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## Molecular Pathways: Revisiting Glycogen Synthase Kinase-3 $\beta$ as a target for the treatment of cancer

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### Abstract

Glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ), a serine/threonine protein kinase, is a complex regulator of numerous cellular functions. GSK-3 $\beta$  is a unique kinase which is constitutively active in resting and non-stimulated cells. GSK-3 $\beta$  has been implicated in a wide range of diseases including neurodegeneration, inflammation and fibrosis, noninsulin-dependent diabetes mellitus, and cancer. It is a regulator of NF- $\kappa$ B-mediated survival of cancer cells, which provided a rationale for the development of GSK-3 inhibitors targeting malignant tumors. Recent studies, many of them reported over the past decade, have identified GSK-3 $\beta$  as a potential therapeutic target in at over 15 different types of cancer. Whereas only active GSK-3 $\beta$  is expressed in cancer cell nucleus, aberrant nuclear accumulation of GSK-3 $\beta$  has been identified as a hallmark of cancer cells in malignant tumors of different origin. This review focuses on the preclinical and clinical development of GSK-3 inhibitors, and the potential therapeutic impact of targeting GSK-3 $\beta$  in human cancer.

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## Keywords

Glycogen Synthase Kinase-3; GSK-3; chemoresistance; metastasis; drug development; combination treatment

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## Background

Glycogen synthase kinase-3 (GSK-3), a serine/threonine protein kinase, was initially described as a key enzyme involved in glycogen metabolism (1). However, it has now been shown to regulate a variety of different cellular functions, including the regulation of many metabolic and signaling pathways as well as the modification of structural proteins (2). There are two highly homologous forms of GSK-3 in mammals, GSK-3 $\alpha$  and GSK-3 $\beta$ , that have different tissue-specific functions and substrates (1, 2). Despite their homology, one isoform cannot compensate for the loss of the other (2). Historically, GSK-3 $\beta$  has been thought of as a potential tumor suppressor due to its ability to phosphorylate pro-oncogenic molecules e.g. c-Jun (3), c-Myc (4), cyclin D1 (5) and  $\beta$ -catenin (6), targeting them for proteosomal degradation. However, a large and ever increasing body of published data has emerged over the past decade demonstrating that GSK-3 $\beta$  is a positive regulator of cancer cell proliferation and survival in advanced cancer (7–35). This suggests that GSK-3 $\beta$  mediates different signaling pathways in early compared to advanced cancer and has led to the credentialing of GSK-3 $\beta$  as a therapeutic target for advanced disease. This has stimulated significant interest in discovering and developing inhibitors of this enzyme (7–35). One of the pioneering studies identified GSK-3 $\beta$  as a potential therapeutic target in human pancreatic cancer (8). Using a number of toolkit inhibitors of GSK-3 $\beta$ , translational studies credentialed GSK-3 $\beta$  as a therapeutic target in multiple tumor types including pancreatic cancer (8, 9), chronic lymphocytic leukemia (10), colon (11), renal (12) and bladder (13) cancer. More recent studies have also shown that the inhibition of GSK-3 $\beta$  suppressed cancer cell viability in models of glioblastoma (14–16), leukemia (17–20), neuroblastoma (21, 22), osteosarcoma (23), melanoma (24), ovarian (25, 26), thyroid (27), prostate (28–30), breast (31–33) and lung cancer (34, 35). Thus, GSK-3 $\beta$  has emerged as a viable therapeutic target for the treatment of a broad spectrum of different cancer types.

NF- $\kappa$ B activation is known to promote human cancer progression, metastasis and chemoresistance (36, 37). Disruption of the GSK-3 $\beta$  gene in mice leads to embryonic lethality due to hepatocyte apoptosis and massive liver degeneration, a phenotype that is similar to the disruption of the NF- $\kappa$ B p65 or IKK $\beta$  genes (38). These findings suggested a link between GSK-3 $\beta$  and the activation of the NF- $\kappa$ B pathway (Fig. 1A). The induction of apoptosis observed when the GSK-3 $\beta$  gene was disrupted raised the possibility that GSK-3 $\beta$  may represent a therapeutic target for the treatment of cancer. Using GSK-3 $\beta$ -deficient mouse embryonic fibroblasts, it was shown that the early steps leading to NF- $\kappa$ B activation following TNF- $\alpha$  treatment (degradation of I $\kappa$ B $\alpha$  and translocation of NF- $\kappa$ B to the nucleus) were unaffected by the loss of GSK-3 $\beta$ , suggesting that NF- $\kappa$ B is regulated by GSK-3 $\beta$  at the level of the transcriptional complex in the nucleus (38). GSK-3 $\beta$  nuclear accumulation has been previously described occurring selectively in pancreatic, bladder and renal cancer cells, as well as malignant B cells, but not in benign cells or tissues (9, 10, 12,

