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Review

Can NF-κB be a target for novel and efficient anti-cancer agents?

Sabine Olivier [a,1], *Pierre Robe* [b,1], *Vincent Bours* [b,*]

[a] *Department of Rheumatology, Centre for Biomedical Integrative Genoproteomics, University of Liège, CHU B35, Sart-Tilman, 4000 Liège, Belgium*

[b] *Department of Human Genetics, Centre for Biomedical Integrative Genoproteomics, University of Liège, CHU B35, Sart-Tilman, 4000 Liège, Belgium*

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ABSTRACT

Since the discovery of the NF-κB transcription factor in 1986 and the cloning of the genes coding for NF-κB and IκB proteins, many studies demonstrated that this transcription factor can, in most cases, protect transformed cells from apoptosis and therefore participate in the onset or progression of many human cancers. Molecular studies demonstrated that ancient widely used drugs, known for their chemopreventive or therapeutic activities against human cancers, inhibit NF-κB, usually among other biological effects. It is therefore considered that the anti-cancer activities of NSAIDs (non-steroidal anti-inflammatory drugs) or glucocorticoids are probably partially related to the inhibition of NF-κB and new clinical trials are being initiated with old compounds such as sulfasalazine. In parallel, many companies have developed novel agents acting on the NF-κB pathway: some of these agents are supposed to be NF-κB specific (i.e. IKK inhibitors) while others have wide-range biological activities (i.e. proteasome inhibitors). Today, the most significant clinical data have been obtained with bortezomib, a proteasome inhibitor, for the treatment of multiple myeloma. This review discusses the preclinical and clinical data obtained with these various drugs and their putative future developments.

Contents

* *Corresponding author*. Tel.: +32 43 66 81 44; fax: +32 43 66 81 46.
E-mail address: vbours@ulg.ac.be (V. Bours).

[1] These authors contributed equally to this review.

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1. Introduction

Since the identification of the NF-κB transcription factor and the cloning of the NF-κB and IκB-coding genes, a large number of experimental evidences have been accumulated demonstrating that this factor plays a major role in the development and progression of various human cancers [1–3]. As discussed in this issue (see review by Keutgens et al., this issue), a constitutive NF-κB activity is observed in many lymphoid or myeloid tumors, including multiple myeloma, Hodgkin diseases and some non-Hodgkin lymphomas. Moreover, various solid tumors, such as for instance breast cancers, glioblastomas and many others, are also characterized by a constitutive and continuous NF-κB nuclear activity [4–7] (and review by Pacifico and Leonardi, this issue). Such a constitutive activity can be linked to genetic rearrangements leading to aberrant NF-κB gene expression, to mutations of the IκBα inhibitor or to increased IKK activity [1,3]. Finally, a genetic demonstration of the oncogenic NF-κB function was obtained by animal models establishing that the development of cancers secondarily to chronic inflammations was dependent on IKK activity and NF-κB activation [8,9].

In addition to this constitutive activity, it has been clearly demonstrated that many anti-cancer agents activate NF-κB in cancer cells (see review by Habraken and Piette, this issue).

In both situations, constitutive or treatment-induced activity, NF-κB functions mainly as an inhibitor of apoptosis. Indeed, the inhibition of NF-κB by genetic or chemical inhibitors induces the apoptosis of various tumor cells and/or restores the apoptotic response after treatment with ionizing radiations or chemotherapeutic agents, thus reversing NF-κB-linked radio- or chemoresistance in many models [7,10–13]. Consequently, the development of novel agents blocking the NF-κB activity has become a major goal for numerous laboratories and companies. Moreover, the mechanisms of action of several more ancient drugs have been re-evaluated and some of them were discovered to act at least partially through NF-κB inhibition. However, the role of NF-κB in the control of apoptosis is not unambiguous and this factor is also, in some experimental conditions, required for the induction of apoptosis either of mutated cells or in response to anti-cancer agents [14–19]. Therefore, a precise knowledge of the signalling pathways controlling NF-κB as well as of the NF-κB target genes is essential in order to define precisely the field of application of anti-NF-κB drugs for the treatment of human cancers.

2. Old drugs, new tricks

Several known therapeutic agents were recently identified as NF-κB inhibitors: some of them have been largely used for cancer treatment while novel therapeutic indications might be considered for a few drugs in the light of such an activity. This review will focus on some of these drugs and try to delineate their potentiality as anti-cancer agents.

2.1. *Anti-inflammatory agents*

Because epidemiological and genetic studies have shown that chronic inflammation predisposes to some cancers, non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, have been extensively studied, mostly as chemopreventive agents. The protective effects of these drugs in colorectal cancer are particularly marked. Indeed, NSAIDs inhibited the growth of colorectal cancer cells *in vitro* and *in vivo* [20] and regular aspirin uptake is associated with a reduced risk of colorectal cancer [21–23]. Aspirin and other NSAIDs prevent prostaglandin synthesis through the inhibition of the cyclooxygenase (COX) activity and consequently, reduced inflammation-associated cancer and exert COX-2-related anti-tumor activities.

