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Review

Interleukin 6/Interleukin 6 Receptor Interaction and its Role as a Therapeutic Target for Treatment of Cachexia and Cancer

ULRICH H. WEIDLE[1], STEFAN KLOSTERMANN[1], DANIELA EGGLE[1] and ACHIM KRÜGER[2]

[1]*Roche Diagnostics GmbH, Division Pharma, D-82372 Penzberg, Germany;*
[2]*Institute of Experimental Oncology and Therapy Research, Klinikum rechts der Isar, Technical University Munich, D-81675 Munich, Germany*

Abstract. *Interleukin 6 (IL6) mediates pleiotropic physiological functions through its interaction with the IL6 receptor (IL6R). Signal transduction can occur via cis- and trans- signaling. The role of IL6/IL6R interaction via autocrine and paracrine loops in tumor proliferation and progression is discussed. The potential role of IL6/IL6R interaction in different experimental systems and tumor entities is summarized while the focus is on inhibition of IL6 signaling with monoclonal antibodies directed against IL6 or IL6R and their potential impact for treatment of tumor-associated cachexia and as antitumoral agents as monotherapy and in combination with small molecule compounds.*

Various observations point to an interplay between the tumor stroma, inflammation and the growth and dissemination of primary tumors (1-3). Immune cells, including B and T lymphocytes, macrophages, dendritic cells, neutrophils, mast cells and fibroblasts are found to be concentrated in tumors compared to surrounding tissue. Tumor-associated macrophages (TAM) are exposed to factors which polarize them toward M2 type macrophages (4). M1 macrophages are activated by microbial products and are involved in killing microorganisms and in the production of reactive oxygen and nitrogen species (ROS and RNS). M2 macrophages suppress adaptive immunity and promote matrix remodeling, tumor growth and survival, invasion and metastasis as well as angiogenesis (4). The M1 phenotype is typically interleukin (IL)12 high and IL10 low, whereas M2 macrophages are IL10 high and IL12 low. Inflammatory mediators often activate oncogenic transcription factors such as nuclear factor κB (NFκB) and signal transducer and activator of transcription 3 (STAT3), whereas oncogenes such as rat sarcoma (ras) and avian myelocytosis virus oncogene cellular homolog (myc) can initiate an inflammatory response. Autoimmunity may also contribute to tumor development. Inflammatory bowel disease increases the risk of colitis-associated cancer (CAC). Inflammatory cytokines released by cancer cells can lead to activation of tumor-infiltrating macrophages that secrete cytokines which activate oncogenic transcription factors in the remaining cancer cells stimulating their survival and proliferation. Inflammation can produce ROS and RNS and induce mutagenic enzymes such as activation-induced cytidine deaminase (AID) and can inactivate DNA gatekeeper pathways. It remains to be established whether inflammation alone can result in tumor initiation. Inflammation-associated factors such as IL6 and tumor necrosis factor alpha (TNFα) can serve as mitogens and survival factors for pre-malignant and fully established cancer cells.

The interplay between tumor and stromal cells and its impact on tumor growth and dissemination has been substantiated by several findings. In a mouse model of squamous cell carcinogenesis (K14-HPV16 model) B-cells and humoral immunity fostered cancer development by activating fragment crystallizable γ (Fcγ) receptors on resident and recruited myeloid cells. Auto-antibodies accumulating in the stroma in premalignant skin interacted with the Fcγ receptors leading to the recruitment of leukocytes into neoplastic tissue resulting in activation of chronic inflammatory programs that promote *de novo* carcinogenesis (5). B-Cells, humoral immunity and activating Fcγ receptors on myeloid cells are required for establishing chronic inflammatory programs that promoted *de novo* carcinogenesis. This results in regulation of the composition of the leukocytes recruited to premalignant tissues and their bioeffector functions. Cluster of differentiation 4 positive ($CD4^+$) T-cells have been shown to be implicated in pulmonary metastasis of mammary carcinomas by enhancing

Correspondence to: Ulrich H. Weidle, Roche Diagnostics, Division Pharma, Im Nonnenwald 2, D-82372 Penzberg, Germany. Tel: +49 8856602801, e-mail: ulrich.weidle@roche.com

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the pro-tumoral properties of macrophages (6). The role of B-cells in the development of castrate-resistant prostate cancer has been shown in a mouse model (7). Tumor cells killed by androgen deprivation released pro-inflammatory factors which lead to infiltration of B- and T-cells. A series of studies has specifically implicated lymphotoxin β (LTβ) secreting B-cells as promoters of castration-resistant prostate cancer. Mice with a B-cell-specific deficiency in LTβ showed delayed growth of castrate-resistant prostate cancer in contrast to mice with a T-cell-specific deficiency of LTβ expression (7). Cancer-associated fibroblasts (CAF) have been implicated in skin carcinogenesis (8). They were shown to stimulate angiogenesis, cancer cell proliferation and invasion and to promote macrophage recruitment. Angiogenesis and tumor growth can be decreased by inhibition of NFκB. A proinflammatory gene signature has been found in CAFs from dysplastic skin and this signature was also found in mammary and pancreatic tumors. It has been shown that alterations in the stroma could control carcinogenesis (9). Genes expressed in stromal cells were shown to discriminate pre-invasive from invasive disease, predict outcome and point at inflammatory pathways in digestive carcinomas. Supervised clustering of gene expression profiles from micro-dissected stroma between Barrett's esophagus (BE) and esophageal carcinoma identified a signature which could distinguish between BE metaplasia, dysplasia and esophageal adenocarcinoma (9). Gene ontology analysis identified a strong inflammatory component in BE disease progression and key pathways included cytokine-cytokine receptor interactions and transforming growth factor beta (TGFβ).

IL6/IL6R-mediated Signaling

IL6 is a pleiotropic cytokine which is released into the circulation upon injury or infection. IL6 is involved in processes such as hematopoiesis, neural development, inflammation, immunity, reproduction and bone metabolism (10). In addition, involvement in the induction of B-cell, T-cell and astrocyte differentiation and the induction of acute phase proteins in hepatocytes, such as C-reactive protein (CRP) have been reported (11). Involvement in oncology-related processes will be discussed below. IL6 belongs to the family of IL6-type cytokines that includes IL11, ciliary neurotrophic factor (CTNF), leukemic inhibitory factor (LIF), oncostatin M (OSM) and cardiotrophin-like factor (CLF). All of these cytokines share a four-helix bundle protein motive (12). This family of proteins signals *via* receptor complexes which contain glycoprotein 130 (gp130), the common signal transducing protein of the IL6 family of cytokines (13). Murine IL6 acts in a species-specific manner, whereas human IL6 is also active on IL6R-positive murine cells. Sequence alignments between murine and human IL6 and IL6R are shown in Figures 1 and 2. The critical sites of individual amino acid substitutions in human IL6 and IL6R which lead to more than 70% compromised ligand binding affinity (according to Swissprot protein data base) are indicated in red. Amino acid identity and similarity between murine and human IL6 are 41.6% and 65%, for the IL6R the corresponding scores are 53.4 and 65.8%. IL6 binds to its receptor (IL6R) and this complex recruits two molecules of gp130, which is ubiquituously expressed, in contrast to IL6R which is expressed on defined cell types such as hepatocytes and leukocytes (14). A soluble form of the IL6R (sIL6R) can be produced by processing of the receptor by proteases such as a disintegrin and metalloproteinase 17 (ADAM17) or by differential splicing (15, 16). IL6R by itself is not a signal-transducer, its function is to present IL6 to the signal-transducer gp130. This results in phosphorylation of gp130 by janus kinase 2 (JAK2) and subsequent recruitment of signal transducers and activators of transcription (STAT1 and STAT3) which subsequently dimerize and after phosphorylation they are translocated into the nucleus and mediate transcription of defined gene signatures (17). This type of signaling is referred to as *cis*-signaling (Figure 3A). sIL6R can bind its ligand IL6 and induce signaling in cells which express gp130 and not IL6R. This kind of signal transduction is referred to as trans-signaling (18) (Figure 3B). In contrast to most soluble receptors, the IL6-sIL6R complex can act as an agonist. A soluble fusion protein consisting of the extracellular domain of gp130 and Fc moiety of human IgG has been shown to inhibit *trans*-signaling due to binding of the IL6-sIL6R complex, whereas *cis*-signaling was not affected because this fusion protein cannot bind IL6 (19). The impact of *cis*- and *trans*-signaling for cancer-related processes will be discussed below. The interactions of IL6R with different types of proteins based on the Ingenuity software for pathway analysis of canonical pathways such as cytokines, proteases, transmembrane receptors, kinases and other enzymes as well as transporters, growth factors and other proteins are displayed in Figure 4. In addition to the activation of STAT3 signaling, the activation of mitogen-activated protein kinase (MEK/ERK) signaling resulting in transcription mediated by SRE (serum response elements) and IL6 RE (IL6-response elements) on promoters was highlighted by the Ingenuity analysis (Figure 5).

