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Interleukin-6 as a Therapeutic Target on Human Cancer

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ABSTRACT

Interleukin-6 (IL-6) is a proinflammatory cytokine produced by various cells. Emerging evidence shows that IL-6 plays critical roles in cancer development, progression and metastasis by regulating the tumor microenvironment and cancer stem cells. In the past few years, anti-IL-6 has become a hot spot for cancer research. Several monoclonal antibodies (mAbs) developed for anti-IL-6/IL-6 receptor (IL-6R) therapy such as siltuximab (anti-IL-6), have shown to have potential benefits treating various human cancers either as a single agent or in combination with other chemotherapy drugs. In clinical studies on tocilizumab (anti-IL-6R) for treatment of cancer, combination therapy with IL-6 blockade and conventional drugs achieved better treatment efficacy and patient responses compared to monotherapy. New strategies such as combination of IL-6 blockade and epidermal growth factor receptor **(EGFR)** inhibition or other targeted therapy may be helpful to improve IL-6 targeted immunotherapy of human cancer.

Keywords: Interleukin-6 (IL-6); Interleukin-6 receptor (IL-6R); Monoclonal antibody (mAb); human cancer

Interleukin-6 (IL-6) is a proinflammatory cytokine that has pleiotropic effects. To date, a role for IL-6 has been implicated in almost every cancer examined [1]. It is mainly produced by stroma cells, hematopoietic cells, epithelial cells, or muscle cells. Upon binding of IL-6 to IL-6 receptor (IL-6R, also called the IL-6Rα), the IL-6/IL-6R complex recruits glycoprotein 130 (gp130, sometimes called the IL-6Rβ) to form a hexameric IL-6/IL-6R/gp130 complex composed of two IL-6, two IL-6R, and two gp130 subunits that initiate downstream signaling, including JAK/STATs, Ras/MEK/ERK and PI3K/Akt [2, 3]. This IL-6 signaling via the membrane IL-6R and gp130 has been termed classic-signaling. An alternative to classic-signaling has recently been described, termed trans-signaling, in which a complex is formed between IL-6 and a soluble form of IL-6R, sIL-6R, which then joining events [4]. Classic-signaling is limited to a few cell types since membrane IL-6R is only expressed on hepatocytes and immune cells. In contrast, sIL-6R, which is generated by alternative splicing and/or proteolysis, can bind to IL-6 and elicit trans-signaling in all cells due to ubiquitous expression of membrane gp130 [5-7]. IL-6 plays an important role in immune responses and repair processes through classic-signaling, and may be involved in the pathogenesis of inflammatory diseases and cancers through trans-signaling. However, the full range of biological functions of IL-6 mediated by classic and trans-signaling remains to be elucidated [8].

Emerging evidence shows that IL-6 plays critical roles in cancer development, progression and metastasis by regulating the tumor microenvironment and cancer stem cells [7]. Therefore, the IL-6 signaling pathway represents an attractive target for therapeutic or preventive intervention. In agreement, anti-IL-6 therapies can decrease inflammation, hepatic acute phase proteins, and anemia as well as have antiangiogenic effects. The inhibition of C-reactive protein (CRP) production is a trustworthy surrogate marker of anti-IL-6 therapy efficacy [9]. The use of murine or humanized monoclonal antibodies (mAbs) in clinical investigations on cancers started in the early 1990's. Clinically registered IL-6 inhibitors include monoclonal antibodies (mAbs) to IL-6 (siltuximab) or IL-6R (tocilizumab), and other inhibitors are being investigated in clinical trials. Current IL-6 targeted therapies of cancer focus on monotherapy using mAbs against IL-6/IL-6R, and combination therapy of IL-6/IL-6R mAbs with conventional hemotherapy agents. In this review, we detail the progress of the current anti-IL-6 therapy for various human cancers.

HEMATOLOGIC MALIGNANCIES

Multiple Myeloma (MM)

IL-6 was recognized in the late 1980s as an important growth factor for MM cells, being produced mainly by the tumor environment and also by MM cells [10-12]. However, current data show that insulin-like growth factor-1 **(IGF-1)** is the major growth factor for MM cells, and that IL-6 is active mainly in CD45-positive MM cells [13, 14]. The phosphatase CD45 dephosphorylates IGF-1 receptor **(IGF-1R)** in MM cells and weakens IGF-1R signaling, making MM cells more dependent on IL-6 [13]. Besides IGF-1 and IL-6, other MM cell growth factors have been described, including mainly BAFF/APRIL, produced by osteoclasts [15], heaptocyte growth factor **(HGF)** and vascular

endothelial growth factor **(VEGF)** [16]. Moreover, CRP, whose production is under IL-6 control, also enhances IL-6 production in MM cells, promotes their proliferation under stress conditions, and protects them from chemotherapy [17]. Regarding signaling pathways, IL-6 drives an antiapoptotic response through increase of the MCL1 protein and activates the cell cycle in MM cells by triggering the JAK/STAT3 [18] and ERK1/2 [15] signaling pathway, respectively. The PI3K/Akt pathway is also important to promote MM cell growth. The Akt is activated in MM cells [19]. However, IL-6 does not activate this pathway, unlike IGF-1 or other MM cell growth factors [18]. This may explain the additive effects between IL-6 and other growth factors to support MM cell growth.