However, Aspirin, sulindac, and selective COX-2 inhibitors also mediate their effects by COX-independent mechanisms. Several studies suggest that these drugs can exert a pro-apoptotic effect on cancer cells by targeting the NF-κB pathways. NF-κB pathways can be inhibited at multiple levels, including IKK activity, proteasome-mediated degradation of IκB, nuclear translocation of NF-κB, DNA-binding and transcriptional activity of NF-κB complex. Twelve years ago, E. Kopp and S. Ghosh demonstrated that high concentrations of aspirin and sodium salicylate inhibited the LPS-induced NF-κB-dependent transcription by preventing IκBα degradation [24]. This effect was later confirmed on TNFα-induced activation of NF-κB and attributed to the specific inhibition of IKKβ kinase activity. IKKβ inhibition is due a competitive effect of aspirin and salicylates with adenosine triphosphate (ATP) binding to IKKβ [25]. Recently, several other NSAIDs were shown to suppress NF-κB activation by inhibiting the IKK kinase activity, although with highly variable efficacy. Indeed, sulindac can prevent IκBα degradation and subsequent NF-κB nuclear translocation while indomethacin or ibuprofen failed to inhibit TNF-induced NF-κB activation in colon cancer cell lines [26]. In contrast, indomethacin and ibuprofen might be more efficient than aspirin for the inhibition of TNF-induced NF-κB activation in the human leukaemia cell line KMB-5 [27].

Ibuprofen inhibited also the constitutive activation of IKKα and NF-κB in androgen-independent prostate tumor cells [28]. These results suggest that the NSAIDs effects on NF-κB activity are cell type and drug specific.

In addition, a recent report identified a novel mechanism for aspirin-regulated NF-κB-driven transcription and apoptosis in colon cancer cells. According to this study [29], the aspirin long-term inhibitory effects on NF-κB in the absence of additional cytokines were associated with IκBα degradation and relocalization of RelA into the nucleolus. Furthermore, the nucleolar targeting of RelA was also required for aspirin induced apoptosis in SW480 cells suggesting that, by sequestering RelA away from target promoters, aspirin decreased the transcription of NF-κB-dependent anti-apoptotic genes and, consequently, induced apoptosis [29]. Moreover, long-term diclofenac treatment could induce IκBα degradation and RelA nuclear translocation in HCT116 cells. This effect might contribute to the inhibition of Wnt/β-catenine signalling by diclofenac through RelA interaction with β-catenine [30]. These data suggest that the activation of NF-κB pathways by diclofenac treatment could be part of a chemopreventive effect in colon cancer. However, there are no data on the transcriptional activity of NF-κB in response to diclofenac.

All these studies indicate that aspirin and other NSAIDs exhibit a potential chemopreventive activity against various cancers such as colon cancer, lung cancer, Hodgkin's lymphoma, oesophageal cancer or prostate cancer and that such an effect could be mediated at least partially by regulation of NF-κB activity [31–37].

2.2. *Sulfasalazine*

Sulfasalazine (SSZ) is a synthetic anti-inflammatory drug made of an aminosalicylate, 5-amino salicylic acid (5-ASA), linked to sulfapyridine (SP) and routinely used for the treatment of inflammatory bowel disease and rheumatoid arthritis.

Following oral intake, the bioavailability of SSZ only reaches 15%. The remaining drug is split by bacterial azoreductases in the colon into 5-ASA and sulfapyrydine (SP) that are also both partially absorbed. Further catabolism of SSZ takes place in the liver and also yields SP and 5-ASA. SP and 5-ASA are themselves predominantly acetylated, a process that for SP involves the enzyme *N*-acetyltransferase 2 (NAT2) [38]. The NAT2 genotype conditions the plasma concentration of sulfasalazine [39] and its metabolites SP and acetyl-SP.

Sulfasalazine was recently shown to inhibit NF-κB activation [40] via a direct inhibition of ATP binding to IKKα and β [41]. Its metabolite 5-ASA, which lacks any IKK-inhibitor property, was further shown to inhibit interleukin-1 induced p65 phosphorylation and NF-κB-dependent transcription in Caco-2 cells [42].

Sulfasalazine is also known to inhibit the uptake of cysteine [43–45], and competitively inhibit gluthatione-S-transferase [46], which may result in increased sensitivity to reactive oxygen species [43]. It is a weak agonist of NMDA-type glutamate receptors and thereby reduce ischemic neuronal death [47], and induces a NF-κB p50-independent adenosine release that can activate A2 receptors and mediate some of its anti-inflammatory effects [48].

Finally, resistance to sulfasalazine in human T-lymphocytes has been associated with the expression of the multidrug resistance protein ABCG2/BRCP [49].

Epidemiological evidence suggests that SSZ prevents the development of colon cancer in patients with chronic inflammatory bowel disease, although to a lesser extent than 5-ASA [50]. In long-term toxicologic studies run by the US National Toxicology Program, SSZ was found to decrease the incidence of leukemias and forestomach squamous cell papillomas in B6C3F1mice. These same studies however found that SSZ increased the occurrence of hepatic cancers in mice and bladder transitional epithelium neoplasms both in F344/N rats and B6C3F1 mice, which could result from the *p*-amino aryl sulfonamide nature of its derivate sulfapyridine (SP) [51]. Sulfasalazine-induced bladder cancer has also been suspected in humans [52].