Experimental Models Linking Inflammation to Cancer

A causal link between inflammation and cancer was first proposed by Virchow in the 19th century (20). Important molecular players in this context are NFκB and STAT-mediated signaling, as well as IL6 (2). STAT3 as well as NFκB signaling can up-regulate IL6. NFκB is constitutively active in activated B-cell-like (ABC)-DLBCL (diffuse large B-cell lymphoma), but not in germinal center B-cell-like (GCB)-DLBCL (21). Caspase recruitment domain-containing protein 11(CARD11)

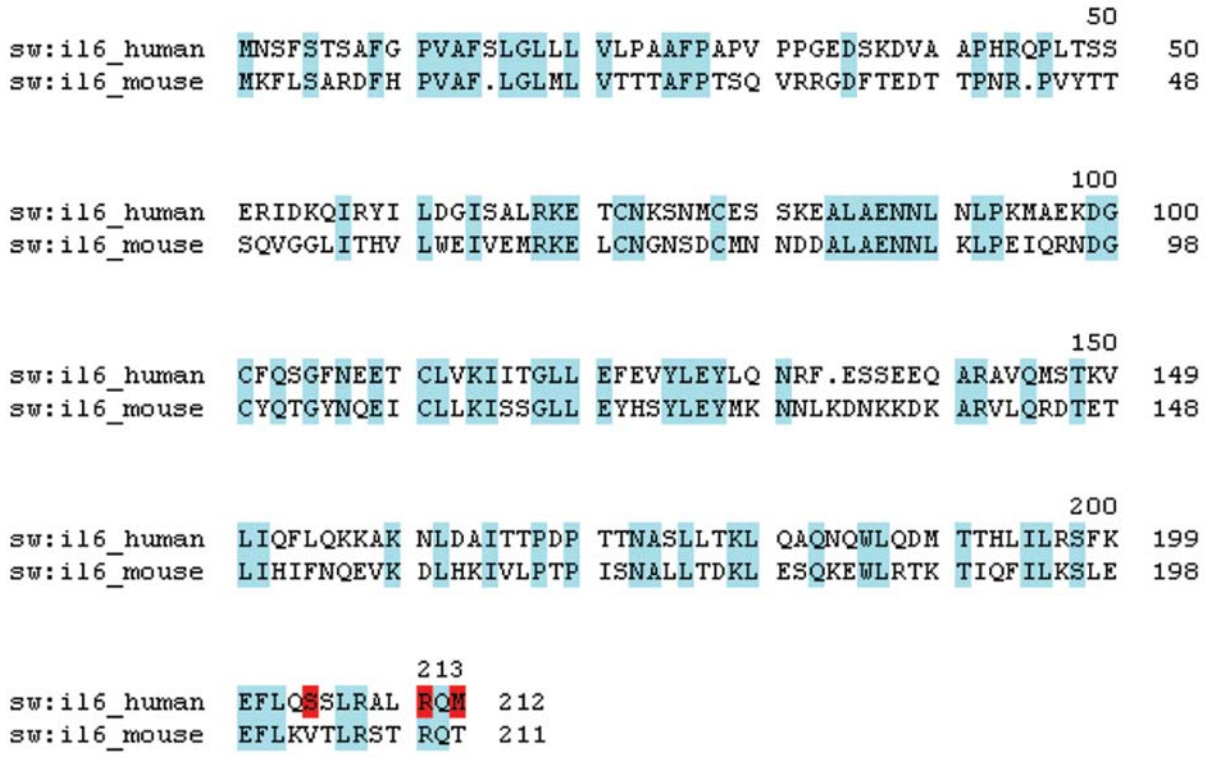

Figure 1. *Amino acid sequences of murine and human IL6. Identical amino acids are boxed in blue and critical residues for IL6/IL6R interaction are marked by a red background.*

has been identified as the driver of constitutive NFκB activity in ABC-DLBCL (22). NFκB is also activated in multiple myeloma by stabilization and accumulation of NFκB inducing kinase (NIK) (23, 24). Large deletions affecting closely linked cellular inhibitor of apoptosis (cIAP1) and cIAP2 loci resulting in the absence of their protein products preventing degradative polyubiquitinylation of NIK have been observed (23, 24).

The function of NFκB signaling has been assessed in a model of CAC. Mice were treated with the procarcinogen azoxymethane (AOM) and concomitant induction of colonic inflammation by repeated administration of the irritant dextrane sulfate sodium (DSS). Conditional disruption of the NFκB activating kinase IKB kinase β (IKKβ) in the mice revealed that NFκB activation in intestinal epithelial cells is essential for the development of colonic adenomas (25). During CAC development, IL6 is produced by lamina propria macrophages and dendritic cells. The ablation of IL6 reduced multiplicity and size of colonic adenomas in these mice (1). In contrast, in a diethylnitrosamine (DEN)-induced model of hepatocellular carcinoma (HCC) in mice, it was found that the hepatocyte-specific deletion of the IKKβ subunit of the nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor (IκB) kinase (IKK) complex dramatically enhanced HCC induction (26). It was shown that DEN administration to IKKβ deficient mice resulted in elevated ROS accumulation, hepatocyte death and compensatory proliferation (26, 27). DEN-induced HCC depends on the production of the NFκB regulated IL6 (28, 29). Kupffer cells are activated by IL-1α released by dying tumor cells. In a transplant system for chemically induced HCC it was found that IKKβ suppressed malignant transformation by preventing the accumulation of ROS that could lead to activation of STAT3, the oncogenic transcription factor involved in HCC development (30). An inverse correlation between NFκB and STAT3 has been found in a large subfraction of human HCC. In contrast in other mouse models of HCC that depend on chronic inflammation and not on liver damage and death-driven compensatory proliferation, hepatocyte IKKβ and NFκB were found to promote tumor development (31).

IL6 Signaling and Cancer

As outlined in a preceeding section, IL6 signaling can be exerted by interaction of IL6 with gp130/IL6R complex (cis-signaling) as well as by interaction of soluble IL6R/IL6 complex with gp130 (*trans*-signaling). These interactions result in JAK/STAT3 activation and MEK/ERK signaling, respectively. The involvement of IL6/IL6R interaction in

