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INTERLEUKIN-6 AS A TARGET IN CANCER

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SUMMARY

Interleukin-6 (IL-6) is a multifunctional cytokine which is implicated in tumorigenesis and progression in several human cancers. Whereas in prostate cancer both growth-stimulatory and -inhibitory effects could be induced by IL-6, this cytokine is a positive growth factor in hematological malignancies, including myeloma and chronic lymphocytic leukemia. Elevated IL-6 levels were measured in sera from patients with various malignancies. IL-6 could stimulate Janus kinase (JAK)/signal transducer and activator of transcription (STAT), mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K/Akt) signaling pathways in a nonexclusive manner. The action of IL-6 is regulated through endogenous inhibitors, such as suppressors of cytokine signaling and protein activators of activated STAT. Several options for targeting IL-6 are available. These include anti-IL-6 or anti-IL-6 receptor antibodies, antisense oligonucleotides and superantagonists. The chimeric monoclonal anti-IL-6 antibody CNTO-328 has been tested in various experimental models, in particular in prostate cancer and myeloma. In prostate cancer, it was shown that CNTO-328 could inhibit the growth of PC-3 xenografts or prevent the acquisition of a therapy-resistant phenotype in LuCaP 35 xenografts. In myeloma models, CNTO-328 may potentiate the effect of bortezomib or sensitize the cells to dexamethasone. At present, limited data are available to judge the clinical usefulness of CNTO-328, but ongoing experimental studies may provide the basis for future improvement of clinical treatment with the anti-IL-6 antibody.

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IL-6 SIGNAL TRANSDUCTION

Interleukin-6 (IL-6), a 26-kDa protein, is a multifunctional cytokine that may activate different signaling pathways in target tissues (reviewed in Ref. 1). Its role has been mostly investigated in relation to inflammation. During inflammation, IL-6 upregulates the expression of C-reactive protein (CRP). IL-6 expression may also be elevated in other nonmalignant diseases, such as osteoporosis, autoimmune arthritis, Paget's disease and Alzheimer's disease. However, its biological function is not limited to the regulation of immune responses. The plethora of biological effects of IL-6 include regulation of T- and B-cell differentiation, induction of the expression of acute-phase proteins, terminal (e.g., neuroendocrine) differentiation and regulation of cellular events (proliferation, apoptosis, angiogenesis) in hematological, urological, skin and gastrointestinal tumors. More recently, there has been an increased interest in the role of IL-6 in the process of epithelial to mesenchymal transition, frequently described in cancer. This transition is characterized by a switch in the expression of E-cadherin to N-cadherin (the latter is increasingly produced). Other mesenchymal markers, such as vimentin, may be upregulated. IL-6 may be produced by benign epithelial, stromal and tumor cells.

The IL-6 receptor contains two subunits: the ligand-binding subunit gp80 and the signal transduction subunit gp130. The presence of gp80 determines ligand-specific effects, whereas signal transduction through gp130 could be initiated through other cytokines, such as IL-11, oncostatin-M (OSM), leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF) and cardiotrophin-1 (CT-1). In addition to the membrane receptor, several effects of IL-6 could be mediated through the soluble receptor, the expression of which may increase in certain diseases. Agonist signaling through the soluble receptor is known as trans-signaling. Following ligand binding to the IL-6 receptor and homo- or heterodimerization of the receptor, activation of Janus kinase (JAK)/signal transducer and activator of transcription (STAT), mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt kinase signaling pathways in a nonexclusive manner has been described (1). Nuclear phosphorylation and translocation of STAT3 is relatively specific for the effect of IL-6, whereas MAPK and Akt could be activated by several other factors and their increased phosphorylation is common in human cancers (2, 3) (Fig. 1).