Animal models and *in vitro* cell culture experiments however vastly demonstrated the anti-cancer properties of SSZ. Human breast carcinoma [45], lymphoma [53], colon carcinoma [50], and malignant glioma [6] cell growth are all inhibited by SSZ. SSZ also induces apoptosis in chronic lymphocytic leukaemia cells [54], Jurkat human T-leukemia and normal human T-lymphocytes [55], mouse Raw 264.7 leukaemic monocytes cells [56] and malignant glioma cells [6,43]. *In vivo*, SSZ was shown to reduce the growth of human experimental pancreatic tumors – albeit transiently [57] – and glioblastomas [6,43].

SSZ also experimentally modulates the effect of some cancer chemotherapies. It thus enhances the anti-tumoral effect of etoposide on human pancreatic Capan-1 cells [57]. Interestingly, SSZ preferentially increases topoisomerase inhibitor-induced apoptosis in pancreatic Capan-1 and 818-4 cells that exhibit a constitutive NF-κB activity with respect to other pancreatic carcinoma cell lines that only transiently activate NF-κB in response to these drugs [58]. Sulfasalazine also potentiates the cytotoxicity of cisplatin in human small-cell lung cancer lines [46]. Gene therapy strategies can also be modulated by SSZ treatment: TRAIL-mediated glioma cell death is indeed enhanced by SSZ, although in an apparently NF-κB-independent fashion [59], and SSZ increases the effect of HSV-TK/ganciclovir gene therapy in these tumors (Robe et al., unpublished data) just as the IκBα super-repressor [60]. A pioneer clinical study of sulfasalazine as an enhancer of melphalan chemotherapy for miscellaneous recurrent tumors also showed some possible benefit in ovarian cancer [61].

NF-κB inhibition by sulfasalazine obviously accounts for at least some of its anti-tumor effects, especially in glioblastomas where these can be mimicked by IκBα super-repressor expression [6] as well as RelA antisense oligonucleotides [62]. Although sulfasalazine analogues with enhanced NF-κB inhibitory activity are more cytotoxic than SSZ itself in some malignancies [54], the relative extent to which other pharmacological properties of SSZ, such as cysteine uptake inhibition [43], account for this enhanced anti-tumor effect is not known at present. Meanwhile, a phase 1–2 clinical trial of sulfasalazine for the treatment of recurrent malignant gliomas in humans has been initiated [63] and is likely the first of a line of clinical trials assessing the anti-cancer potential, alone or in combination, of this still intriguing 40-year-old veteran drug.

2.3. Glucocorticoids

Glucocorticoids (GCs) such as dexamethasone or prednisolone are another class of widely prescribed drugs with well-established anti-inflammatory and immunosuppressive activities associated to their inhibitory effects on AP-1 and NF-κB pathways [64]. These drugs are also commonly administered for the treatment of lymphoid leukemias, Hodgkin or non-Hodgkin lymphomas and multiple myeloma. Interestingly, these glucocorticoid-responsive cancers are characterized by a constitutive NF-κB activity, thus suggesting a direct link between the NF-κB inhibition and GC therapeutic efficacy in such tumors.

Several molecular mechanisms were reported for NF-κB inhibition by GCs. Dexamethasone could induce, through the glucorticoid receptor (GR), the transcription of the IκBα gene in lymphocytic and monocytic cells, therefore increasing IκBα protein levels and NF-κB cytoplasmic sequestration [65,66]. However, this mechanism seems to be cell type and even possibly target gene dependent. Indeed, the upregulation of IκBα expression is not involved in dexamethasone effect on NF-κB-dependent gene expression in endothelial cells [67–69] nor in the regulation of ICAM-1 expression by GCs in U937 monocytic cells [70]. In these last studies, dexamethasone did not prevent nuclear translocation of NF-κB or its DNA-binding activity. The authors suggested that the decrease of NF-κB transcriptional activity in response to dexamethasone might be due to a direct interaction between GR and p65 leading to histone deacetylation or methylation. This interaction can also cause a decreased phosphorylation of RNA polymerase II, thus reducing its transcriptional activity. This effect was demonstrated on the IL-8 and ICAM-1 promoters but not at the IκBα promoter, confirming that the mechanism of GR-repressed NF-κB activity is promoter-specific [71,72].

2.4. SERMs

Similarly to GCs, the agents that target estrogen receptors (ERs) such as estrogen and selective estrogen receptor modulators (SERMs) can also modulate the NF-κB activity and might be relevant for the treatment of some cancers [73]. Preliminary data demonstrated that high concentration of the SERM tamoxifen reduces TNF-α induced NF-κB activation in KBM5 and A293 cells. This effect was mediated through the inhibition of IKK activity and the suppression of IκBα degradation but was ER-independent [27]. More recently, we reported that the SERMs raloxifene and tamoxifen are efficient NF-κB inhibitors in multiple myeloma cells. Raloxifene could indeed block the NF-κB constitutive activity through a modulation of ER association with p65. In parallel, raloxifene and tamoxifen induced apoptosis and increased vincristin and arsenic trioxide cytotoxic response in several ER-positive multiple myeloma cell lines. Therefore the inhibition of NF-κB activation may be responsible for these SERM effects on myeloma cells and contribute to overcome drug resistance [74]. Based on tamoxifen activity in *in vitro* multiple myeloma models, clinical trials were initiated to evaluate its therapeutic activity in refractory or relapsed multiple myeloma [75,76].