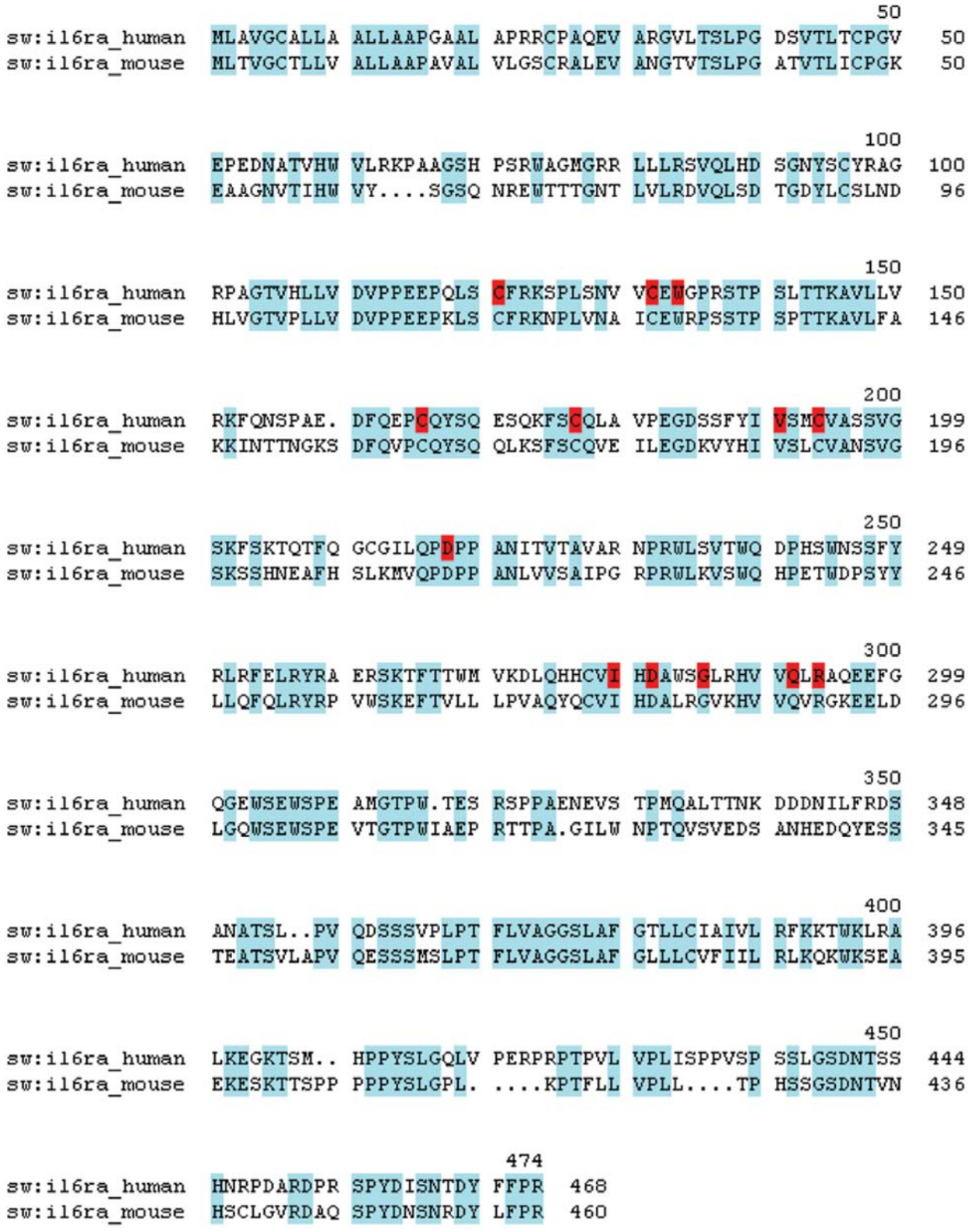

Figure 2. *Amino acid sequences of murine and human IL6R. Identical amino acids are boxed in blue and critical residues for IL6/IL6R interaction are marked by a red background.*

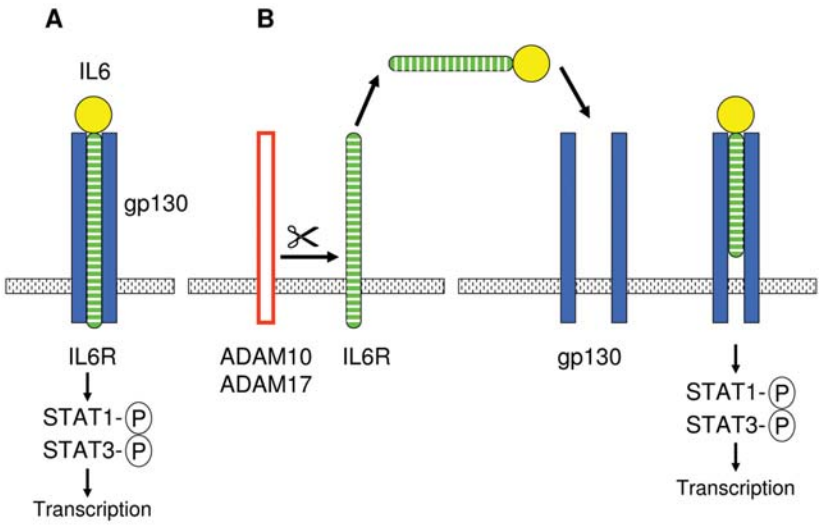

Figure 3. *IL6/IL6R-mediated signaling. A: Cis-type signaling; B: trans-type signaling. ADAM: a disintegrin and metalloproteinase; STAT: signal transducer and activator of transcription; gp130: glycoprotein 130.*

inflammation-based cancer related disease models has been outlined above.

IL6 has been shown to be expressed in mammospheres (multicellular spheroids composed of anchorage-dependent self-renewing cells and their derivatives) originating from breast cancer tissue and IL6 blocking antibodies suppressed tumor formation (32). High IL6 expression has been observed in basal-like breast carcinoma tissues which are enriched in mammosphere and stem cell markers.

Mutant variants of epidermal growth factor receptor (EGFR) have been identified as inducers of IL6 in lung adenocarcinomas resulting in activation of STAT3 (33). An autocrine loop between IL6 and IL6R is implicated in lung adenocarcinoma.

Cross-talk of signals between EGFR and IL6R through JAK2/STAT3 mediate epithelial–mesenchymal transition in ovarian carcinoma (34). EGF treatment of OvCA433 and SKOV3 cell lines results in increased steady state IL6 mRNA levels and increased IL6 secretion into serum-free medium. Exogenous addition of IL6 stimulates STAT3 activation and enhanced migration. Blocking antibodies against IL6R inhibit IL6 production and EGF- and IL6-induced migration. Specific inhibition of STAT3 activation by JAK2 specific inhibitor AG490 blocked STAT3 phosphorylation, cell motility, induction of N-cadherin, as well as vimentin and IL6 expression. These data suggest that the activated status of STAT3 in these cell lines may occur directly through EGFR or IL6R or indirectly through IL6 signaling. We have investigated the expression of IL6 and IL6R at the RNA level in ovarian carcinoma as outlined in Figure 6. IL6 and IL6R were co-expressed in clear cell ovarian carcinoma, however, with a very low expression level of the IL6R.

The expression of IL6 and IL6R in prostate cancer as well as the role of IL6 as a growth factor in prostate cancer is well documented (35). A correlation between IL6 protein levels and more advanced stages of the disease and poor prognosis has been established (36-38). In a model system for advanced prostate cancer, LNCaP $IL6^+$ cells, which produce IL6 due to transfection, it was shown that an autocrine IL6/IL6R loop is responsible for resistance to apoptosis and increased levels of Mcl-1 (myeloid leukemia-cell protein 1), an anti-apoptotic member of the Bcl-2 family (39). Treatment of the cells with CNTO328, a chimeric IL6 monoclonal antibody, led to the induction of apoptosis and a decrease of Mcl-1 protein. Specific knockdown of Mcl-1 by RNAi also caused an increase of apoptosis in LNCaP $IL6^+$ cells. *Vice versa*, inactivation of the IL6 autocrine loop had no influence on the apoptotic level in the absence of Mcl-1, thus suggesting this molecule as a mediator of the survival action. Involvement of p38 and ERK1/2 mitogen-activated protein kinase pathways in IL6-dependent regulation of Mcl-1 has been shown by making use of selective kinase inhibitors. It was shown that IL6 induces the growth of neuroendocrine cells which are increased in castrate-resistant prostate cancer (CRPC) representing a change in the prostate cell microenvironment. Neuroendocrine cells are present at low levels in the normal prostate and signify the transition phase of normal hormone-sensitive to hormone-refractory cells.

