding protein 3 in women with squamous intraepithelial lesions and cervical cancer. Biomedica 2006;26:258-68

62. Serrano ML, Sanchez-Gomez M, Bravo MM. Cervical scrapes levels of insulin-like growth factor-II and insulin-like growth factor binding protein 3 in women with squamous intraepithelial lesions and cervical cancer. Horm Metab Res 2010;42:977-81

63. Mathur SP, Landen CP, Datta SM, et al. Insulin-like growth factor II in gynecological cancers: a preliminary study. Am J Reprod Immunol 2003;49:113-19

• **Evaluated IGF-2 expression in gynecologic cancers and found significantly high levels in cervical cancer.**

64. 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

65. Serrano ML, Sanchez-Gomez M, Bravo MM, et al. Differential expression of IGF-I and insulin receptor isoforms in HPV positive and negative human cervical cancer cell lines. Horm Metab Res 2008;40:661-7

66. 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

67. Shen MR, Hsu YM, Hsu KF, et al. Insulin-like growth factor 1 is a potent stimulator of cervical cancer cell invasiveness and proliferation that is modulated by alphavbeta3 integrin signaling. Carcinogenesis 2006;27:962-71

68. Klopp AH, Jhingran A, Ramdas L, et al. Gene expression changes in cervical squamous cell carcinoma after initiation of chemoradiation and correlation with clinical outcome. Int J Radiat Oncol Biol Phys 2008;71:226-36

69. 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

•• **Identified significant correlations between the IGF-1R system and cervical cancer death and recurrence.**

70. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2009. CA Cancer J Clin 2009;59:225-49

71. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol 1983;15:10-17

72. Lax SF. Molecular genetic pathways in various types of endometrial carcinoma: from a phenotypical to a molecular-based classification. Virchows Arch 2004;444:213-23

73. Boruta DM II, Gehrig PA, Groben PA, et al. Uterine serous and grade 3 endometrioid carcinomas: is there a survival difference? Cancer 2004;101:2214-21

74. Soliman PT, Wu D, Tortolero-Luna G, et al. Association between adiponectin, insulin resistance, and endometrial cancer. Cancer 2006;106:2376-81

75. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 2004;4:579-91

76. Arai Y, Kojima T, Takayama M, Hirose N. The metabolic syndrome, IGF-1, and insulin action. Mol Cell Endocrinol 2009;299:124-8

77. Belfiore A, Frasca F. IGF and insulin receptor signaling in breast cancer. J Mammary Gland Bio Neoplasia 2008;13:381-406

78. Belfiore A, Malaguarnera R. The insulin receptor and cancer. Endocr Relat Cancer 2011; [Epub ahead of print]

79. Nagamani M, Stuart CA. Specific binding and growth-promoting activity of insulin in endometrial cancer cells in culture. Am J Obstet Gynecol 1998;179:6-12

80. Hemkens LG, Grouven U, Bender R, et al. Risk of malignancies in patients with diabetes treated with human insulin or insulin analogues: a cohort study. Diabetologia 2010;53:209-11

81. Jonasson JM, Ljung R, Talback M, et al. Insulin glargine use and short-term cancer incidence-a population based follow-up in Sweden. Diabetologia 2009;52:1745-54

82. Colhoun H; SDRN Epidemiology Group. Insulin glargine use and cancer rates in Scotland. Diabetologia 2009;52:1755-65

83. Mawson A, Lai A, Carroll JS, et al. Estrogen and insulin/IGF-1 cooperatively stimulate cell cycle progression in MCF-7 breast cancer cells through differential regulation of c-Myc and cyclin D1. Mol Cell Endocrinol 2005;229:161-73

84. De Marco P, Bartella V, Vivacqua A, et al. Insulin-like growth factor-I regulates GPER expression and function in cancer cells. Oncogene 2012; [Epub ahead of print]

85. Mendoza RA, Enriquez MI, Mejia SM, et al. Interactions between IGF-I, estrogen receptor-alpha (ERalpha), and ERbeta in regulating growth/apoptosis of

MCF-7 human breast cancer cells. J Endocrinol 2011;208:1-9

86. Hirano S, Ito N, Takahashi S, Tamaya T. Clinical implications of insulin-like growth factors through the presence of their binding proteins and receptors expressed in gynecological cancers. Eur J Gynaecol Oncol 2004;25(2):187-91

87. McCampbell AS, Broaddus RR, Loose DS, Davies PJ. Overexpression of the insulin-like growth factor I receptor and activation of the AKT pathway in hyperplastic endometrium. Clin Cancer Res 2006;12(21):6373-8

88. Attias-Geva Z, Bentov I, Kidron D, et al. p53 Regulates insulin-like growth factor-I receptor gene expression in uterine serous carcinoma and predicts responsiveness to an insulin-like growth factor-I receptor-directed targeted therapy. Eur J Cancer 2012;48:1570-80