2.5. PPAR ligands

The regulation of NF-κB by the peroxisome proliferator-activated receptor γ (PPAR-γ) agonist is a promising effect that might represent a new strategy in inflammatory diseases and cancer. The endogenous ligand 15 desoxy-PGJ2 and the synthetic agonists thiazolidinediones, a class of antidiabetic drugs, have demonstrated a NF-κB-dependent pro-apoptotic effect in B cell malignancies and colon cancer cells. Indeed, 15d-PGJ2 and ciglitazone have shown a pro-apoptotic effect on B lineage cells by upregulation of IκBα and IκBβ expression and inhibition of constitutive NF-κB activity [77]. In HT-29 colon cancer cells, long ciglitazone treatment led to an inhibition of NF-κB activity through an increased interaction between p65 and PPARγ and induced HT-29 cell apoptosis [78]. In parallel, 15d-PGJ2 treatment decreased IL-8 expression by preventing IL-1β-induced NF-κB activity via an IκBα-dependent mechanism in CaCo-2 colon cancer [79]. It is therefore possible that inhibition of NF-κB signalling by these endogenous or synthetic compounds is mediated by several mechanisms, either PPAR-γ-dependent or -independent. In this respect, the 15d-PGJ2 treatment induced a decrease in TNF-α-induced IKK activity through covalent modification of IKKβ in HeLa cells which do not express PPAR-γ [80]. In addition, a recent study indicates that troglitazone and pioglitazone significantly inhibit tumor growth and have an anti-angiogenic effect in a mouse model of lung cancer. These effects could be associated with the inhibition of NF-κB transcriptional activity by troglitazone as it results in a decreased angiogenic chemokines production (CXCL1, CXCL5 and CXCL8) by A549 lung cancer cells [81].

2.6. Thalidomide and immunosuppressive drugs

Thalidomide and its analogues, classified as immunomodulatory drugs (IMiDs), have also shown promising results in multiple myeloma treatment [82]. Some of these immunomodulatory activities, together with their anti-angiogenic, anti-proliferative and pro-apoptotic properties, are believed to mediate anti-tumor responses in multiple myeloma. Of course, these drugs, as those described above, do not exclusively target NF-κB; however, the inhibition of NF-κB contributes to explain their anti-angiogenic and pro-apoptotic effects in multiple myeloma models. Indeed, pretreatment of Jurkat T-cells with thalidomide blocked TNF-α or IL-1β-induced NF-κB activation through a mechanism that involves the suppression of IKK activity and, consequently, reduced the expression of pro-angiogenic and anti-apoptotic molecules such as IL-8 or c-IAP2 [83]. The expression of c-IAP2 and FLIP, two other NF-κB-regulated proteins, was also decreased in response to the immunomodulatory derivative lenalidomide (IMiD3/CC5013/Revlimid) in myeloma cells. The down-regulation of these caspase inhibitors by IMiDs may contribute to enhanced myeloma cell sensitivity to FAS and TRAIL-induced apoptosis [84]. In addition, thalidomide and its immunomodulatory analogues decreased the secretion in the bone marrow environment of cytokines such as TNF-α, IL-6 and VEGF mediating growth, survival and drug resistance of myeloma cells [85,86]. Of course, anti-cancer activities of thalidomide and the IMiDs result of the combination of non-immunomodulatory

with immunomodulatory properties. For example, thalidomide and its analogues have been shown to cause also a stimulation of T helper 1 response with an increased IL-2 and IFN-γ secretion. Elevated levels of these cytokines stimulated the activity and number of natural killer cells leading to the lysis of tumor cells [87].

In agreement with *in vitro* data, lenalidomide has shown interesting responses in clinical trials, including some complete responses even in patients with refractory or relapsing MM [88]. Thalidomide and IMiDs have shown promising activity in other haematological malignancies such as myelodysplastic syndrome or chronic lymphocytic leukemia as well in some solid tumors (renal cancer, melanoma, progressive brain tumor or prostate cancer) [89].

Several macrolides, prescribed as antibiotics or immunosuppressants, have recently been shown to exert an anticancer effect that is partially mediated through the inhibition of NF-κB pathways. Recent data showed that everolimus (RAD), a rapamycin derivative, had an anti-proliferative activity *in vitro* and *in vivo* against Hodgkin lymphoma (HL) and anaplastic large cell lymphoma (ALCL) [90]. Rapamycin and its derivatives compounds, in complex with their cellular receptor FK506-binding protein (FKBP12), act on cell growth and proliferation mainly by inhibiting the mammalian target of rapamycin (mTOR) [91]. However, this study showed that RAD inhibited NF-κB DNA-binding activity in L540cy HL and Karpas 299 ALCL cells through stabilization of IκBα and that overexpression of p65 rescued HL cells from RAD-induced cell cycle arrest [90].

Moreover, rapamycin might be a potent agent to overcome drug resistance through the inhibition of NF-κB. In melanoma cells, rapamycin inhibited IKK activity and decreased NF-κB nuclear translocation induced by doxorubicin treatment. Rapamycin effect on IKK activity was independent to PI3K/Akt/mTOR pathways but might be mediated by the inhibition of FKBP51 isomerase activity, leading to a down-regulation of Bcl-2 and c-IAP1 anti-apoptotic protein expression and thus enhancing the doxorubicin cytotoxic effect [92].