It has been shown that IL6 stimulates androgen synthesis and the androgen receptor (AR) in prostate cancer cells (40). IL6 is a potent inducer of protein encoded by the S100P gene (*S100P*) which is up-regulated in CRPC and metastatic prostate

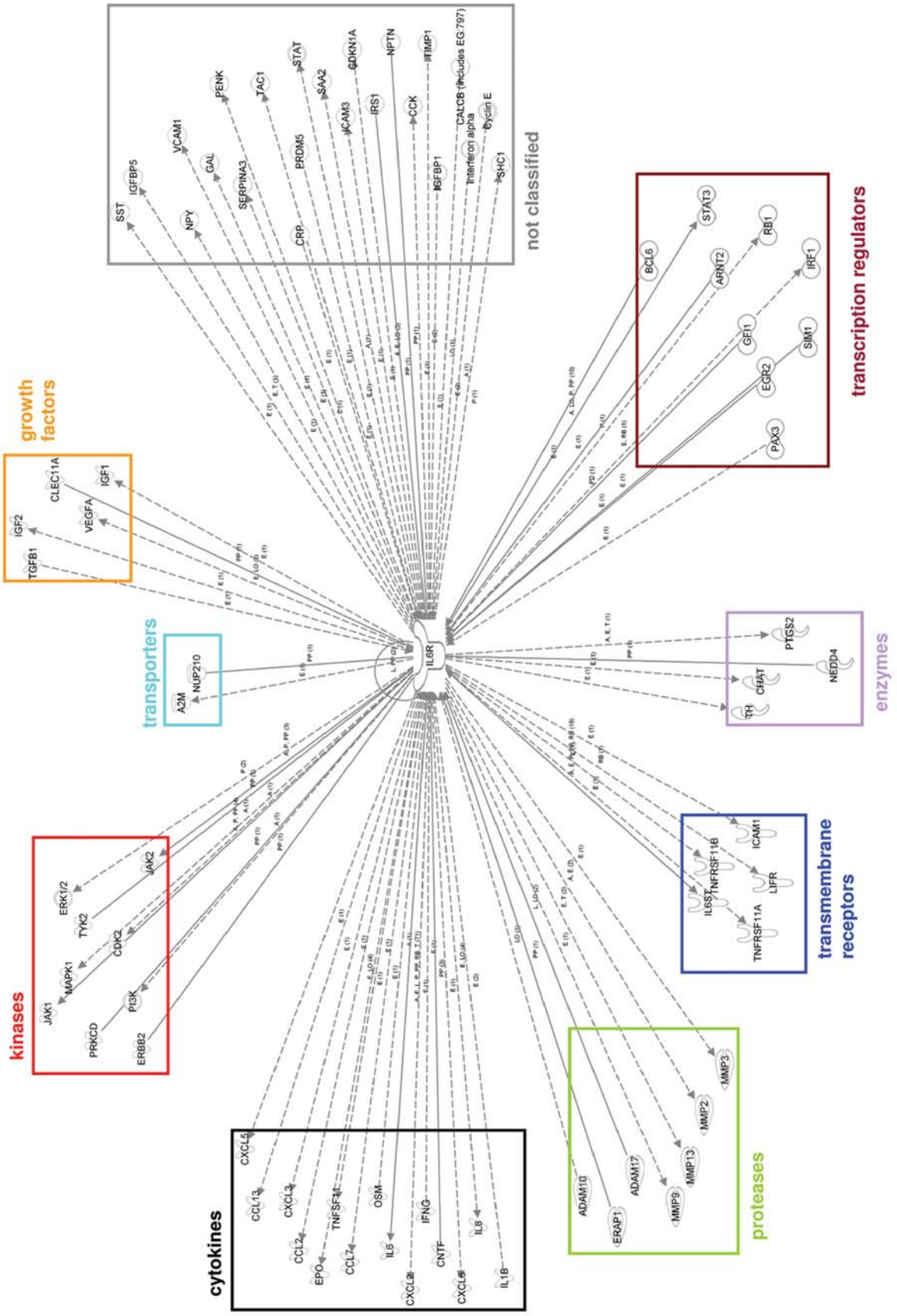
not classified
SST
IGFBP5
NPY
VCAM1
GAL
SERPINA3
PENK
TAC1
CRP
PRDM5
STAT
SAA2
ICAM3
CDKN1A
IRS1
NPTN
CCK
ITIMP1
IGFBP1
CALCB (includes EG:797)
Interferon alpha
Cyclin E
SHC1
growth factors
IGF2
TGFB1
CLEC11A
VEGFA
IGF1
transporters
A2M
NUP210
IL6R
transcription regulators
BCL6
STAT3
ARNT2
RB1
GFI1
IRF1
EGR2
SIM1
PAX3
enzymes
PTGS2
NEDD4
TH
CHAT
transmembrane receptors
IL6ST
TNFRSF11B
ICAM1
LIFR
TNFRSF11A
kinases
JAK1
ERK1/2
TYK2
JAK2
MAPK1
CDK2
PRKCD
PI3K
ERBB2
proteases
ADAM10
ADAM17
ERAP1
MMP9
MMP13
MMP2
MMP3
cytokines
CXCL5
CCL13
CXCL3
CCL2
EPO
TNFSF11
CCL7
OSM
IL6
CXCL2
IFNG
CNTF
CXCL6
IL8
IL1B

Figure 4.

cancer and therefore is implicated in the transition of androgen-sensitive to CRPC (41). IL6 contributes to the development of androgen independence. IL6 inhibition prevented conversion to androgen independance and prolonged survival in a mouse model (42). Elevated circulating IL6 and up-regulated S100P in prostate cancer specimens correlate independently with progression to androgen-independent and metastatic prostate cancer. Studies with LNCaP/C4-2B (functional androgen receptor) and PC3 cells (lacking functional androgen receptor) have suggested that IL6 may require a functional AR receptor for *S100P* induction. IL6 regulates androgen synthesis in prostate cancer cells (43). Androgen, deprivation therapy reduces testosterone concentrations, but intra-prostatic androgen levels have been measured at concentations sufficient to activate AR suggesting that prostate cancer cells may survive androgen deprivation therapy by increasing intracrine androgen synthesis. LNCaP IL6$^+$ cells were inoculated orthopically into the prostates of castrated nude mice and it was found that IL6 increased the levels of intracrine androgens through enhanced expression of genes mediating androgen metabolism in prostate cancer cells. It has been shown that IL6-stimulated growth of prostate cancer cells *in vitro* and *in vivo* occurs through activation of the AR (44). Growth of M.D. Anderson human prostate cancer cell line 2b (MDAPCa2b) xenografts in castrated animals was similar to that in non-castrated animals. Bicalutamide showed an inhibitory effect on IL6-regulated growth *in vivo*.

We have recent evidence that alteration in tissue homeostasis in the host may evoke significant up-regulation of IL6 expression which can then, in a paracrine fashion, increase the metastatic potential of circulating tumor cells: ablation of matrix metalloproteinase 9 (MMP9) in the host tissue of mice led to up-regulation of IL6 in the bone marrow and concomitant IL6 serum levels (45). Increased levels of IL6 significantly increased migration and invasion of CT26 murine colon carcinoma cells *in vitro* (45). When MMP-9 ablated mice exhibiting increased levels of IL6 were inoculated intravenously with gene encoding for β-galactosidase (lacZ)-tagged C26 cells, a dramatic induction of liver metastasis was found (45). Therefore, IL6 is an important mediator of invasion and metastasis as well as a signaling molecule that communicates alterations in tissue homeostasis over a distance, to other entities such as a tumor cell population or a target organ of metastasis such as the liver (45). Such impact of IL6 may even alter the so-called pre-metastatic niche (46) and could be an undesired side-effect of other metastasis-directed therapy approaches (47) and underlines the importance of IL6 as a pro-invasive and pro-metastatic signaling molecule.