The BE-8 **(elsilimomab)** murine anti-IL-6 mAb was the first antibody used in studies on IL-6 blockade. In a clinical trial of 10 patients with advanced and progressive MM treated with BE-8, three patients showed marked inhibition of plasmablastic proliferation, but none of them showed significant responses to this therapy [20]. A combination therapy of BE-8, dexamethasone and high-dose melphalan, followed by autologous stem cell transplantation, has been shown to significantly inhibit IL-6 activity in advanced MM patients without toxic or allergic reactions [21]. In a series of 24 newly diagnosed patients, the BE-8 was given together with 140 mg/m2 of melphalan and autologous stem cell transplantation for 21 days [22]. This combination was shown to be as active as 200 mg/m2 of melphalan to decrease the tumor mass, based on historical comparison, and without impairment on the hematopoietic recovery [22]. The inhibition of IL-6 signaling using the chimeric anti-IL-6 mAb silxutimab **(CNTO328)** can enhance the antitumor activity of the proteasome inhibitor bortezomib in MM by attenuating inducible chemoresistance [23]. For example, treatment of both IL-6-dependent and IL-6-independent MM cell lines with silxutimab enhanced the cytotoxicity of bortezomib. Silxutimab enhanced bortezomib-mediated activation of caspase-8 and caspase-9, and attenuated bortezomib-mediated induction of antiapoptotic hsp-70 [23,24]. A Phase II multicentre study of siltuximab, alone or in combination with dexamethasone, was completed for patients with relapsed or refractory MM. Decreased serum CRP levels were observed. There were no responses to siltuximab monotherapy, but combination therapy yielded a partial/minimal response rate of 23%. Further study of siltuximab in modern corticosteroid-containing myeloma regimens is ongoing [25]. Siltuximab was evaluated in an open-label, seven-cohort, Phase I study in patients with B-cell nonHodgkin's lymphoma **(NHL)**, MM, or Castleman disease. Two of fourteen evaluable NHL patients had a partial response; 2/13 MM patients had complete response and 12/36 evaluable Castleman disease patients had radiologic response. CRP suppression was most pronounced at a dose of 12 mg/kg administered 3 times a week. There was no dose-limiting toxicities **(DLT)**, antibodies to siltuximab, or dose-toxicity relationship observed [26]. In another case report, complete remission was achieved with siltuximab monotherapy in relapsed and refractory myeloma [27]. Most recently, two randomized trials showed a lack of effect of anti-IL-6 therapy. In one trial, the silxutimab was given to transplant-ineligible patients in association with a regimen of bortezomib-melphalan-prednisone compared with bortezomib-

melphalan-prednisone and then as a maintenance therapy for 18 months or until relapse [28]. In another trial, siltuximab was given in relapsed/refractory MM in association with bortezomib compared with bortezomib alone and then as maintenance therapy until progression [29]. These two trials showed no benefit for event-free or overall survivals. In the first study, an improvement of response was found. How to explain this lack of benefit in randomized trials with anti-IL-6 mAb despite the major role of IL-6 to control healthy plasmablast proliferation [30,31] and despite the blockage of myeloma cell proliferation by anti-IL-6 mAb in patients with fulminant disease [32,33] ? In these randomized trials, siltuximab was able to block CRP production, indicating its neutralization of IL-6 bioactivity in the liver. Thus, a lack of antimyeloma effect of siltuximab was not due to an inability to block a too large IL-6 production. In line with this, median CRP levels are not higher in patients with MM compared with those with rheumatoid arthritis for whom anti-IL-6 therapy had benefit [9]. Several mechanisms could explain this lack of effect of anti-IL-6 therapy. First, the efficacy of anti-IL-6 therapy was compared with drug combination (bortezomib or bortezomib- melphalan-prednisone), which already reduces IL-6 production and inhibits CRP production [28]. Second, other factors could stimulate MM cell growth in the absence of IL-6, in particular IGF1 in CD45-negative/low MM cells, and favor the emergence of MM cell subclones independent of IL-6 [12,13]. This emergence of IL-6-independent subclones could not be investigated in the initial short-term treatments in patients with fulminant disease. Finally, other cytokines of the IL-6 family can activate the gp130 IL-6 transducer and the growth of MM cells and could substitute for IL-6 to promote MM cell survival and growth in vivo [34].