Macrolides antibiotics as clarithromycin or roxithromycin could also inhibit NF-κB pathways in some models. Roxithromycin has been shown to decrease constitutive activation of NF-κB in a rat model of hepatocellular carcinoma and reduce liver tumor formation [93]. Clarithromycin treatment reduced TNF-α-induced NF-κB transcriptional activity in Jurkat or U937 cells [94]. This effect could be involved in this drug anti-inflammatory properties and contribute to the treatment of inflammation-associated cancer.

2.7. Other drugs

The action of digitoxin and structurally related cardiac glycosides drugs such as oleandrin or UNBS1450 on the NF-κB pathways was also shown to have potential implications in inflammation and tumorigenesis. Oleandrin treatment is able to block TNF-α-induced NF-κB DNA-binding activity in a variety of cells such as U937, ovarian CaCOV3, HeLa and Jurkat cells. Moreover, oleandrin inhibited TNF-α-induced activation of JNK and AP-1 [95]. A recent report demonstrated that digitoxin and oleandrin inhibit TNF-α signalling pathways leading to NF-κB and AP-1 activation by blocking recruitment of the TNF receptor associated death domain (TRADD) protein to TNF receptor 1 [96]. Oleandrin can also suppress the activation of NF-κB by the C2-ceramide and enhance the ceramide cytotoxic effect in HeLa cells [97]. Finally, the cardenolide UNBS1450 showed anti-growth effects *in vitro* and *in vivo* in A549 non-small-cell lung cancer cells [98]. The *in vitro* study demonstrated that this compound was able to reduce the phosphorylation of IκBα and subsequently decrease the basal NF-κB DNA-binding activity, suggesting that this inhibitory effect was involved in the drug anti-tumor effect.

Finally, several other agents, including many plant-extracted chemicals or dietary agents, can inhibit NF-κB and thus possibly exert anti-tumors activities [99,100]. Such compounds include parthenolide, flavonoids, epoxyquinomycin C, antioxidants, curcumin and many others.

3. Novel anti-NF-κB agents

3.1. Proteasome inhibitors and bortezomib

The proteasome is a 26S multiprotein complex that is located in the cytoplasm, nucleus and SER. It consists of a 19S regulatory ubunit and a 20S catalytic subunit that contains six unique ATP-dependent serine protease sites, two of each with chymotrypsin, trypsin, and caspase-like activities [101,102]. Ubiquitinated proteins are recognized by the 19S unit, which results in the liberation of ubiquitin chains that are recycled and the formation of a denatured protein that is transferred to the outer ring or the 20S core unit. This outer ring only allows such denatured proteins to enter the catabolic site of the proteasome where they are hydrolyzed into small polypeptides [101,103].

26S proteasome-mediated protein degradation is a central metabolic and regulatory process in cell physiology. Besides its role in scavenging damaged proteins, the 26S complex is an important regulator of cell life and fate. For instance, specific ubiquination of key proteins like cyclins A, B, D and E, eEF2-kinase, c-Myc, Notch, c-Jun, $p21^{WAF/CIP1}$, p27, p53, topoisomerases I and IIα, apoptosis modulators XIAP, Bik/NBK, Bad and Bid, transcription-coactivator β-catenin and the NF-κB regulator IκBα targets these proteins towards proteasome degradation [104–115]. Through its regulation of protein turnover, the 26S proteasome is thus involved in cell-cycle progression, apoptosis, and other processes like angiogenesis [116,117] and cell motility [118,119] that are important in cancer progression. While proteasome defects may favour tumor progression in some cell lines [120,121], other tumors present an increased proteasome function that participates in their unrestrained cell-cycle progression and apoptosis resistance [122]. Increased proteasome function can for instance result from the enhanced transcription of its subunits [123,124], or from increased ubiquitin-conjugating activities [125]. Interestingly, the effect of proteasome inhibition on apoptosis appear, at least in some cell types such as lymphocytes and monocytes, to affect tumor cells more than their normal counterparts [101,126].

The active sites of the human proteasome can be reversibly inhibited by the peptidyl aldehydes chymostatin and leupep-

tin [127]. Other aldehyde peptides have been developed but they inhibit both serine and cysteine proteases and somewhat lack specificity [128]. Lactacystin is a streptomyces metabolite that can be chemically synthetized and irreversibly inhibits the proteasome by alkylating several subunits of its 20S core [129,130]. Very potent specific and reversible proteasome inhibitors were obtained with the coupling of boronic acid to dipeptides [131,132]. PS-341, or bortezomib, is one such dipeptide boronate and is the only proteasome inhibitor to date that have entered clinical development. New allosteric peptidic inhibitors of the proteasome are also being developed that can preferentially inhibit the degradation of specific proteasome targets, such as IκBα or HIFα for instance [133,134]. Finally, a new orally bioactive proteasome inhibitor, NPI-0052, was recently described that can induce apoptosis in bortezomib-resistant cells [135].

The proteasome inhibitor bortezomib is an IV drug that disappears from the plasma compartment almost completely within 15 min of injection [101] but has a long elimination half life (>40 h) [136]. Clinical dose monitoring is thus inferred from the measure of residual proteasome activity in blood cells [101,137]: doses of 1.3 mg/m^2 body surface inhibit 65% of proteasome activity, with a peak inhibition at 1 h and a return to baseline activity within 48–72 h. Primate experiments have also shown that bortezomib is distributed evenly in most organs with the notable exception of the CNS in areas protected by the blood–brain barrier (D. Nix et al., cited in [136]).