Besides IL-6, epidermal growth factor (EGF) is known to increase STAT3 activity. In addition to STAT3, IL-6 may induce phosphorylation of other STAT factors, although not to the same extent. Activation of STAT factors is endogenously controlled by suppressors

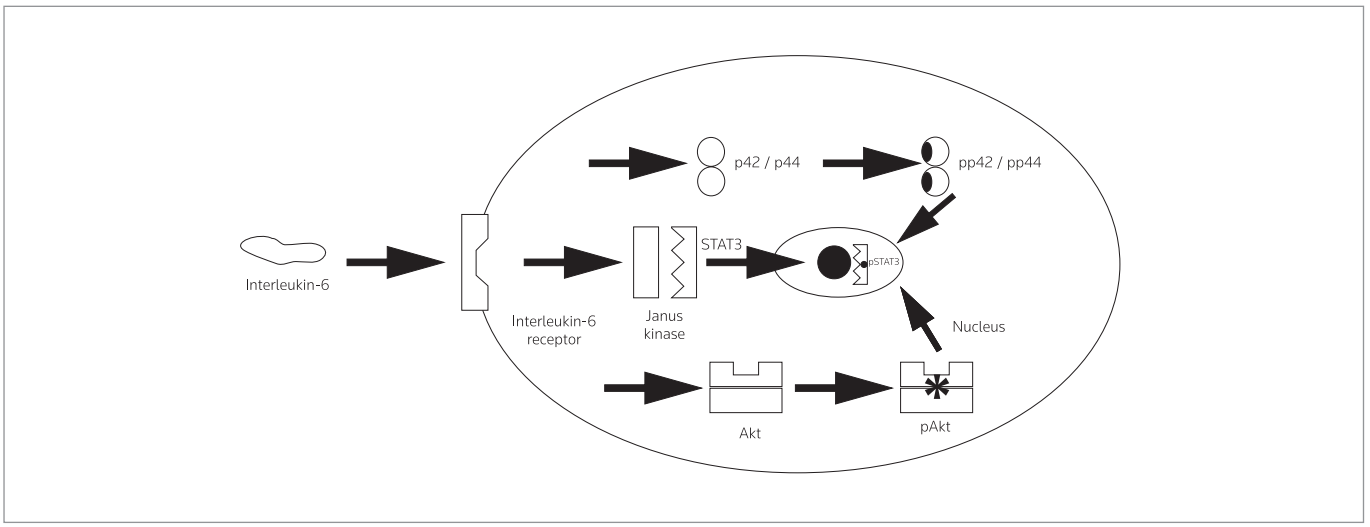

Figure 1. IL-6 may induce activation and nuclear translocation of elements of signaling pathways of JAK/STAT3, MAPK and Akt in target tissues. This activation regulates cellular events in a cell type-specific manner.

of cytokine signaling (SOCS) and protein inhibitors of activated STAT (PIAS) (4, 5). These inhibitors antagonize continuous activation of the JAK/STAT pathway, and in this way may contribute to the prevention of chronic inflammation. The function of IL-6 inhibitors is cell type-specific.

Activation of the JAK/STAT pathway per se could not be associated with either proliferation or inhibition of cell growth. Although there is a large body of evidence suggesting the association of STAT3 phosphorylation with malignant transformation, inhibition of proliferation and terminal differentiation was also reported (6, 7).

IL-6 has a role in the self-renewal of stem cells and their progenitors. Inflammatory cytokines are frequently elevated in cells in which stem cell markers are detectable (8). Because of an increased interest in the role of stem cells in cancer development and progression to therapy resistance, it is felt that more emphasis should be put on investigation of the role of IL-6 and other cytokines in the conversion of premalignant to malignant lesions. Possible antitumor effects of IL-6 in prostate cancer may also be anticipated. For example, IL-6 could exert antitumor effects by stimulation of the activity of macrophages and killer cells in the immune system, and activation of the complement system, which may result in the lysis of tumor cells.

In this review, the focus will be on the effect of IL-6 in several human malignancies in which this cytokine is an established therapeutic target. Importantly, the potential of the antibody CNTO-328 in experimental cancer therapy will be addressed.

IL-6 EXPRESSION IN PROSTATE CANCER AND HEMATOLOGICAL MALIGNANCIES

IL-6 measurements in sera from patients with prostate cancer represented the first investigation into the role of this cytokine in this malignancy (9). Prostate cancer can be cured if detected in the early stage and the possibilities for therapeutic intervention include radical surgery or radiotherapy. An increasing number of localized prostate cancers are diagnosed, although many small prostate tumors do not become clinically significant. Non-organ-confined tumors are in most cases treated with either orchiectomy or chemical castration and/or antiandrogens. If endocrine treatment fails, chemotherapy with docetaxel is an approved option for these patients. However, treatment with docetaxel prolongs the patients' survival for only a few months.