89. Amichay K, Kidron D, Attias-Geva Z, et al.1 is expressed in uterine serous carcinoma (USC) and controls insulin-like growth factor I receptor (IGF-IR) gene expression in USC cell lines. Int J Gynecol Cancer 2012;22(5):748-54

90. 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

91. 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

• **Demonstrated a significant association of IGF-1 and IGF-2 serum levels with endometrial cancer.**

92. 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

93. Weiderpass E, Brismar K, Bellocco R, et al. Serum levels of insulin-like growth factor-I, IGF-binding protein 1 and 3, and insulin and endometrial cancer risk. Br J Cancer 2003;89:1697-704

94. McCampbell AS, Broaddus RR, Loose DS, Davies PJ. 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

95. 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

•• **Showed the association of IGF-1, IGF-2 and their receptors with the progression of endometrial adenocarcinomas.**

96. Pengchong H, Tao H. Expression of IGF-1R, VEGF-C and D2-40 and their correlation with lymph node metastasis in endometrial adenocarcinoma. Eur J Gynaecol Oncol 2011;32:660-4

97. McGrath M, Lee IM, Buring J, De Vivo I. Common genetic variation within IGFI, IGFII, IGFBP-1, and IGFBP-3 and endometrial cancer risk. Gynecol Oncol 2011;120:174-8

98. Attias-Geva Z, Bentov I, Fishman A, et al. Insulin-like growth factor-I receptor inhibition by specific tyrosine kinase inhibitor NVP-AEW541 in endometrioid and serous papillary endometrial cancer cell lines. Gynecol Oncol 2011;121:383-9

• **Reports the efficacy of IGF-1R targeting therapy in endometrial cancer.**

99. Attias-Geva Z, Bentov I, Ludwig DL, et al. Insulin-like growth factor-I receptor (IGF-IR) targeting with monoclonal antibody cixutumumab (IMC-A12) inhibits IGF-I action in endometrial cancer cells. Eur J Cancer 2011;47:1717-26

100. Mendivil A, Zhou C, Cantrell LA, et al. AMG 479, a novel IGF-1-R antibody, inhibits endometrial cancer cell proliferation through disruption of the PI3K/Akt and MAPK pathways. Reprod Sci 2011;18:832-41

101. Shu S, Li X, Yang Y, et al. Inhibitory effect of siRNA targeting IGF-1R on endometrial carcinoma. Int Immunopharmacol 2011;11:244-9

102. Shu S, Yang Y, Li X, et al. Down-regulation of IGF-1R expression inhibits growth and enhances chemosensitivity of endometrial carcinoma in vitro. Mol Cell Biochem 2011;353:225-33

103. du Bois A, Luck HJ, Meier W, et al. A randomized clinical trial of cisplatin/paclitaxel versus carboplatin/paclitaxel as first-line treatment of ovarian cancer. J Natl Cancer Inst 2003;95:1320-9

104. Perren TJ, Swart AM, Pfisterer J, et al. A phase 3 trial of bevacizumab in ovarian cancer. N Engl J Med 2011;365:2484-96

105. Aghajanian C, Blank SV, Goff BA, et al. OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. J Clin Oncol 2012;30:2039-45

106. Yee D, Morales FR, Hamilton TC, Von Hoff DD. Expression of insulin-like growth factor I, its binding proteins, and its receptor in ovarian cancer. Cancer Res 1991;51:5107-12

107. 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

• **Reported anti-neoplastic activity of IGF-1R inhibitor and suggested that the IGF-1R is a potential therapeutic target in ovarian cancer.**

108. Lukanova A, Lundin E, Toniolo P, et al. Circulating levels of insulin-like growth factor-I and risk of ovarian cancer. Int J Cancer 2002;101:549-54

• **Found a strong direct relationship between circulating IGF-1 levels and risk of developing EOC before age 55.**

109. Bese T, Nomir SK. The importance of serum insulin-like growth factor-I level determination in the follow-up of patients with epithelial ovarian cancer. Eur J Gynaecol Oncol 2001;22:372-6

110. Serin IS, Tanriverdi F, Yilmaz MO, et al. Serum insulin-like growth factor (IGF)-I, IGF binding protein (IGFBP)-3, leptin concentrations and insulin resistance in benign and malignant epithelial ovarian tumors in postmenopausal women. Gynecol Endocrinol 2008;24:117-21

111. Tworoger SS, Lee IM, Buring JE, et al. Insulin-like growth factors and ovarian cancer risk: a nested case-control study in three cohorts. Cancer Epidemiol Biomarkers Prev 2007;16:1691-5

112. Terry KL, Tworoger SS, Gates MA, et al. Common genetic variation in IGF1, IGFBP1 and IGFBP3 and ovarian cancer risk. Carcinogenesis 2009;30:2042-6