IL6 has been shown to activate the phosphoinosite 3-kinase/cellular homolog of the oncogene of retrovirus Akt B (PI3K/Akt) pathway and to regulate cyclin A1 to promote prostate cancer survival (48). Treatment of cells with a PI3K inhibitor or cotransfection with a vector expressing wild-type phosphatase and tensin homolog (PTEN) reduced cyclin A1 promoter activity. LNCaP cells overexpressing cyclin A1 are resistant to campothecin-induced apoptosis. Xenograft tumors generated from LNCaP-IL6$^+$ cells showed higher levels of cyclin A1 and rapid tumor growth compared to LNCaP xenograft tumors. Induced Mcl 1 is regulated by IL6 and mediated the survival activity of cytokines in a model of late-stage prostate carcinoma (49). The involvement of IL6 in the growth of colorectal cancer has been demonstrated (50). Alteration of TGFβ signaling in colorectal cancer triggers the production of sIL6R and the activation of STAT3. In parallel,

Figure 4. *IL6 neighborhood analysis. Ingenuity Pathway Analysis (IPA) was used to create the neighborhood of genes with a reported relationship to IL6R. Neighboring molecules manually grouped and annotated according to their molecule type. A2M, Alpha-2-macroglobulin; ADAM10/17, a disintegrin metallopeptidase domain 10/17; ARNT2, aryl-hydrocarbon receptor nuclear translocator 2; BCL6, B-cell CLL/lymphoma 6; CALCB, calcitonin-related polypeptide beta; CCK, cholecystokinin; CCL2/7/13, chemokine (C-C motif) ligands 3/7/13; CDK2, cyclin-dependent kinase 2; CDKN1A, cyclin-dependent kinase inhibitor 1A; CHAT, choline acetyltransferase; CLEC11A, C-type lectin domain family 11, member A; CNTF, ciliary neurotrophic factor; CRP, C-reative protein, pentraxin-related; CXCL2/3/5/6, chemokine (C-X-C motif) ligands 2/3/5/6; EGR2, early growth response 2; EPO, erythropoietin; ERAP1, endoplasmic reticulum aminopeptidase 1; ERBB2,v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian); ERK1/2, mitogen-activated protein kinase 1/3; GAL, galanin prepropeptide; GFI1, growth factor independent 1 transcription repressor; ICAM1/3, intercellular adhesion molecule 1/3; IFNG, interferon, gamma; IGF1/2, insulin-like growth factor 1/2; IGFBP1/5, insulin-like growth factor binding protein 1/5; IL1B/6/8, Interleukin 1, beta/6/8; IL6ST, interleukin 6 signal transducer; IRF1, interferon regulatory factor 1; IRS1, insulin receptor substrate 1; JAK1/2, janus kinase 1/2; LIFR, leukemia inhibitory factor receptor alpha; MAPK1, mitogen-activated protein kinase 1; MMP2/3/9/13, matrix metallopeptidase 2/3/9/13; NEDD4, neural precursor cell expressed, developmentally down-regulated 4; NPTN, neuroplastin; NPY, neuropeptide Y; NUP210, nucleoporin 210kDa; OSM, oncostatin M; PAX3, paired box 3; PENK, proenkephalin; PI3K, phosphatidylinosital 3-kinase; PRDM5, PR domain containing 5; PRKCD, protein kinase C, delta; PTGS2, prostaglandin-endoperoxide synthase 2; RB1, retinoblastoma 1; SAA2, serum amyloid A2; SERPINA3, serpin peptidase inhibitor, clade A, member 3; SHC1, SHC transforming protein 1; SIM1, single-minded homolog 1; SST, somatostatin; STAT3, signal transducer and activator of transcription 3; TAC1, tachykinin, precursor 1; TGFB1, transforming growth factor beta 1; TH, tyrosine hydroxylase; TIMP1, tissue inhibitor of matrix metallopeptidase 1; TNFRSF11A/B, tumor necrosis factor receptor superfamily, member 11a/b; TNFSF11, tumor necrosis factor (ligand) superfamily, member 11; TYK2, tyrosine kinase 2; VCAM, vascular cell adhesion molecule 1; VEGFA, vascular endothelial growth factor A; Arrows: relationship type: A, activation; E, expression; L, proteolysis; LO, location; PD, protein-DNA interaction; P, phosphorylation; PP, protein–protein interaction; RB, regulation of binding; T, transcription. Number of journal references supporting the relationship are shown in brackets.*

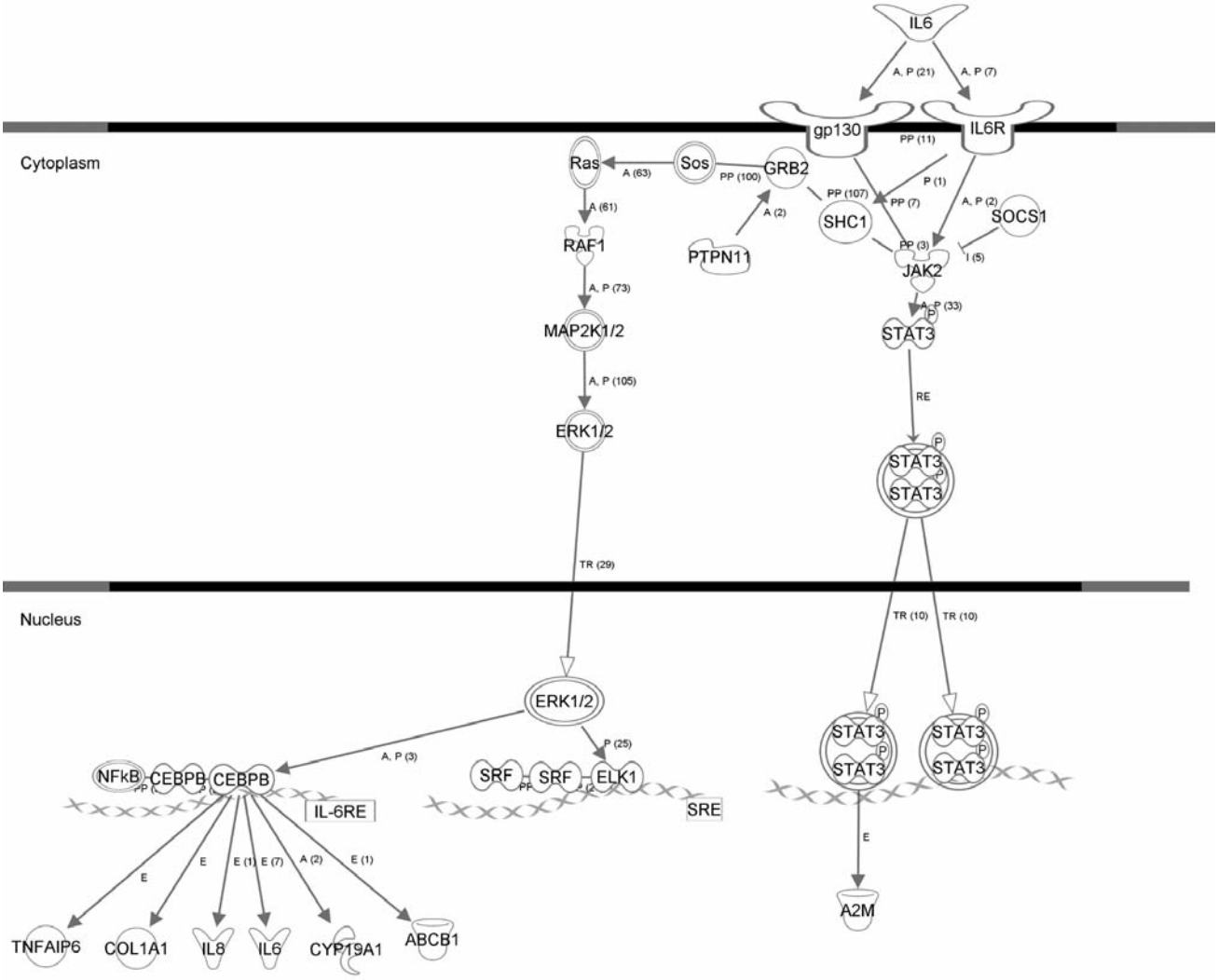

Figure 5. *IL6 signaling. Pathway derived from the Ingenuity database of canonical pathways devised using Ingenuity Pathway Designer. A2M, alpha-2-macroglobulin; ABCB1, ATP-binding cassette, sub-family B (MDR/TAP), member 1. IL-6RE, IL6 response element; CEBPB, CCAAT/enhancer binding protein (C/EBP), beta; COL1A, collagen, type I, alpha 1; CYP19A1, cytochrome P450, family 19, subfamily A, polypeptide 1; ELK1, member of ETS oncogene family; ERK 1/2, mitogen-activated protein kinase 1/3; gp130, glycoprotein 130; GRB2, growth factor receptor-bound protein 2; IL6, interleukin 6; IL6R, interleukin 6 receptor; IL8, interleukin 8; JAK2, janus kinase 2; MAP2K1/2, mitogen-activated protein kinase kinase 1/2; NFkB, group of nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 proteins; PTPN11, protein tyrosine phosphatase, non-receptor type 11; RAF1, v-raf-1 murine leukemia viral oncogene homolog 1; Ras, group of Ras proteins; SHC1, SHC transforming protein 1; Sos, group of son of sevenless homolog 1 proteins; SRE, serum response element. SRF, serum response factor; STAT3, signal transducer and activator of transcription 3; TNFAIP6, tumor necrosis factor, alpha-induced protein 6; Arrows: relationship type: A, activation; E, expression; P, phosphorylation; PP, protein–protein interaction; TR, translocation. Number of journal references supporting the relationship are shown in brackets.*

loss of membrane IL6R and increase of ADAM17 activity was observed (51). Tumor growth was inhibited by IL6R antibody or sgp130 Fc pointing to an important role of IL6 *trans*-signaling.