PS-341, or bortezomib, experimentally inhibits the proliferation and/or induces apoptosis in cells from a variety of solid tumors such as breast [138], gastric [139], colon, ovarian [115], lung [140], head and neck [141], prostate [117], and pancratic [142,143] cancers as well as in malignant gliomas [144] and neuroblastomas [145]. Some of its most striking *in vitro* effects were observed on hematologic malignancies such as myeloma, Hodgkin [146] and non-Hodgkin lymphomas and other leukemias. The anti-proliferative and pro-apoptotic actions of PS-341 largely correlate with its ability to block the degradation of the NF-κB inhibitor IκBα [138,141,147–149]. Specific NF-κB inhibitors such as the IKK inhibitor PS-1145, however, fail to completely reproduce all bortezomib activities [150], thus demonstrating the importance of other pathways that are also regulated by proteasome inhibitors [151]. Moreover, another proteasome inhibitor, MG-132 can induce apoptosis in Hodgkin cells without affecting the activation of NF-κB [152]. Indeed, a recent phase 2 trial in colon carcinoma did not find any alteration of phospho-p65 or IκBα expression in tumor biopsies taken from patients receiving the treatment, but evidenced a modulation of the HIF-1α pathway [153]. Moreover, bortezomib and other proteasome inhibitors can even induce apoptosis and at the same time activate NF-κB in endometrial carcinomas cells [154]. Interestingly, most of the bortezomib side effects that are observed in experimentally treated animals also appear to be NF-κB-independent [101,155]. Taken together, results from preclinical studies show that bortezomib can induce apoptosis in a number of otherwise resistant tumor cells, and sensitize such cancer cells to other chemotherapies and radiation therapy [156–160]. Resistance to bortezomib does not appear to involve the MDR proteins [161] (Steiner et al., cited in [101]), but can be found in Hsp27-overexpressing cells [162] and may depend in specific cell types on the p53 status [142].

To date, at least 155 clinical trials using bortezomib alone or in combination have been registered in the US (source: clinical trials.gov database, accessed 27 April 2006). Almost all resistant cancers are currently being assessed for their sensitivity to bortezomib, including solid tumors such as melanoma, malignant glioma, kidney, colon, breast, lung, pancreas and head or neck cancers, as well as hematological malignancies. Phase 1–3 trials have notably proven the efficacy of bortezomib for the treatment of multiple myeloma, prompting an accelerated FDA approval for its use in myelomas that progress after at least two prior chemotherapies (source: FDA web site). In a recently published prospective, international, multicenter, randomized phase 3 trial comparing bortezomib and dexamethasone (APEX) treatment for resistant multiple myeloma (MM), the proteasome inhibitor yielded a rate of 6% complete and 32% partial responses versus 1% and 17%, respectively, for dexamethasone. The median time to progression was significantly increased from 106 days with dexamethasone to 189 days with bortezomib and the 1-year overall survival was also higher in the bortezomib group (80% versus 66%) [163]. Although some critics may apply to the APEX trial design [164], this report and all other clinical trials of bortezomib clearly demonstrate its efficacy in inducing complete and partial responses as well as prolonging survival in otherwise chemoresistant MM [165,166]. Bortezomib is also being evaluated as a co-treatment with other chemotherapies such as melphalan [167], thalidomide [168], or doxorubicin [169] in progressive or *de novo* MM. Bortezomib has also proven some efficacy in non-small-cell lung cancer [170,171] and mantel cell lymphoma [172], although pancreatic malignancies, renal cancers and metastatic melanoma did not appear to respond to this drug [173–175].

3.2. *IKK inhibitors*

Since the activation and nuclear translocation of the most commonly expressed NF-κB dimers require an IKKβ-dependent phosphorylation of their cytoplasmic IκB partner, IKK constitutes a key target for the development of small NF-κB inhibitors. IKK consists of two catalytic (IKKα and IKKβ) and a regulatory (IKKγ or NEMO) subunits. The activity of IKKα and β depends on their phosphorylation at serine residues located in their respective kinase domains that also contains a critical cysteine residue. As mentioned in the first part of this review, a variety of 'old' drugs, such as aspirin, salicylates, sulindac, sulfasalazine and thalidomide, inhibit the IKKs catalytic activity. Thiol-reactive drugs such as arsenic trioxide (As_2O_3) and the gold compound auranofin alter IKKβ Cys-179 and also inhibit IKK-dependent NF-κB activation [176,177]. As_2O_3 has recently been used in clinical trials against promyelocytic leukaemia and solid tumors [178–180], with the nuance that several signalling pathways may be involved in its anti-tumor effect [181]. Auranofin is also known to induce apoptosis in promyelocytic leukaemia cells [182].

Another class of IKK inhibitors alter the assembly of the IKK complex and impede the downstream transduction of NF-κB-

activating signals. Mamumycin A, an epoxyquinoid farnesyltransferase inhibitor, induces a covalent dimerization of IKKβ, inhibits this kinase and prevents the association of NEMO with the IKKα/β complex [183]. Jesterone is another epoxyquinoid IKK inhibitor [184]. Interestingly, epoxyquinoids have been shown to promote apoptotic cell death in a variety of tumor cell lines [185,186].