IL-6 was found to be increased in the sera of patients who present with metastases and failed hormonal therapy (9). These findings were extended by Ittmann's group, who reported that IL-6 and IL-6 receptor levels increased in tissue extracts obtained from patients who underwent radical prostatectomy (10). On immunohistochemistry, IL-6 and its receptor were detectable in the majority of prostate tumor cells (11). This finding is consistent with data showing that IL-6 is secreted by most prostate cancer cell lines. High levels of IL-6 were detectable in the supernatants from the androgen receptor-negative cell lines DU 145 and PC-3 (9).

Similar findings were obtained in patients with chronic lymphocytic leukemia (CLL). An increase in IL-6 serum levels was evident in a subgroup of patients and it was proposed that IL-6 and soluble IL-6 receptor are markers for this disease. In addition, IL-6 expression levels in the plasma of these patients increased with the disease stage, and thus, IL-6 could be considered a prognostic factor (12). High IL-6 levels in serum were measured in patients with aggressive non-Hodgkin's or large cell lymphoma (13, 14). It has been proposed that elevation of IL-6 expression occurs due to stimulation by IL-1, which is produced by tumor cells themselves and in the microenvironment. In multiple myeloma, bone marrow stromal cells show high expression of IL-6. A considerable percentage of myeloma cells are IL-6- and IL-6 receptor-positive, so that an autocrine loop could be postulated in these cases (15). IL-6 trans-signaling may be particularly important for the development and progression of multiple myeloma, since expression levels of the soluble receptor markedly increase in this disease (16).

An increase in serum levels of IL-6 was reported in patients with metastatic breast cancer (17), gastrointestinal cancer (18), extensive small cell lung cancer (19), melanoma (20), ovarian cancer (21) and pancreatic cancer (22). In some of these tumors, increased IL-6 expression is associated with the existence of a paraneoplastic syndrome.

PROSTATE CANCER – EVIDENCE FOR MULTIFUNCTIONAL EFFECTS OF IL-6

Basic science studies carried out recently in prostate cancer models revealed differences in the expression and function of IL-6. Androgen receptor-positive LNCaP cells express neither IL-6 mRNA nor protein (9). In several publications, LNCaP cells were treated with IL-6 and contrasting results were reported (6, 10, 23). IL-6 may either inhibit or stimulate their proliferation in vitro. These differences may be explained by different numbers of cells seeded or other variations in cell culture conditions. Inhibition of LNCaP proliferation by IL-6 is associated with enhanced STAT3 phosphorylation and terminal neuroendocrine differentiation (7). Neuroendocrine cells are growth-arrested, although they secrete peptides which affect the proliferation of adjacent cells in a paracrine manner. There may be several factors which contribute to a lack of expression of IL-6 in LNCaP cells, such as the presence of the androgen receptor, which may inhibit the activation of the IL-6 upstream regulator nuclear factor NF-kappa-B (NF-κB) or the expression of the functional tumor suppressor retinoblastoma-associated protein (Rb), which is a repressor of IL-6 expression (24, 25). In other tumor models, IL-6 expression may be enhanced through upregulation of transforming growth factor β (TGF-β), a peptide that is growth-inhibitory in vitro and -stimulatory in vivo (26). Thus, it should be kept in mind that there is a subgroup of patients with prostate cancer in whom IL-6 is not a target for therapy.

The differences in the sensitivity of prostate cancer cells to IL-6 are comparable to those observed in human melanoma (27). In contrast, LNCaP cells transfected with IL-6 cDNA acquire a growth advantage and the ability to grow under androgen-deprived conditions (28). Similar findings were obtained after prolonged treatment of LNCaP cells, which upregulate endogenous IL-6 expression and grow faster in vitro and in vivo (23, 25). This growth advantage is associated with upregulation of cyclin-dependent kinases, reduced expression of tumor suppressors and increased activation of the MAPK signaling pathway. In a subline established in the presence of IL-6, i.e., LNCaP-IL-6$^+$ cells, there is also an upregulation of vascular endothelial growth factor (VEGF), thus suggesting angiogenesis as an underlying mechanism of accelerated growth in vivo (29). Thus, IL-6, which acts as a paracrine growth inhibitor in some cells, may, during tumor progression, function as an autocrine growth stimulator (30). The cells in which IL-6 is upregulated also express higher levels of the survival molecule Mcl-1 (31). Mcl-1 may be a common mediator of the anti-apoptotic effects of IL-6 in various other malignancies.