A very recent study in Japan evaluated the safety, pharmacokinetics, immunogenicity, and antitumor effect of siltuximab in combination with bortezomib and dexamethasone with relapsed or refractory MM patients [35]. A open-label, phase I, dose-escalating study used two doses of siltuximab for nine patients: 5.5 and 11.0 mg/kg (administered on day 1 of each 21-day cycle). In total, nine patients were treated. The most common grade 3/4 adverse events, lymphopenia (89 %) and thrombocytopenia (44 %), occurred in patients receiving both doses of siltuximab; however, no DLT were observed. Following intravenous administration of siltuximab at 5.5 and 11.0 mg/kg, the maximum serum concentration and the area under the curve from 0 to 21 days and from 0 to infinity increased in an approximately dose-proportional manner. Mean half-life, total systemic clearance, and volume of distribution were similar at doses of 5.5 and 11.0 mg/kg. Across both doses, six of the nine patients had complete or partial response (22 and 44 %, respectively). In conclusion, as no DLT was observed, the recommended dose for this combination is 11.0 mg/kg once every 3 weeks [35].

In addition, because blockade of IL-6R may be effective in suppressing MM cell growth, the tocilizumab humanized anti-IL-6R mAb is now being evaluated in open-label Phase I (USA) and II (France) trials as monotherapy in MM patients.

Other Hematological Malignant Tumors

IL-6 and/or CRP are prognostic factors for several B-cell malignancies, particularly malignant lymphoma [36]. IL-6 is a biomarker in other lymphoid malignancies, including Hodgkin disease, mantle cell lymphoma, cutaneous CD30-positive lymphomas, KSHV-associated malignancies and systemic mastocytosis **(SM)** [37-41]. Polymorphisms of different cytokine genes, especially IL-6 gene **(IL-6-174GG genotype)** are related to treatment failure in Hodgkin disease [42]. Most B-cell tumors expressed the IL-6R, in particular mantle cell lymphoma [43]. BE-8 was used in 11 patients with human immunodeficiency virus **(HIV)**-associated lymphoma and showed a clinical benefit, especially on B lymphoma [44] and in one patient with acute monoblastic leukemia [45]. Another study showed that IL-6 could increase the severity of SM, and cause a counteracting transcriptional induction of the antioxidant protein DJ-1 **(PARK-7)** which would protect malignant mast cells from oxidative damage [46]. A mouse model of mastocytosis recapitulated the biphasic changes in DJ-1 and the increasing IL-6, reactive oxygen species **(ROS)** and DJ-1 levels as mast cells accumulate, findings which were reversed with anti-IL-6R blocking antibody. These findings provided evidence of increased ROS and a biphasic regulation of the antioxidant DJ-1 in variants of SM and implicate IL-6 in DJ-1 induction and expansion of mast cells with the receptor tyrosine kinase KIT mutations, suggesting that IL-6 blockade is a potential adjunctive therapy in the treatment of patients with advanced mastocytosis, as it would reduce DJ-1 levels making mutation-positive mast cells vulnerable to oxidative damage [46]. Another study suggested that tocilizumab could be effective molecular targeting therapies for Primary effusion lymphoma **(PEL)** [47]. PEL is an invasive subtype of NHL that shows malignant effusion mainly seen in advanced acquired immunodeficiency syndrome **(AIDS)** patients. Although the production of VEGF and IL-6, and the expression of IL-6Rα in PEL cell lines, tocilizumab did not inhibit the proliferation of PEL cells in vitro but decreased VEGF mRNA and protein by inhibiting STAT3 phosphorylation and STAT3 binding to VEGF promoter [47]. Tocilizumab also significantly inhibited ascites formation in vivo and improved the overall survival of treated mice [47]. Thus, IL-6 and more generally gp130 IL-6 transducer signaling play an important role in B-cell neoplasias, and new clinical programs could be developed in such diseases.

HORMONE-DEPENDENT CANCERS

Prostate Cancer (PC)

PC is one of the most prevalent cancers in men worldwide. IL-6 acts as a paracrine and autocrine growth factor for both benign and cancer prostate cells. The levels of IL-6 and its receptor are elevated during prostate carcinogenesis and tumor progression [48]. It's also reported that a correlation between increased serum IL-6 and sIL-6R levels with aggressiveness of the disease [49]. Castration resistance usually leads to the death of the patient during the treatment of PC. In the cancer-resistance phenotype acquisition and cancer progression, the signaling pathway mediated by IL-6 represents an alternative pathway [48,49]. Aberrant IL-6/STAT3 signaling and

loss of p53 occur during PC progression to metastatic disease. The abnormality of the IL-6/STAT3/p53 axis is frequently accompanied by other genetic alterations; however, its potential role as an important mediator of oncogenic reprogramming, invasion and metastatic transformation remains unknown. The failure of anti-IL-6 treatments may be due to an incomplete understanding of the mechanism of the in vivo role of IL-6/STAT3 in PC. The identification of the alternative reading frame protein **(ARF)**/murine double minute protein **(MDM2)**/p53 tumour suppressor pathway potentially involving the IL-6/STAT3 axis as a restricting factor in PC deficient in the tumour suppressor phosphatase and tensin homologue **(PTEN)** opened new avenues to currently available therapies [50]. During androgen deprivation therapy, a regulation loop may emerge between sex steroids and IL-6, with a strong positive correlation with total testosterone, androstenedione, and estradiol levels [51]. Therefore, these data suggest that the importance of the IL-6/IL-6R pathway in the regulation of growth and drug resistance of PC cells.