In addition, a cell permeable peptide corresponding to the NEMO binding domain of IKKβ is able to block the association of NEMO to IKKβ and TNF-α-induced NF-κB activation. Although this peptide slightly increases the baseline activity of IKKβ itself, it inhibits the NF-κB activation in response to inflammatory cytokines [187]. More recently, the NBD peptide has been shown to be effective *in vitro* in a model of breast cancer. Indeed, in heregulin-stimulated ErbB2-positive breast cancer cells, the inhibition of NF-κB activation with the NBD peptide blocked cell-cycle progression and induced apoptosis of proliferating cells [188]. *In vivo*, the beneficial effect of this IKK inhibitor has been shown in various models of inflammation [189]. For instance, in a rheumatoid arthritis animal model, the local inhibition of IKK following intra-articular injections of the NBD peptide decreased the inflammatory cell infiltration and the expression of the pro-inflammatory cytokines TNF-α and IL-1β in synovial tissues [190].

Novel, selective small-molecule inhibitors of the IKK complex have finally been developed by the pharmaceutical industry. The majority of these products inhibit the fixation of ATP to the enzyme complex. All of them target the IKKβ kinase preferentially although some also weakly inhibit IKKα, which is important to control the alternative pathway of NF-κB activation. Among the dozen of IKK inhibitors that have been described by pharmaceutical companies so far (for a review, see [99]), few have undergone published preclinical studies. PS-1145 (Milllenium Pharmaceuticals) is a β-carboline derivative [191] that has shown some anti-proliferative potential in multiple myeloma cells [150]. BMS-345541 is a potent and specific allosteric IKKβ inhibitor [192] that is able to induce apoptosis in malignant melanoma cells [193]. The cyanoguanidine CHS-828 (Leo Pharma) may have IKK-inhibitor effects [99] and is being evaluated as an anti-cancer drug in experimental tumors [194] as well as in humans [195–197]. ACHP, or 2-amino-6-[2-(cyclopropylmethoxy)-6-hydroxyphenyl]-4-piperidin-4-yl nicotinonitrile was described in 2004 as an orally active anti-inflamatory agent [198] and was subsequently shown to inhibit the growth of multiple myeloma cells [199] and adult T-cell leukaemia [200]. Finally, the AS602868 compound (Serono International) is active against acute myeloid leukaemia cells, including primary cultures, and increases their response to conventional chemotherapeutic agents [10,201]. This last drug will soon enter phase I clinical trials (M. Dreano, personal communication).

3.3. *Nucleic acid-based treatments*

Initially, small interfering RNAs (siRNA) or decoy oligonucleotides blocking NF-κB protein synthesis or DNA-binding have been developed as experimental tools to study the role of this factor in various models. More recently, several reports suggested that blocking NF-κB-induced transcription through these molecular approaches could have an anti-tumor effect *in vivo*. Indeed, intratumoral injection of decoy oligonucleotides containing NF-κB binding sequences inhibited tumor-associated symptoms such as cachexia in murine adenocarcinoma colon26 model [202], probably as a consequence of a decreased local cytokine production. In a second study, intravenous injection of the same NF-κB decoy inhibited hepatic metastasis in a M5076 murine reticulosarcoma model and reduced M5076-induced expression of IL-1β, TNFα and ICAM-1 in liver [203]. More recently, the inhibition of p65 expression with a p65 siRNA enhanced *in vitro* and *in vivo* colon cancer cell response to irinotecan [204]. These data suggest that these technologies could offer other potential therapeutic strategies to target NF-κB in cancer treatment.

4. Difficulties and caveat

As for most anti-cancer drugs, anti-NF-κB agents are not fully specific and therefore there are concerns about the risk of side effects due to their action on normal cells. Indeed, normal B lymphocytes also show a constitutive NF-κB activity and this activity is required for their survival, while the role of NF-κB at various stages of B lymphopoiesis and B and T immune response has been largely demonstrated [205,206]. Therefore, chronic administration of such drugs could exert a profound immunosuppressive effect. Moreover, in response to a variety of stress, including DNA-damage, the NF-κB activation protects cells from apoptotic cell death. Therefore, one could fear that, when administered together with chemotherapy or radiotherapy, NF-κB inhibitors could significantly increase side effects and that physicians would rapidly reach dose-limiting toxicities. Indeed, a phase I clinical trial combining the proteasome inhibitor bortezomib and radiotherapy in patients with recurrent head and neck carcinoma led to dose-limiting grade 3 hypotension and hyponatriemia [149]. Other side effects associated with bortezomib treatment include, when the drug is administered alone to heavily pretreated patients, nausea, diarrhea, thrombocytopenia, anemia, neutropenia and peripheral neuropathy but such toxicities are manageable [163,165].