In contrast to LNCaP cells, in androgen receptor-negative PC-3 and DU 145 cells IL-6 is secreted in an autocrine manner (9). In PC-3 cells IL-6 promotes tumor cell survival through activation of the PI3K/Akt signaling pathway (32). Contrasting results regarding activation of STAT3 were reported for DU 145 cells (6, 33).

IL-6 is a known activator of the androgen receptor (AR) in prostate cancer. Nonsteroidal activation of the AR may be relevant to tumor progression after androgen withdrawal therapy. A classic example of the growth-supportive effect is regulation of the AR by erbB-2/NEU (34). The effects of IL-6 on the activation of the AR were investigated in LNCaP and MDA-PCa-2b cells. Interestingly, in LNCaP cells AR activation by IL-6 leads to differentiation, as evidenced by increased expression of the prostate-specific antigen (PSA) gene (*KLK3*), whereas in the MDA-PCa-2b cell line the IL-6/AR interaction promotes proliferation and growth in vivo (35, 36). The processes which may lead to metastatic spread are in part mediated through the soluble IL-6 receptor. In this context, it is important to note that the tumor suppressor maspin is downregulated by IL-6 in the presence of the soluble IL-6 receptor (37).

In recent publications it has been documented that SOCS-3 expression is inversely correlated with phosphorylation of STAT3 in prostate cancer (38). Moreover, downregulation of SOCS-3 by specific siRNA leads to an increase in phosphorylation of STAT3. However, it is also evident that the role of SOCS-3 is not limited to interference with IL-6/STAT3 signaling. This member of the SOCS family inhibits tumor cell death in androgen-insensitive cells (39). On the other hand, induction of SOCS-3 by androgen leads to inhibition of proliferation and secretion (40). Another member of the SOCS family, SOCS-1, is ubiquitously expressed in prostate cancer cells and clinical specimens. Interestingly, a tumor suppressor role has been documented for SOCS-1 (41).

Although there is an indication that chronic prostate inflammation is associated with carcinogenesis in a subset of patients, it is difficult to establish a causative role for IL-6 in that process. The major obstacle in achieving a better understanding of the processes that link inflammation, premalignant status and cancer is the lack of appropriate models relevant to human disease.

THE ROLE OF IL-6 IN HEMATOLOGICAL MALIGNANCIES

Glucocorticoid steroids cause cell death in multiple myeloma and leukemia cell lines. Resistance to glucocorticoids develops and the factors that antagonize the effect of IL-6 are under investigation. The effect of IL-6 on the development of a therapy-resistant phenotype has been investigated. Therapy resistance may be largely mediated through the MAPK rather than the Akt pathway, and IL-6 contributes to its activation. The growth of several plasma cell lines is IL-6-dependent. IL-6 may be induced in response to IL-1, a cytokine that regulates the growth of myeloma cells in an autocrine manner (42). The survival of myeloma cells was promoted through overexpression of Mcl-1 and Bcl2-L-1 (43). Mcl-1 could also be induced by other oncogenes, such as VEGF (44). The importance of IL-6 in the development of B-lineage neoplasms was confirmed in an animal model (45). Mice homozygous for the IL-6-null allele are completely resistant to the development of B-lineage tumors. Consistent with these findings, downregulation of IL-6 receptors by the prodifferentiation agent retinoic acid leads to retardation of the growth of myeloma cell lines (46).

The pleiotropic nature of IL-6 regulation was observed in B-chronic leukemia (B-CLL) cells in which IL-6 antagonized the proliferative effect of TNF (47). However, at the same time, IL-6 may prolong survival in chronic leukemia cell lines (48). An important regulator of

cytokine signaling, SOCS-1, is frequently silenced by methylation in multiple myeloma (49). Thus, the action of IL-6 and related cytokines may be enhanced in this disease. SOCS-1 may, for example, inhibit cell cycle progression through direct inhibition of cell cycle proteins and cyclin-dependent kinases (41). Consistent with these findings, adenoviral expression of SOCS-1 may lead to inhibition of IL-6-induced myeloma proliferation (50).