No adverse events related to siltuximab treatment were observed in a Phase I study of PC [52]. A decrease in phosphorylation of STAT3 and ERK1/2 was observed in this study. Gene analysis in tumor specimens also indicated down-regulation of genes downstream of IL-6 signaling and important enzymes of the androgen signaling pathway [52]. A phase II study of siltuximab was completed in chemotherapy-pretreated patients with castration-resistant PC, showing a prostate-specific antigen **(PSA)** response rate of 3.8% and a RECIST (Response Evaluation Criteria in Solid Tumors) stable disease rate of 23%. Declining CRP levels during treatment may reflect biological activity. Despite evidence of siltuximab-mediated IL-6 inhibition, elevated baseline IL-6 levels portended a poor prognosis [53]. The combination of mitoxantrone/prednisone and siltuximab was not associated with a clinical improvement in a randomized phase II study, as compared with chemotherapy alone [54]. In a recent phase I study, siltuximab in combination with docetaxel appears to be safe and shows preliminary efficacy in patients with castration-resistant PC. Of the 17 patients with measurable disease, 2 confirmed and 2 unconfirmed radiologic partial responses were achieved. CRP concentrations were suppressed throughout treatment in all patients. DLT was reported in 1 of 11 patients [55]. Other molecules such as polyphenols have been shown efficacious on tumorigenesis in PC by inhibiting IL-6/STAT3 signaling [56].

Breast Cancer (BC)

High levels of circulating IL-6 are associated with metastatic BC and correlated with poor survival of patients [57]. Overexpression of IL-6 up-regulates serum VEGF in BC patients, which may promote angiogenesis and contribute to the metastasic potential of BC [58]. IL-6 expression is more abundant in aggressive tumors and is inversely associated with estrogen receptor **(ER)** levels in samples from patients with BC [59]. BC is commonly classified by receptor expression and can be categorized into ER-positive, HER2-positive, or triple-negative ER **(TNBCs)** [60]. The three BC subtypes rely on IL-6 signaling to varying degrees. It has been shown that exogenous IL-6 dose dependently increases the growth rate and migration of ERα-positive BC cells [61,62] and also drives epithelial to mesenchymal transition [63,64]. In a recent study on TNBCs, inhibition

of IL-6 and IL-8 expressions dramatically inhibited tumor cell survival in vitro and suppressed tumor engraftment in vivo. A multivariable analysis of patient specimens revealed that IL-6 and IL-8 expressions are predictive factors for TNBC patient survival [65]. IL-6 is elevated in HER2-positive BC where IL-6 activated STAT3 and induced an autocrine loop of IL-6/STAT3 expression [66]. Further evidence revealed that the growth of HER2-positive BC in vivo was dependent on the HER2/IL-6/STAT3 signaling pathway [66]. Drug resistance is a critical problem in BC therapy, and autocrine production of IL-6 by breast tumor cells promotes resistance to multi-drug chemotherapy [67]. Recently, IL-6 has been suggested as a major factor influencing resistance to trastuzumab, a therapeutic HER2 antibody, in BC [68]. Trastuzumab resistance in HER2-overexpressing BC cells is shown to be mediated by the IL-6 inflammatory loop, leading to expansion of BC stem cells **(BCSCs)** [68]. Blockade of this IL-6 loop by an IL-6 antagonist, tocilizumab, reduced BCSCs, resulting in decreased tumor growth and metastasis in mouse xenografts [68]. These data suggest that IL-6 plays a critical role in the oncogenic transformation, growth and metastasis of BC cells, renewal of BC stem cells **(BCSCs)**, and drug resistance of BCSCs, making anti-IL-6/IL-6R/gp130 therapies promising options for the treatment and prevention of BC [69].

A study evaluated the apoptotic activity of anti-IL-6 as a novel candidate for BC treatment strategy compared its effects with those obtained using tumour necrosis-related apoptosis-inducing ligand **(TRAIL)** as an established apoptotic agent. Their results revealed that levels of either anti-IL-6- or TRAIL-induced apoptosis in the tumour or normal tissue cultures were significantly higher than those in their corresponding untreated ones ($P<0.001$). Recombinant anti-IL-6 monoclonal antibodies could represent a novel effective element of immunotherapeutic treatment strategy for BC [70].