It has been shown that the genome of human herpes virus 8 (HHV8) encodes a viral version of IL6 (vIL6) with 25% homology to human IL6 (52). This virus was found to be associated with 90% of Kaposi sarcoma lesions and in patients with primary effusion lymphoma and Castleman disease (53). 3T3-mouse fibroblasts transfected with v-IL6 were able to promote angiogenesis and hematopoiesis after injection into mice (54). It was shown that vIL6 interacted with gp130 without a requirement for sIL6R and was able to stimulate cells which express gp130 and IL6R (55). This finding has implications for the extended range of target cells for vIL6.

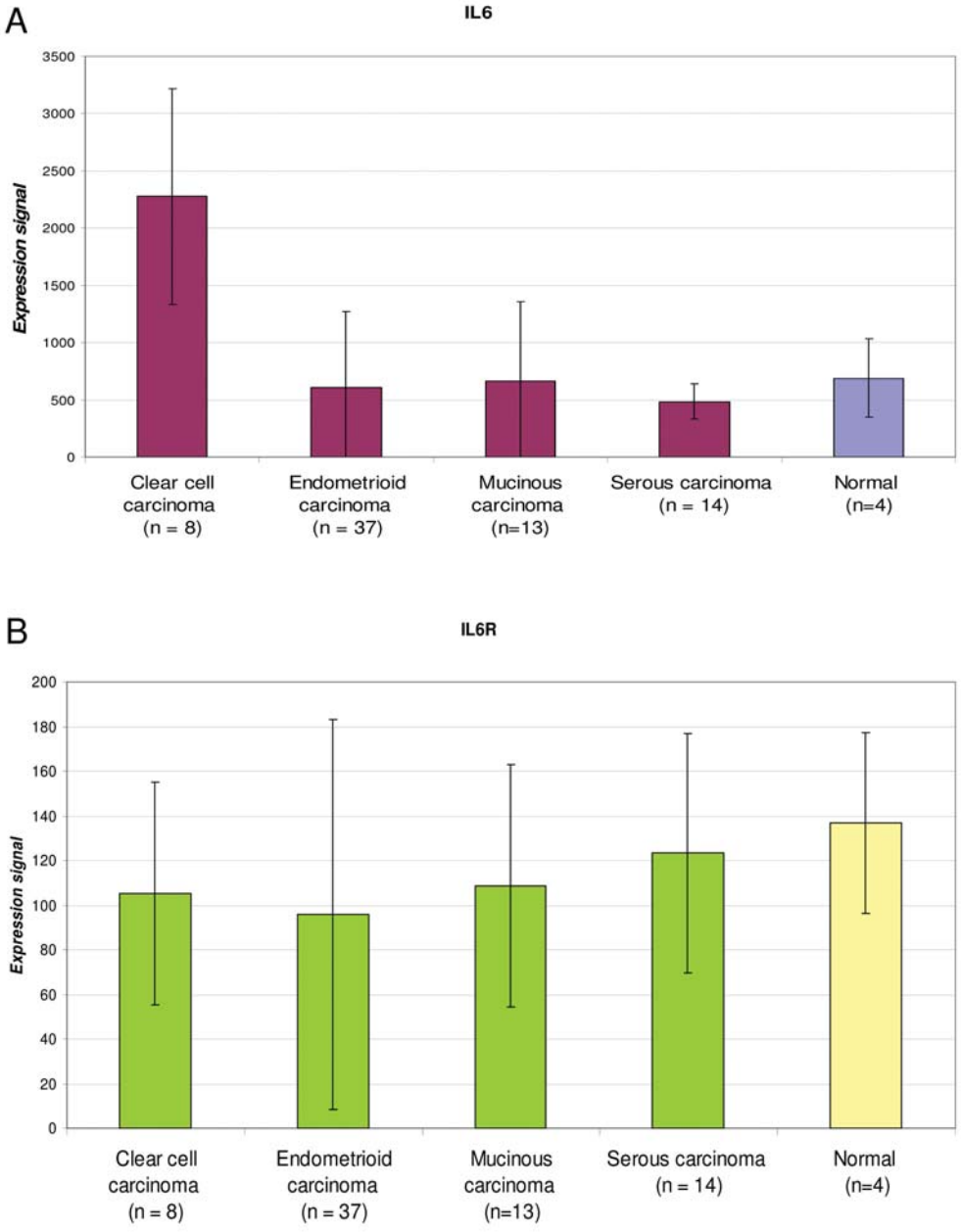

Figure 6. *Comparison of IL6 (A) and IL6R (B) gene expression signals. Signals from patient tumor and normal samples derived from ovary are shown. Gene expression data was derived from the GEO database (GSE6008). n depicts the number of biological samples within each group.*

Cachexia

High levels of IL6 mRNA have been observed in tumor tissues of pancreatic carcinoma patients with weight loss and increasing IL6 levels were noted during progression of disease. Symptoms of cachexia such as anemia, abnormalities of liver function, fatigue and vomiting can be induced by the administration of IL6. Elevated serum IL6 in patients with pancreatic cancer and correlation with cachexia has been observed (56). Cachexia worsens prognosis in patients with resectable pancreatic cancer (57). Life expectancy in patients with pancreatic carcinoma inversely correlates with serum IL6 levels (58). Involvement of IL6 in nerve-invasion is a key prognostic factor always associated with pancreatic cancer (100%) (55). IL6 was shown to be highly expressed at sites of nerve invasion and increased motility and chemotaxis