REGULATION OF GROWTH OF RENAL CANCER BY IL-6

IL-6 is an autocrine and/or intracrine growth factor for renal cancer cells (51, 52). Thus, it was shown that interferon-γ (IFN-γ) interferes with the IL-6 autocrine loop in this disease. Takenawa and colleagues have confirmed that there is an increased expression of IL-6 in tumors obtained from patients with renal cancer (53). Consistent with its role in disease progression, patients with stage IV renal cell cancer presented with the highest IL-6 levels (54). The autocrine IL-6 loop in patients with renal cell cancer could be explained by frequent downregulation of the tumor suppressor p53 (55). As expected, NF-κB subunits p65 and p50 and activity are elevated in patients with renal cell carcinoma (56). Expression of the inhibitor I-kappa-B-alpha (IκBα) is, in contrast, decreased. It was suggested that the major pathway that contributes to tumor cell proliferation in renal cells is JAK/STAT3 (57).

In experimental studies, renal cancer cells were sensitized to cisplatin by monoclonal antibodies against IL-6 or the IL-6 receptor (58). Interestingly, in cells treated with the anti-IL-6 antibodies glutathione *S*-transferase P, which is antiproliferative, mRNA expression decreased. Patients with renal cell carcinoma frequently present with a paraneoplastic inflammatory syndrome, which is characterized by a decrease in platelets, polymorphonuclear neutrophils and monocytes (59).

EFFECTS OF THE ANTI-IL-6 ANTIBODY CNTO-328 IN PRECLINICAL MODELS OF PROSTATE CANCER

Since most prostate cancer cell lines respond to IL-6 by proliferation or inhibition of apoptosis, IL-6 may be an appropriate target for novel therapies. In vitro and in vivo studies were performed in order to establish an experimental basis for the new treatment. Some possibilities to interfere with the oncogenic effects of IL-6 in various tumors, including prostate cancer, were previously mentioned. The results from basic science studies imply that there is a heterogeneous responsiveness of prostate cancer to treatment with an anti-IL-6 antibody. CNTO-328 is a chimerized murine monoclonal antibody to IL-6. This antibody contains the variable antigen-binding region of the murine anti-IL-6 antibody and the constant region of the human $IgG_{1\kappa}$ immunoglobulin (60). The use of other anti-IL-6 antibodies in clinical studies in oncology was reviewed elsewhere (61). An overview of the effects of CNTO-328 in preclinical experimental models is provided in Table I.

On the basis of basic science studies, PC-3, DU 145 and LNCaP-IL-6$^+$ cells are considered models for anti-IL-6 therapy. Consistent with the increased phosphorylation of Akt by IL-6 in that cell line, CNTO-328 inhibited the growth of PC-3 xenografts via induction of apoptosis. Etoposide-induced apoptosis was potentiated by CNTO-328 (62). However, a less pronounced effect of CNTO-328 was noted with the more metastatic variant of PC-3, PC-3M (63). On the other hand, treatment of experimental animals with CNTO-328 inhibited the paraneoplastic syndrome, which is frequently observed in tumors in which IL-6 is overexpressed. In contrast, there was little effect of CNTO-328 on the viability of LNCaP-IL-6$^+$ cells (64).

The LuCaP 35 xenograft is considered a good model for studying the progression of prostate cancer. It was possible to achieve prolonged monitoring of animals treated with the anti-IL-6 antibody. Treatment with CNTO-328 delayed progression of this tumor to the therapy-resistant stage (65). Although CNTO-328 is potentially useful in prostate cancer treatment, at this stage it is difficult to identify patients who will benefit from this type of treatment. More research is needed to identify other downstream targets of IL-6 which are overexpressed in prostate cancer. It will also be important to discuss the most appropriate stage and combination therapy for anti-IL-6 intervention in prostate cancer.