The selective estrogen receptor modulators **(SERMs)**, raloxifene and bazedoxifene, are used clinically to treat or prevent ER-positive invasive BC and osteoporosis [71, 72]. Recently, raloxifene and bazedoxifene were discovered to be inhibitors of the IL-6/gp130 interface [73]. The interaction of these drugs with gp130 was demonstrated indirectly via docking studies and a drug affinity responsive target stability assay. Both agents inhibited IL-6-induced STAT3 phosphorylation in the pancreatic cancer cell line PANC-1 [73]. A more recent study showed that recombinant IL-6 increased phosphorylation of tyrosine 705 of STAT3 in eight of nine ERα-positive BC cell lines. Differential gene expression analysis identified 17 genes that could be used to determine IL-6 pathway activation by combining their expression intensity into a pathway activation score. The gene signature included a variety of genes involved in immune cell function and migration, cell growth and apoptosis, and the tumor microenvironment. Validation of the IL-6 gene signature in 36 matched human serum and ERα-positive breast tumor samples showed that patients with a high IL-6 pathway activation score were also enriched for elevated serum IL-6 (≥10 pg/mL). When human IL-6 was provided in vivo, MCF-7 cells engrafted without the need for estrogen supplementation, and addition of estrogen to IL-6 did not further enhance

engraftment. Subsequently, prophylactical treatment mice at MCF-7 engraftment with anti-IL-6 mAb siltuximab, ER antagonist fulvestrant, or combination therapy. Siltuximab alone was able to blunt MCF-7 engraftment. Similarly, siltuximab alone induced regressions in 90% (9/10) of tumors, which were established in the presence of human mesenchymal stem cells **(hMSC)** expressing human IL-6 and estrogen. Therefore, given the established role for IL-6 in ERα-positive BC, these data demonstrate the potential for anti-IL-6 therapeutics in BC [74]. To date, no clinical data are available for BC therapy. However, these SERMs may have potential effects against BC via IL-6 signaling in addition to ER modulating mechanisms.

Ovarian Cancer (OVCA)

OVCA remains the most lethal gynecologic cancer and new targeted molecular therapies against this miserable disease continue to be challenging. It has been widely reported that IL-6 is overexpressed in the serum and ascites of OVCA patients, and elevated IL-6 level correlates with poor prognosis and survival [75,76]. Results of multiple studies indicate a pathogenic role of this cytokine in the malignant transformation, progression and chemotherapy resistance of OVCA [77-82]. The results of our previous studies indicated that the autocrine production of IL-6 by OVCA cells contributes to anchorage-independent growth, proliferation, adhesion and invasion of these cells by inducing IL-6 signal transduction [81], and promotes these cells resistance to cisplatin and paclitaxel through down-regulation of proteolytic activation of caspase-3 [82]. About 40-60% of OVCA cases express ERα, but only a small proportion of patients respond clinically to anti-estrogen treatment with ER antagonist tamoxifen **(TAM)** [83]. Recently, we demonstrated that IL-6 secreted by OVCA cells may contribute to the refractoriness of these cells to TAM via ER isoforms and steroid hormone receptor coactivator **(SRC)-1**[84]. Subsequently, we investigated another potential mechanism involved in IL-6-mediated TAM resistance in OVCA cells. We found that IL-6 may also confer TAM resistance in OVCA cells via the crosstalk between ER and IL-6-mediated intracellular signal transduction cascades such as MEK/ERK and PI3K/Akt pathways. Our studies suggest that TAM-IL-6-targeted adjunctive therapy may lead to a more effective intervention than TAM alone [85].

The potential roles of IL-6/STAT3 signaling pathway in tumorigeneis [86, 87] and drug resistance [88] have been reported in OVCA. Inhibition of this signaling pathway and downregulation of Axl and Tyro3 receptor tyrosine kinases **(RTKs)** expression might be a therapeutic strategy to overcome taxol resistance in OVCA cells [89]. In in vitro studies of OVCA cells, the anti-IL-6 antibody siltuximab has shown significant effects on apoptosis, survival, and resistance to drugs by blocking IL-6 signaling pathway [90]. Siltuximab also shows promise for OVCA in clinical trials [91]. In this trial, the primary endpoint was response rate as assessed by combined RECIST and CA125 criteria. One patient of eighteen evaluable had a partial response, while seven others had periods of disease stabilization. In patients treated for 6 months, there was a significant decline in plasma levels of IL-6-regulated CCL2, CXCL12, and VEGF. Gene expression levels of factors that were reduced by siltuximab treatment in the patients significantly correlated