However, a major concern about the use of anti-NF-κB agents for the treatment of cancer comes from the heterogeneous NF-κB function in cancer progression and in the apoptotic response to DNA-damaging agents. Indeed, Ras-induced transformation requires NF-κB inhibition in human epidermal cells [16]. Moreover, although it is clearly established that NF-κB contributes to radio- and chemoresistance in many cancer models, a few reports showed that, at least in some experimental conditions, NF-κB was required for chemotherapy-induced apoptosis. Indeed, in response to UV light or anthracyclins, a repressive form of RelA/p65, associated with histone deacetylases, is expressed and can actively repress anti-apoptotic gene expression [15]. Several groups thus surprisingly reported that NF-κB was required for daunomycin/doxorubincin cytotoxic effect [14,15,17] while others demonstrated that NF-κB inhibition significantly enhanced the apoptotic cell death induced by the very same compounds [12,13], thus suggesting an influence of the cancer cell origin and/or genotype. It has also been suggested that NF-κB could be required for p53-induced

apoptosis [18] or could be a factor destabilizing and inhibiting p53 [207,208]. Obviously, these contradictory preclinical data need to be considered with great attention and will have to be kept in mind for the design and the interpretation of clinical trials. Hopefully, the growing knowledge on the NF-κB signalling networks should deliver new data that could allow scientists and physicians to understand the molecular mechanisms triggering NF-κB pro- or anti-apoptotic activities.

Another concern relates to the efficacy of NF-κB inhibition in some models. It is indeed possible that a complete NF-κB inhibition would be required for an efficient therapeutic effect, at least in some tumor types. We reported that the inhibition of NF-κB with an IκBα super-repressor in several cancer cell lines could not be sufficient to increase the response to cytotoxic agents and such a lack of effect is probably due to an incomplete biological inhibition of the transcription factor activity [209]. The nature of the NF-κB inhibitor will also influence the response: for instance IKKβ inhibitors will probably not be efficient for the treatment of tumors characterized by an activation of the alternative pathway. Therefore, the choice of an appropriate NF-κB inhibitor might have to be carefully considered according to the type of cancer and possibly to genetic and epigenetic events, as inhibiting the proteasome, IKKβ or p65 does not necessarily act on the same NF-κB target genes and could therefore demonstrate distinct clinical activities.

5. Conclusion

A very large number of experimental data accumulated during the last two decades clearly indicate that NF-κB plays an important role in the development or progression of several human cancers. Researchers established that well-known and widely used agents, such as glucocorticoids, exert their anti-tumoral activities at least partially through NF-κB inhibition (Table 1). Meanwhile, clinical trials are being performed with several novel drugs that block the NF-κB activity (Table 1) but the most significant clinical data have so far been obtained with bortezomib, a proteasome inhibitor that exerts NF-κB-dependent and -independent biological effects.

Despite of these first successes, the place for NF-κB inhibition in cancer treatment remains to be determined. With a very few exceptions, it is highly unlikely that such a

Table 1 – Partial list of the NF-κB inhibitors with their identified sites of action and their reported clinical activities

Drug	Molecular targets (NF-κB-linked)	Clinical activity
NSAIDs		
Aspirin	IKKβ inhibition P65 localization COX-2 inhibition	Chemoprevention: colon, lung, HK, prostate; oesophagus
Sulfasalazin	IKKβ inhibition Cystéine uptake	Trial in progress
5ASA	P65 phosphorylation	NA
Glucocorticoids	IκBα transcription GR interaction with p65	Lymphoid leukemias, NHL, HK, MM
SERMs	IKK inhibition ER interaction with p65	Trials in progress
PPAR-γ ligands	Increased IκB expression PPARγ interaction with p65 IKKβ inhibition	NA
Immunosuppressants		
Thalidomide/lenalidomide	IKKβ inhibition	MM, MDS, CLL, various solid tumors
Macrolides	IκBα stabilization IKKβ inhibition	NA
Proteasome inhibitors		
Bortezomib	Proteasome inhibition	MM, NSCLC, MCL.
NPI-0052	Proteasome inhibition	NA
IKK inhibitors		
Arsenic trioxide	IKKβ inhibition	PML, solid tumors
Manumycin A	IKKβ inhibition	NA
PS-1145	IKKβ inhibition	NA
BMS-345541	IKKβ inhibition	NA
CHS-828	IKKβ inhibition	NA
ACHP	IKKβ inhibition	NA
AS602868	IKKβ inhibition	NA
Blocking peptides	IKK complex assembly	NA
Gene therapy		
siRNA	Decreased p65 expression	NA
Decoys	Inhibition of DNA-binding	NA

NA: not available; HK: Hodgkin's disease; NHL: non-Hodgkin's lymphoma; MM: multiple myeloma; MDS: myelopdysplastic syndrome; CLL: chronic lymphocytic leukaemia; NSCLC: non-small-cell lung cancer; MCL: mantle cell lymphoma; PML: promyelocytic leukaemia.

strategy could be used alone and these novel, or more ancient, inhibitors will thus have to be combined with agents from distinct classes. Given the broad role of NF-κB in the response to various cellular stresses, it is not clear today whether such combinations will be clinically tolerable, especially in heavily pretreated patients.

In the future, it would be essential to pursue the precise identification of the mechanisms controlling NF-κB target gene expression. Possibly, groups of target genes are controlled by the same activating pathways and/or by similar DNA-binding complexes including NF-κB subunits and cofactors. Possibly, it will thus be possible to act downstream of the general NF-κB switch, such as the IKK complexes, to target specific groups of regulated genes, hoping that the gain is specificity will not be associated with a loss in efficacy.

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