In the clinic, a study on the use of CNTO-328 in patients with metastatic prostate cancer is ongoing. This is a nonrandomized, open-label safety and efficacy study. The outcomes measured are the safety and tolerability of multiple dosing regimens of the antibody in combination with docetaxel, as well as the collection of pharmacokinetic and pharmacodynamic data (ClinicalTrials.gov Identifier NCT00401765). At the ASCO annual meeting in 2009, it was reported that 59% of the study participants showed a prostate-specific antigen (PSA) response, whereas tumor response was observed in a smaller number of patients.

POTENTIAL ROLE OF CNTO-328 IN THE THERAPY OF MYELOMA

Previous therapeutic strategies against IL-6 included potent super-antagonists designed on the basis of substitutions that abolish

Table I. *Experimental models used for studies with the anti-IL-6 antibody CNTO-328.*

Cell line	Origin	Effect	Reference
PC-3	Prostate	Apoptosis Tumor regression	60
PC-3M	Prostate	Inhibits tumor-induced cachexia	61
LuCaP 35	Prostate	Inhibits conversion to a therapy-resistant phenotype	63
LNCaP-IL-6$^+$	Prostate	Modest inhibition of tumor growth	62
Multiple cell lines	Myeloma	Potentiation of effect of bortezomib	70
	Myeloma	Sensitization in preclinical models to dexamethasone	74

interactions with the receptor subunit gp130 (66). These superantagonists are potent inducers of myeloma cell death (67). The superantagonist Sant-7 was successfully used to overcome bone marrow-mediated resistance to therapy (68). Interestingly, the vitamin D_3 derivative EB-1089 (seocalcitol) was used for the treatment of IL-6-dependent myeloma (69). This compound inhibits upregulation of the gp80 subunit of the IL-6 receptor. These findings may also be of interest for several other malignant tumors in which IL-6 is involved in pathogenesis. Similarly, dexamethasone may act in concert with the prodifferentiation compound retinoic acid to decrease the expression of IL-6 and its receptor in myeloma cells (62).

In the last decade, it has become obvious that proteasome inhibitors could be used in myeloma therapy. For example, the proteasome inhibitor bortezomib (PS-341) inhibits the proliferation and paracrine growth of myeloma cells by decreasing their adherence to bone marrow stromal cells and stimulation of NF-κB and downstream cytokines. Bortezomib induces downregulation of gp130, a process which is caspase-dependent (70). Treatment of myeloma cells with CNTO-328 enhances the antitumor effect of the proteasome inhibitor bortezomib (71).

Van Zaanen and colleagues have treated patients with progressive multiple myeloma with CNTO-328 in two cycles (60, 72). Different doses were applied, with total doses ranging between 140 and 560 mg. In the first study reported by this group, it was evident that disease stabilization could be achieved, although a clinical response was not evident. When three additional patients were enrolled, one clinical response was observed after the second course of treatment. The half-life of CNTO-328 was determined and found to be 17.8 days, and the antibody may be suitable for chronic administration. Allergic or toxic reactions were not seen.

CNTO-328 has also been used for the treatment of Castleman's disease. This is a rare B-cell proliferative disorder described predominantly in Asian men. The proliferation of B cells is IL-6-dependent. The appearance of multiple erythema is a typical sign of the disease. In a phase I study CNTO-328 was administrated at a dose of 12 mg/kg every 3 weeks. A response to therapy was reported in a 42-year-old female patient very early after the beginning of therapy (74).

OUTLOOK AND FUTURE PERSPECTIVES

Clinical trials with CNTO-328 are ongoing, for example in ovarian cancer and metastatic prostate cancer. In a meeting report, it was indicated that CNTO-328 reduces the levels of surrogate markers in patients with renal cell cancer. Preclinical studies clearly indicated that there is potential for the drug, mostly in prostate cancer and multiple myeloma. Several questions which became evident in preclinical studies have not yet been resolved. For prostate cancer, it will be important to establish a basis for selectivity and identification of patients who will benefit from this therapy. The fact that there are divergent responses of prostate cancer cell lines to IL-6 implies that molecular events downstream to the IL-6 receptor are still not completely understood. Most probably, the complex network between transcription factors and endogenous inhibitors of IL-6 signaling may provide some clues for a better understanding of the basis for a positive or negative growth-regulatory response in prostate cancer.

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