with high IL-6 pathway gene expression and macrophage markers in microarray analyses of OVCA biopsies. The investigators noted that the percentage of women who received clinical benefit from siltuximab is an unusually high proportion for an experimental cancer drug study. Typically, only 5-20% of participants secure any benefit from taking untried treatments, according to the investigators [91]. A recent study showed that a high level of IL-6R expression but not IL-6 expression in OVCA cells is an independent prognostic factor. In in vitro analyses using OVCA cell lines, while six (RMUG-S, RMG-1, OVISE, A2780, SKOV3ip1 and OVCAR-3) of seven overexpressed IL-6R compared with a primary normal ovarian surface epithelium, only two **(RMG-1, OVISE)** of seven cell lines overexpressed IL-6, suggesting that IL-6/IL-6R signaling exerts in a paracrine manner in certain types of OVCA cells. OVCA ascites were collected from patients, and showed that primary CD11b+CD14+ cells, which were predominantly M2-polarized macrophages, are the major source of IL-6 production in an OVCA microenvironment. When CD11b+CD14+ cells were co-cultured with cancer cells, both the invasion and the proliferation of cancer cells were robustly promoted and these promotions were almost completely inhibited by pretreatment with anti-IL-6R antibody

tocilizumab. These data suggest a rationale for anti-IL-6/IL-6R therapy to suppress the peritoneal spread of OVCA, and represent evidence of the therapeutic potential of anti-IL-6R therapy for OVCA treatment [92].

Colorectal Cancer (CRC)

Inflammation and IL-6/sIL-6R expression play important roles in the pathogenesis of CRC [93]. Elevated levels of IL-6 have detected in the serum and tumor tissues of patients with CRC [94]. Levels of IL-6 in CRC patients are associated with tumor stage, tumor burden, metastasis, and overall survival [93, 95]. IL-6 plays an important role in modulating Th17 and Treg cells in CRC, and also in recruitment of immune cells that produce pro-inflammatory cytokines [96]. Furthermore, IL-6 was shown to be localized at the sites of macrophage infiltration, suggesting an interaction between IL-6 and immune cells in the tumor microenvironment [97]. IL-6 also promotes the survival of cholangiocytes by altering microRNAs including let-7a, which contribute to the constitutive activation of STAT3 [98]. In a study of 46 patients with CRC, multivariate analysis showed that the serum IL-6 level and the blood granulocyte/lymphocyte ratio were independent risk factors for poor prognosis, suggesting that both factors may be significantly predictive for CRC cancer progression [99].

It has been shown anti-cancer effects of tocilizumab in a colon cancer xenograft model [100]. A recent study showed IL-6 treatment stimulated aerobic glycolysis, upregulated key genes in aerobic glycolysis and promoted cell proliferation and migration in SW480 and SW1116 CRC cells [101]. In colitis-associated CRC mouse, anti-IL-6R antibody treatment reduced the incidence of CRC and decreased the expression of key genes in aerobic glycolysis, whereas the plasma concentrations of glucose and lactate were not affected. Further analysis in human samples

revealed overexpression of 6-phoshofructo-2-kinase/fructose-2,6- bisphosphatase-3 **(PFKFB3)** in colorectal adenoma and adenocarcinoma tissues, which was also associated with lymph node metastasis, intravascular cancer embolus and TNM stage [101]. These results indicate that chronic inflammation promotes the development of CRC by stimulating aerobic glycolysis and IL-6 is functioning, at least partly, through regulating PFKFB3 at early stage of CRC and provide reference for further clinical basis [101]. Another recent study evaluated that IL-6 released from CRC cells and associated with CRC pathogenesis and metastasis modulates the phagocytic capacity and migratory ability of macrophages, using a monocyte-macrophage THP-1 cell model and human peripheral monocytes. They found that CRC cells enhanced the phagocytic capacity and migration of THP-1 cells and human peripheral monocytes. CRC cell culture supernatants and recombinant IL-6 neutralized with anti-IL-6 and anti-gp130 antibodies considerably decreased IL-6-mediated phagocytosis by and migration of THP-1 cells and human peripheral monocytes, via the phosphorylation of STAT3 [102]. These data suggest that CRC cells secreting IL-6 via STAT3 phosphorylation can enhance the phagocytic capacity and migration of macrophages in the tumor microenvironment [102]. However, so far no clinical study has been reported in CRC.