113. Pearce CL, Doherty JA, Van Den Berg DJ, et al. Genetic variation in insulin-like growth factor

2 may play a role in ovarian cancer risk. Hum Mol Genet 2011;20:2263-72

114. Qian B, Katsaros D, Lu L, et al. IGF-II promoter specific methylation and expression in epithelial ovarian cancer and their associations with disease characteristics. Oncol Rep 2011;25:203-13
- **Suggested that DNA methylation regulates IGF-2 promoter-specific expression in ovarian EOC and is associated with tumor progression.**

115. Ouban A, Muraca P, Yeatman T, Coppola D. Expression and distribution of insulin-like growth factor-1 receptor in human carcinomas. Hum Pathol 2003;34:803-8

116. Weigang B, Nap M, Bittl A, Jaeger W. Immunohistochemical localization of insulin-like growth factor 1 receptors in benign and malignant tissues of the female genital tract. Tumour Biol 1994;15:236-46

117. Beck EP, Russo P, Gliozzo B, et al. Identification of insulin and insulin-like growth factor I (IGF I) receptors in ovarian cancer tissue. Gynecol Oncol 1994;53:196-201

118. Zhang F, Liu J, Lin B, et al. Increase in docetaxel-resistance of ovarian carcinoma-derived RMG-1 cells with enhanced expression of Lewis Y Antigen. Int J Mol Sci 2011;12:7323-34

119. Waksmanski B, Dudkiewicz J, Kowalski T. Changes in insulin-like growth factor I, 17-b-estradiol, and progesterone in postmenopausal women with benign and malignant ovarian tumours. Med Sci Monit 2001;7:919-23

120. Resnicoff M, Ambrose D, Coppola D, Rubin R. Insulin-like growth factor-1 and its receptor mediate the autocrine proliferation of human ovarian carcinoma cell lines. Lab Invest 1993;69:756-60

121. Sayer RA, Lancaster JM, Pittman J, et al. High IGF-2 gene expression is an independent predictor of poor survival for patients with advanced stage serous epithelial ovarian cancer. Gynecol Oncol 2005;96:355-61
- •• **Showed significantly high expression of IGF-2 in ovarian cancer is associated with grade, stage and survival in EOC tumors. They suggested that IGF-2 is a molecular marker and potential therapeutic target in ovarian cancer.**

122. King ER, Zu Z, Tsang YT, et al. The insulin-like growth factor 1 pathway is a potential therapeutic target for low-grade serous ovarian carcinoma. Gynecol Oncol 2011;123:13-18

123. 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

124. 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
- **Reported that tumor gene expression patterns are prognostic of survival in patients with EOC.**

125. Eckstein N, Servan K, Hildebrandt B, et al. Hyperactivation of the insulin-like growth factor receptor 1 signaling pathway is an essential event for cisplatin resistance of ovarian cancer cells. Cancer Res 2009;69:2996-3003
- **Suggested that targeting the IGF-1R can reduce platinum resistance in ovarian cancer cells.**

126. Huang GS, Brouwer-Visser J, Ramirez MJ, et al. Insulin-like growth factor 2 expression modulates Taxol resistance and is a candidate biomarker for reduced disease-free survival in ovarian cancer. Clin Cancer Res 2010;16:2999-3010
- **Showed that IGF-2 tumor expression is correlated with stage and survival in EOC and demonstrated that IGF-II modulates taxol resistance.**

127. Gest C, Mirshahi P, Li H, et al. Ovarian cancer: stat3, RhoA and IGF-IR as therapeutic targets. Cancer Lett 2012;317:207-17

128. Beauchamp MC, Knafo A, Yasmeen A, et al. BMS-536924 sensitizes human epithelial ovarian cancer cells to the PARP inhibitor, 3-aminobenzamide. Gynecol Oncol 2009;115:193-8
- **Reported anti-neoplastic activity of IGF-1R inhibitor and suggested that the IGF-1R is a potential therapeutic target in ovarian cancer.**

129. Shao M, Hollar S, Chambliss D, et al. Targeting the insulin growth factor and the vascular endothelial growth factor pathways in ovarian cancer. Mol Cancer Ther 2012;11:1576-86
- **Reported anti-neoplastic activity of IGF-1R inhibitor and suggested that the IGF-1R is a potential therapeutic target in ovarian cancer.**

Affiliation

Ilan Bruchim[1,2] & Haim Werner[†1] MD
[†]Author for correspondence
[1]Department of Human Molecular Genetics and Biochemistry,
Sackler School of Medicine,
Tel Aviv University,
Tel Aviv, Israel
Tel: +972 3 6408542;
Fax: +972 3 6406087;
E-mail: hwerner@post.tau.ac.il
[2]Gynecologic Oncology Unit,
Department of Obstetrics and Gynecology,
Meir Medical Center,
Kfar Sava, Israel