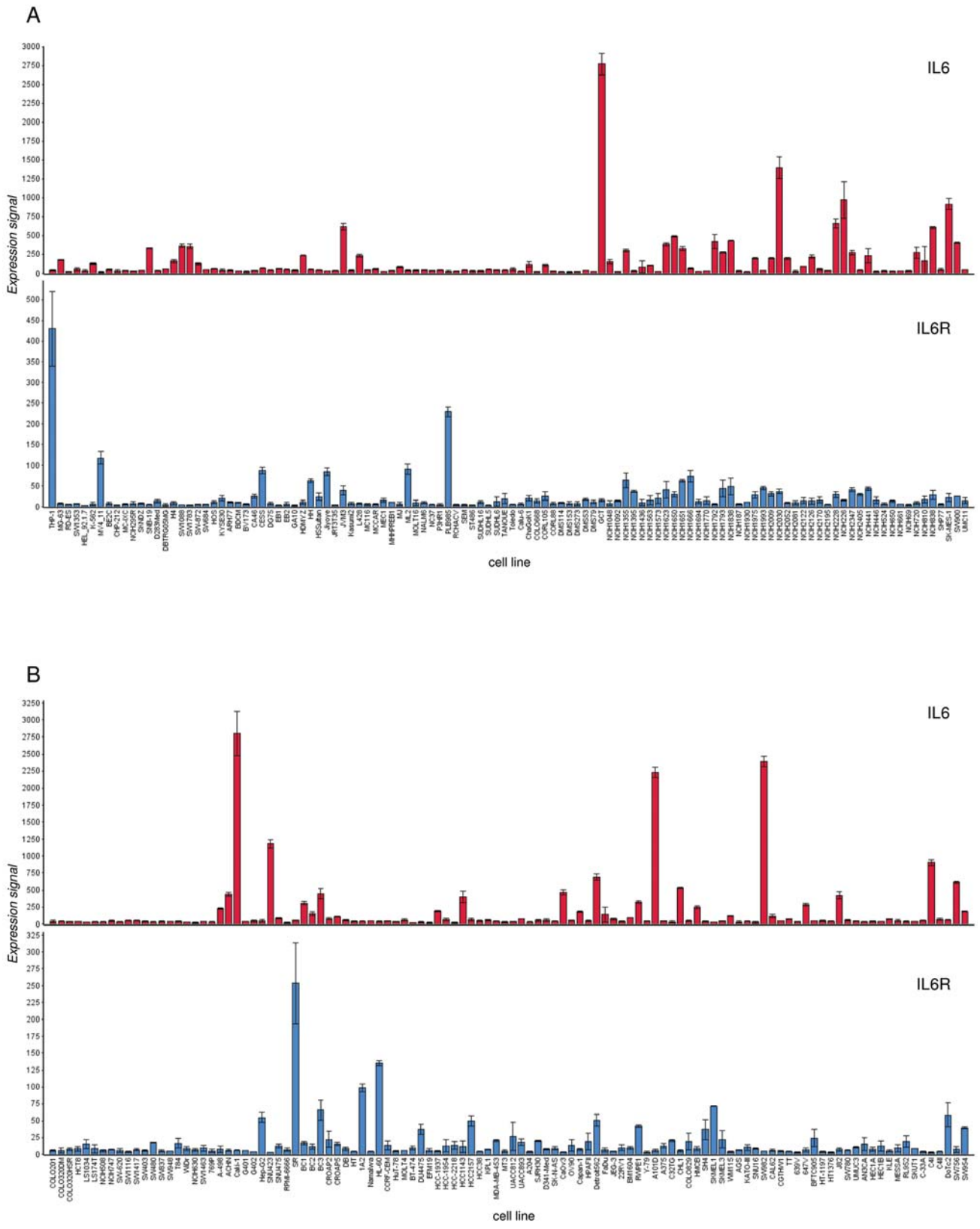

Figure 7. *Gene expression of IL6 and IL6R in 224 tumor cell lines. The first 113 cell lines are shown in (A) and the remainder in (B). Data was derived from ArrayExpress (E-MTAB-37). Shown are only cell lines with at least two replicates.*

accelerating nerve-invasion in a concentration-dependent manner. Inhibition of progression of nerve invasion results in analgesic effects. Pancreatic cancer related cachexia impacts on metabolism, weight loss and pulmonary function (59). A correlation between cytokines with phenotype characteristics and prognosis in pancreatic carcinoma has been established (58). IL6 is implicated in stimulation of VEGF secretion and neuropilin 1 expression in pancreatic cancer cells (60). A correlation of IL6 gene polymorphisms and IL6 serum levels in patients with pancreatic carcinoma and chronic pancreatitis has been observed (61). An independent study revealed an association of IL6 gene polymorphisms and survival times of patients with cachexia susceptibility in patients with pancreatic cancer (62). The expression of cancer cachexia-related factors in human cancer xenografts has been shown by immunohistochemistry (63). IL6 cDNA transfected Lewis lung carcinoma cells showed unaltered net tumor growth rate, but caused weight loss and shortened survival in syngeneic mice (64). The contribution of macrophages to cancer-related cachexia has been highlighted (65).

Treatment of Cancer with Antibodies Directed Against IL6 or IL6R

CNTO 328 is a chimerized anti-IL6 monoclonal antibody (mab) (66) and mab 1339 is a fully human version of murine mab B-E8 directed against human IL6 (67), both neutralizing the function of IL6. Toclizumab is a humanized (IgG1) IL6R mab which inhibits binding of IL6 to the IL6R and competitively inhibits IL6 signaling and completely neutralizes IL6 activity (68). Toclizumab is therapeutically active in rheumatoid arthritis, Crohn's disease, Castleman's disease and juvenile idiopathic arthritis (69-72). B-E8 has been evaluated in several proof-of-concept studies, especially in hematological malignancies (73-75). IL6 and IL6R mabs have been explored in several cancer-related models as outlined below.

CNTO 328 was investigated in cachexia-related *in vivo* models (76). Cachexia is characterized by progressive weight loss and depletion of host reserves of adipose tissue and skeletal muscle. In the A375 human melanoma model in nude mice controle mice lost 19% body weight from day 0 to day 31, wheras CNTO 328-treated mice lost only 1.5% body weight. In this model, CNTO 328 also inhibited the growth of the xenograft. Therefore it was not clear whether small tumor size or reduced secretion of cachectic factors contributed to the anti-cachectic effect of CNTO 328 in this model. In the human PC-3M prostate xenograft model, control treated animals lost 6% body weight whereas CNTO 328-treated animals gained 7% of body weight. In this model, no effect of CNTO 328 on growth was observed, albeit the xenograft secreted IL6. Since CNTO 328 blocks human, but not mouse, IL6, the data indicate that tumor cell secreted IL6 contributes to weight loss and argue in favor of IL6 mabs as anti-cachectic agents.

IL6 is a target for multiple myeloma because it plays an important role in myeloma growth and survival in the microenvironment of the bone marrow. Mab 1339 was shown to inhibit growth of IL6-dependent myeloma cells *in vitro* in a dose-dependent manner (75). The growth promoting effect of bone marrow stromal cells on MM1S, MM1R and U266 cells was significantly inhibited by mab 1339. Inhibition of STAT3, Akt and Erk signaling pathways by mab 1339 was shown. Mab 1339 potentiates the cytotoxicity of anti-myeloma agents such as dexamethasone, velcade, perifosine and revlimide in myeloma cell lines co-cultured with bone marrow stromal cells. *In vivo* efficacy of mab 1339 was shown in a severe combined immunodeficiency (SCID)-mice model in which the human multiple myeloma cell line INA-6 is engrafted into a human fetal bone chip previously implanted into SCID mice and the level of soluble human IL6R produced by INA-6 cells in the murine serum is used as a marker for tumor burden. A combination of mab 1339 and dexamethasone resulted in superior efficacy compared to dexamethasone or mab 1339 alone. CNTO 328 revealed cytotoxicity *in vitro* in H-929, MM1.S, RPM/8226, ABNL-6 and KAS-6/1 multiple myeloma cells with varying sensitivity for different cell lines (77). Isobologram analysis indicated synergistic *in vitro* activity of a CNTO 328/bortezomib combination over a dose-range of bortezomib that exhibited single-agent cytotoxicity in established multiple myeloma cells and in patient-derived multiple myeloma cells. CNTO 328 had no apoptotic activity in H-929 cells, but strongly enhanced the apoptotic activity of bortezomib. These data would support the clinical evaluation of CNTO 328 and bortezomib in patients with multiple myeloma. Although patient outcomes have been improved with the advent of immunomodulatory agents such as thalidomide and lenalidomide and the proteasome inhibitor bortezomib, glucocorticoids remain an important component of multi-agent chemotherapy for myeloma (78). However, resistance to glucocorticoids is a serious clinical problem. It has been shown that CNTO 328 increased the cytotoxicty of dexamethasone in IL6-dependent and -independent cell lines (79). Although CNTO 328 displays minimal cytotoxicity as a single agent, it potentiated dexamethasone mediated apoptosis as shown by the activation of caspases 3, 8 and 9, Annexin V staining and DNA fragmentation. Sensitization was preserved in the presence of bone marrow stromal cells. The p44/42 mitogen-activated protein kinase pathway was identified as a mediator of the IL6-induced glucocorticoid resistance. The increased activity of the combination of CNTO 328 and dexamethsone was also seen in plasma cells from patients with glucocorticoid-resistant myeloma.