RENAL CELL CARCINOMA (RCC)

IL-6 was shown to be an autocrine proliferation factor for tumor cell lines obtained from patients with metastatic RCC [103]. Mutations in the TP53 gene could promote the overexpression of IL-6 in RCC [104]. Many studies have demonstrated that CRP/IL-6 is a prognostic factor in metastatic RCC [105-107]. A significant association between the presence of the IL-6R in tumors and tumor stage, nuclear grade, proliferation index, and serum IL-6 was also demonstrated [108]. Treatment of RCC cells with cisplatinum **(CDDP)** in combination with anti-IL-6 or anti-IL-6R mAbs can overcome their CDDP resistance by decrease of glutathione S-transferase π expression [109]. The combination therapy of anti-IL-6R mAb tocilizumab and interferon-alpha can inhibit RCC cell growth in vitro and in vivo through suppressed suppressor of cytokine signaling-3 **(SOCS3)** expression [110]. In a phase I study, the use of IL-6 and GM-CSF in patients with RCC was associated with inverse clinical effects [111]. Treatment of 18 metastatic RCC patients with BE-8 mAb showed a well clinical efficacy, including abrogation of fever, hypercalcemia, and inflammatory syndrome, reduction of anemia and morphine intake, and weight increase, with objective responses observed (2/18 patients with partial responses, one with a minorresponse, and one with stable disease) [105]. Three patients with RCC were included in a Phase II trial of BE-8 given daily for 21 days. Reductions of CRP, haptoglobin, and serum alkaline phosphatases were observed in all 3 patients during anti-IL-6 administration; toxicity was minimal [112]. In a phase I/II study, siltuximab was shown to stabilize the disease in more than 50% of progressive metastatic RCC patients, with one partial response and a favorable safety profile, a situation that could authorize further evaluation of dose-escalation strategies and/or combination therapy [113]. Nevertheless, to our knowledge, no further studies have been performed.

LUNG CANCER

Inflammatory pathways contribute tomorbidity and mortality associated with lung cancer. IL-6 has been associated with poor prognosis and lung cancer-related symptoms such as fatigue, thromboembolism, cachexia and anemia. A therapy targeting IL-6 may be an effective treatment for the inflammatory microenvironment in lung cancer. Siltuximab has been shown to inhibit the growth of non small cell lung cancer **(NSCLC)** cell lines through suppressing STAT3 phosphorylation [114]. Patients with NSCLC have increased levels of IL-6 and CRP in serum, and high IL-6 expression is associated with weight loss and lower survival [115]. IL-6 has been implicated in resistance of lung cancer to EGF receptor **(EGFR)** inhibitors. In NSCLC cell lines, increased IL-6 secretion can cause drug resistance to EGFR inhibitors, including erlotinib **(Tarceva)** and gefitinib **(Iressa)** [116]. Constitutively activated mutant variants of EGFR can promote IL-6 production and activate JAK/STAT3 signaling pathway in human lung adenocarcinomas. Inhibition of EGFR activity leads to partially blocked transcription of IL-6 and decreased IL-6 production [117]. These data suggest that IL-6 blockade may help treat NSCLC patients who have EGFR mutations and those patients who have relapsed from EGFR inhibitors treatment, and a combined approach blocking both EGFR and IL-6/JAK/STAT3 pathways may benefit cancer therapy. A recent study has reported that the PI3K/Akt1/IL-6/STAT3 signaling pathway regulates generation and stem cell-like properties of NSCLC tumor initiating cells **(TICs)**. Mutant Akt1, mutant PIK3CA or PTEN loss enhances formation of lung cancer spheroids **(LCS)**, self-renewal, expression of stemness markers and tumorigenic potential of human immortalized bronchial cells **(BEAS-2B)** whereas Akt inhibition suppresses these activities in established **(NCI-H460)** and primary NSCLC cells. Inhibition of Akt in NSCLC cells decreases IL-6 level, phosphorylation of IκK and IκB, NF-κB transcriptional activity, phosphorylation and transcriptional activity of STAT3 whereas active Akt1 increases them. Exposure of LCSs isolated from NSCLC cells to blocking anti-IL-6 mAbs, shRNA to IL-6R or to STAT3 markedly reduces the capability to generate LCSs, to self-renew and to form tumors, whereas administration of IL-6 to Akt-interfered cells restores the capability to generate LCSs. These results indicate that aberrant Akt signaling contributes to maintaining stemness in lung cancer TICs through a NF-kB/IL-6/STAT3 pathway and provides novel potential therapeutic targets for eliminating these malignant cells in NSCLC [118]. Another study has confirmed that NADPH oxidase 4 **(NOX4)**, an important source of reactive oxygen species **(ROS)** production in NSCLC cells, functionally interplay with IL-6 to promote NSCLC cell proliferation and survival. NOX4/Akt and IL-6/STAT3 signalings can reciprocally and positively regulate each other, leading to enhanced NSCLC cell proliferation and survival. Therefore, NOX4 may serve as a promising target against NSCLC alone with IL-6 signaling [119]. In addition, hypermethylated in cancer 1 **(HIC1)** is a tumor suppressor through inhibiting the transcription of IL-6 by sequence-specific binding on its promoter. Restoring IL-6 expression could partially rescue these phenotypes induced by HIC1 in vitro and in vivo. IL-6 induced by loss of HIC1 activated STAT3 through IL-6/JAK pathway and was associated with NSCLC progression, and HIC1/IL-6 axis may serve as a prognostic biomarker and provide an attractive therapeutic target for NSCLC [120].

Hepcidin, a key regulator of iron metabolism, is produced mainly by IL-6 during inflammation. Overproduction of hepcidin by IL-6 signaling might be a major factor that leads to functionally iron-deficient cancer-related anemia in the IL-6-producing human lung cancer cell line LC-06-JCK model. Inhibition of the IL-6 signaling pathway by rat anti-mouse IL-6R antibody MR16-1 treatment resulted in significant recovery of iron-deficiency anemia and alleviation of cancer-related symptoms, suggesting that IL-6 signaling might be one possible target pathway to treat cancer-related anemia disorders [121]. In a recent case report in Japan, tocilizumab was used to treat a patient with cancer cachexia. This patient had high levels of IL-6 expression and multiple symptoms characterized by an IL-6-induced inflammatory response. Tocilizumab had a dramatic effect on cachexia induced by lung cancer, and their survival was prolonged for 9 months without chemotherapy [122].

OTHER CANCER TYPES

IL-6 inhibition may have therapeutic indications in other cancers, including pancreatic cancer [73] as above described. Growth and invasive capability of human bladder cancer cells were attenuated when IL-6 signaling was blocked, suggesting that IL-6 could be a potential predictor for clinical stage and prognosis of bladder cancer [123]. Elevated level of IL-6 was significantly associated with poor prognosis in patients with esophageal cancer. When IL-6 expression was inhibited, aggressive tumor behavior and radiation resistance could be overcome in vitro and in vivo [124]. In a study on tumor-initiating cells **(TICs)** in head and neck squamous cell carcinomas **(HNCs)**, suppression of miR-145 or ADAM17 overexpression increased expression and secretion of IL-6 and sIL-6R in these cells. miR-145 appears to suppress a paracrine signaling pathway of IL-6 and sIL-6R in the tumor microenvironment vital to maintain TICs in HNC [125]. IL-6 increases the VEGF production through activation of STAT3 in glioblastoma cells, which may contribute to angiogenesis and metastasis of tumor [126]. Tocilizumab inhibits the growth of U87MG glioma cell through an inhibitory effect of the IL-6/JAK/STAT3 pathway [127]. The amplification of the IL-6 gene was reported in patients with glioblastoma and was associated with shortened survival [128]. STAT3 plays a critical role in IL-6-mediated drug resistance in human neuroblastoma cells. Treatment of neuroblastoma cells with IL-6 and sIL-6R protected them from drug-induced apoptosis, and the protective effect of IL-6 was STAT3-dependent because it was reversed by a STAT3 inhibitor [129].

CONCLUSIONS

IL-6 plays important roles in the pathogenesis of cancer. Components of the IL-6 signaling pathway, including IL-6, IL-6R, sIL-6R, gp130, JAK, and STAT3, have been used as promising targets for cancer therapy. Several mAbs developed for anti-IL-6/IL-6R therapy, either as a single agent or in combination with other chemotherapeutic drugs, have shown promising results in both preclinical studies and clinical trials in human cancer (Table 1). In clinical studies on tocilizumab for treatment of cancer, combination therapy with IL-6 blockade and conventional

drugs achieved better treatment efficacy and patient responses compared to monotherapy. New strategies such as combination of IL-6 blockade and EGFR inhibition or other targeted therapy may be helpful to improve IL-6 targeted immunotherapy of human cancer.

Table 1: Clinical Studies on Ttargeting IL-6 in Cancers.

Compound name	Target/specificity	Indications	Phase/Status	Reference
Elsilimomab (BE-8)	Murine anti-IL-6 mAb	MM		Bataille, 1995[20]
		MM		Moreau, 2000 [21]
		MM		Rossi, 2005[22]
		HIV-associated lymphoma		Emilie D, 1994 [44].
		RCC	Phase II	Blay, 1997[112]
Siltuximab (CNTO328)	Chimeric anti-IL-6 mAb	MM	Phase II	Voorhees, 2007[24]
		MM	Phase II	Voorhees, 2009 [23]
		MM	Phase II	Voorhees, 2013[25]
		MM, B-cell NHL	Phase I	Kurzrock, 2013[26]
		MM	Phase II	San-Miguel, 2014 [28]
		MM	Phase II	Orlowski, 2015 [29]
		MM	Phase I, Japan	Suzuki K,2015[35]
		PC	Phase I	Karkera, 2011[52]
		PC	Phase I	Dorff, 2010[53]
		PC	Phase II	Fizazi K,2012[54]
		PC	Phase II	Hudes, 2013[55]
		OVCA	Phase I+II	Coward, 2011[91]
		RCC	Phase II	Rossi, 2010 [113]
Tocilizumab (Actemra, RoActemra)	Humanized anti-IL-6R mAb	Cancer cachexia in lung cancer MM	Case report, Japan Open-label phase I, USA; Phase II, Franc	Ando, 2013[122]

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