In a preclinical *in vivo* model, it has been shown that IL6 plays a role in the conversion from an androgen-dependent to an androgen-independent state. The model is based on LuCaP35 androgen-sensitive, prostate-specific antigen (PSA)-producing xenografts. It expresses the AR and the response to androgen ablation is similar to that observed in man. An androgen-

insensitive variant of LuCaP35 has been established by making use of recurring LuCaP35 tumors (80). Mice bearing LNCaP 35 human androgen-dependent prostate cancer xenografts were castrated and IL6 activity was blocked with CNTO 328 for a period of 18 weeks (81). IL6 inhibition increased survival of the mice and inhibited tumor growth reflected by decreased tumor volume and prostate-specific antigen levels compared to mice which received an isotype-matching control antibody. It was shown that tumor cells derived from the isotype-treated animals converted to an androgen-independent state, whereas tumor cells from the anti-IL6 mab treated mice were still androgen-dependent *in vitro* and *in vivo*. No difference of AR levels were noted and IL6 mab was shown to promote apoptosis and inhibition of cell proliferation. CNTO 328 has also been studied in LnCaP-IL6+ prostate cancer cells which secrete IL6 and show features of advanced prostate cancer such as accelerated growth and increased levels of vascular endothelial growth factor (VEGF)(82). As outlined previously, CNTO 328 caused a statistically significant inhibition of cell viability and decrease of Bcl-2 levels and phosphorylation of ERK1/2 mitogen-activated protein kinases. The effect of CNTO 328 on tumor growth was not statistically significant, however, the decrease of Ki-67-positive cells in the CNTO 328-treated tumors was statistically relevant. The role of Mcl-1 as a mediator of cell survival in androgen-independent prostate cancer has been discussed previously in this review.

Toclizumab was evaluated in preclinical models of oral squamous cell carcinoma (83). All the cell lines investigated (SA3, HSC2, HSC3 and HSC4) expressed IL6 and IL6R, in contrast to human keratinocytes. IL6 expression was found in 95% (n=58) of oral squamous cell carcinoma (OSCC) samples, whereas almost all of the normal mucosal tissues showed no reactivity. Neither toclizumab nor exogenous IL6 influenced the proliferation rate of all the OSCC cell lines investigated. *In vivo* growth of a SAS (cell line derived from a well-differentiated human OSCC) xenograft was significantly inhibited by Toclizumab with a marked reduction of STAT3 phosphorylation in tumor cells. A significant impairment of angiogenesis was observed in the xenografts. Since Toclizumab does not inhibit murine IL6 and IL6R, the therapeutic effect relies on inhibition of an autocrine loop between human IL6 and human IL6R. It was shown that Toclizumab suppressed VEGF secretion in OSCC cell lines, but the anti-angiogenic effect of Toclizumab also in part may result from modulation of other angiogenic factors such as IL8 and MMP9. As mentioned earlier, it is possible that inhibition of MMP9 may evoke up-regulation of IL6 expression in bone marrow with systemic consequences including promotion of metastasis (45, 47). In essence it is fair to say that the impact of neutralizing the function of IL6 or vIL6R with respect to cell survival and proliferation is strongly context-dependent and the inhibition of autocrine loops between human IL6 and IL6R with mabs in *in vitro* systems results in varying outcomes which are also reflected in *in vivo* systems.

Clinical Studies

Proof-of-concept clinical studies have been summarized for CNTO 328 (chimeric) and BE-8 (murine) (72). Six clinical studies in patients with multiple myeloma, renal cell carcinoma and B lymphoproliferative diseases were discussed (72). Decrease of CRP levels and decrease of incidence of cancer-related anorexia and cachexia were found in a large fraction of patients (73). However, these studies were not optimally designed from a pharmacokinetic (PK) and pharmacodynamic (PD) point of view. BE-4 and BE-8 are murine antibodies and human antibodies directed against BE-4 and BE-8 were detected. In addition, BE-8 could not effectively block daily production levels of IL6 that was >18 μg/day (84). Inhibition of delayed IL6 secretion may not be possible without repeated dosing due to the short half-life of BE-8 (3-4 days) and because murine mabs are generally neutralized by the human anti-mouse response approximately two weeks after treatment. Although there was no evidence of an immune response against CNTO 328 (siltuximab), it is possible that the high serum concentrations of siltuximab may have masked their detection (84, 85). The schedule of administration of CNTO 328, repeated daily dosing for 14 consecutive days at 4 weeks intervals is not optimal for patients and clinicians. Therefore, considering that the half-life of siltuximab was 17.8 days, treatment schedules that would allow for more convenient, less frequent dosing were designed based on the PK/PD properties of siltuximab (86). In this study, PK, PD and PK/PD modeling data were applied in a prospective manner to assist in selection and the dose schedule of treatment regimens for siltuximab in a phaseI/II study in patients with metastatic renal cell carcinoma with the prospect of extending these findings to treatment of different types of tumors. The clinical studies also suggested that CRP levels can be used as a potential biomarker of IL6 bioactivity.

IL6/IL6R System as a Therapeutic Target in Oncology

As outlined in the previous sections, IL6/IL6R interaction has an impact on tumor cell proliferation and survival and can confer an anti-apoptotic and pro-invasive signal to tumor cells. The level of impact on these processes is context-dependent. More detailed investigations of the IL6/IL6R status and cells of the tumor stroma (fibroblasts, macrophages) could reveal the possible interplay between tumor and stromal cells regarding IL6-mediated signaling. These interactions are probably tumor-type-specific and will define the autocrine and paracrine contribution to IL6-mediated signaling. In autocrine systems, the contribution to proliferation, anti-apoptotic and anti-invasive/metastatic function of the IL6 system could be investigated in a panel of tumor cells and *in vivo* models with blocking antibodies directed against IL6 or IL6R. Regarding the interpretation of the results of xenograft models one has to consider that murine IL6 acts in

a species-specific manner, whereas human IL6 is also active on IL6R positive murine cells. The contribution of the autocrine IL6/IL6R loop could be assessed adequately *in vivo*, however the contribution of paracrine loops based on IL6 secreted by stromal cells cannot be assessed in xenograft models, but the use of purely murine tumor models has revealed the paracrine function of host IL6 in communicating changes in the local proteolytic networks to distant entities, what we have defined as the 'proteolytic internet' (45) and suggested the inhibition of such undesired side-effects by employing IL6 specific drugs (47). The preclinical testing of mabs directed against IL6 and IL6R has been restricted to a few tumor cell lines with an appropriate autocrine loop. The monitoring of an additional 224 cell lines for coexpression of IL6 and IL6R at the RNA level did not reveal further candidate cell lines employing an autocrine loop, as shown in Figure 7, emphasizing the importance of paracrine interactions for the activation of the IL6/IL6R pathway. As described, blockage of IL6/IL6R signaling resulted in interference with inflammation-associated disease such as colitis-associated colon cancer and hepatocellular carcinoma. As outlined, blockage of IL6/IL6R signaling with monoclonal antibodies directed against IL6 or IL6R translates into *in vivo* activity in xenograft models with an autocrine IL6/IL6R loop such as multiple myeloma, prostate carcinoma and oral squamous cell carcinoma. The impact of IL6/IL6R interaction with respect to proliferation and cell viability of the cell lines used in the xenografts in the absence of immune effector cells has been documented with the exception of the four squamous cell carcinoma cells (all IL6- and IL6R-positive) for which inhibition of proliferation was not observed after treatment with Tocilizumab (83). The contribution of IL6/IL6R signaling to proliferation and viability of defined tumor cell types seems to be context-dependent since many other factors can activate NFκB and STAT1/3. Therefore an important function of IL6 signaling-blocking agents might be based on their capability to sensitize tumor cells compared to approved agents such as bortezomib, dexamethasone, lenalidomide and others. Assessment of the activation status of STAT3 might be a viable biomarker strategy. Possible parameters for patient stratification are serum IL6 levels, serum sIL6R/IL6 complexes, IL6 and IL6R expression in tumor cells and corresponding stroma (fibroblasts, macrophages and other cells of the immune system). The documented impact of IL6 on cachexia should be the basis for treatment of this wasting syndrome with IL6-signaling inhibitors, not only for palliative treatment of tumors such as pancreatic carcinoma, but also with respect to the improvement of clinical end-points such as survival. Due to the involvement of IL6/IL6R interaction in perineural invasion, pain reduction in patients with pancreatic carcinoma and other types of cancer is also on the list of potential benefits of inhibitors of IL6 signaling. Presently, much attention in translational cancer research is focused on autocrine loops and interactions of the microenvironment with tumors. Therefore, the IL6/IL6R system has gained a remarkable renaissance and it remains to be seen whether IL6-signaling inhibitors will translate into clinical benefit in appropriately designed clinical studies.

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