13). Only the active form of GSK-3 $\beta$  accumulates in the nucleus of pancreatic cancer cells as the inhibition of GSK-3 $\beta$  enzymatic activity depletes its nuclear accumulation via proteosomal degradation (9). In contrast, only weak expression of cytoplasmic GSK-3 $\beta$  was observed in benign pancreatic ducts and pancreatic intraepithelial neoplasia (PanIN) lesions (9). Aberrant nuclear accumulation of GSK-3 $\beta$  was not observed in any of the PanIN lesions but was found in well, moderately, and poorly differentiated pancreatic adenocarcinomas in 2 of 22 (9%), 23 of 59 (39%), and 37 of 41 (90%) cases, respectively (9). These results demonstrate that aberrant nuclear accumulation of GSK-3 $\beta$  is significantly correlated with poorly differentiated pancreatic adenocarcinoma (9). Further studies also demonstrated that nuclear GSK-3 $\beta$  plays an important role in regulating histone modifications, which may contribute to NF- $\kappa$ B p65/p50 binding to its promoters and activation of target genes in cancer cells, leading to increased cancer cell survival (10). These studies led to a hypothesis that targeting nuclear GSK-3 $\beta$  in cancer cells would lead to the subsequent inhibition of NF- $\kappa$ B mediated transcription and decreased cell survival, providing a rationale for discovering and translating novel GSK-3 $\beta$  inhibitors to the clinic for the treatment of cancer.

## Clinical-Translational Advances

### Rationale for therapeutic targeting of GSK-3 $\beta$ in cancer

GSK-3 $\beta$  represents a signaling node at the intersection of multiple pathways implicated in cancer progression (7). Based on data describing the role of NF- $\kappa$ B in mediating radiation and chemoresistance in cancer (36, 37) and the regulation of NF- $\kappa$ B-mediated gene expression by GSK-3 $\beta$  (7, 38), combining radiation or chemotherapy with GSK-3 inhibitors to re-sensitize resistant tumors to these treatments could be a rational approach to developing new GSK-3 inhibitors for the treatment of cancer (Fig. 1A, B). Indeed, several GSK-3 inhibitors in early development are focusing on this approach. Recent data has emerged that combining GSK-3 inhibitors will enhance the antitumor activity of targeted tyrosine kinase inhibitors (39) and immune checkpoint modulators (40). Thus, there will be sufficient opportunity to explore many novel drug combinations with inhibitors of GSK-3 as these agents enter into clinical development. In this section, we briefly summarize progress to date on the development of GSK-3 inhibitors for clinical use.

### Alzheimer's disease (AD): insights into safety and tolerability of targeting GSK-3

GSK-3 plays a key role in AD other neurodegenerative processes, including inflammation (41), apoptosis (42), impaired axonal transport (43), reduced synaptic plasticity (44), and regulation of long-term potentiation (45). Inhibition of GSK-3 reduces both tau phosphorylation (46, 47) and amyloid production (48, 49) *in vitro* and *in vivo*. Several recent early clinical studies have evaluated the safety and efficacy of the irreversible GSK-3 inhibitor tideglusib in the treatment of patients with AD. Tideglusib is a thiazolidinone (50) that reduces tau phosphorylation in murine primary neurons (51). In a pilot, double-blind, placebo-controlled, randomized, escalating dose trial, thirty mild to moderate AD patients were enrolled to receive either tideglusib (400–1000 mg/day) or placebo administered orally at escalating doses for a total of 20 weeks. Overall, tideglusib was well-tolerated with 65% of the patients treated with tideglusib experiencing adverse events attributable to study drug (versus 30% in the placebo group) that were not dose-dependent

(52). Adverse events occurred equally in the treatment and placebo arms (52). A subsequent ARGO double-blind, randomized, placebo controlled phase II trial in patients with AD enrolled 306 patients aged 50 to 85 years old with mild to moderate disease (53). Patients were randomized to active (1000 mg QD: n = 86, 1000 mg QOD: n = 90, and 500 mg QD: n = 50) or placebo (n=85) treatment arms. There were no deaths during the trial and the most common adverse events were: diarrhea in 14–18% of tideglusib patients versus 11% in the placebo arm and a dose-dependent, mild to moderate, reversible increase in transaminases in 9–16% of tideglusib patients versus 3.5% in the placebo arms (53). The ARGO sponsored trial concluded that tideglusib could be given safely over a 26 week treatment period, providing substantial clinical safety data for targeting GSK-3.

## Development of GSK-3 inhibitors for the treatment of cancer

### Tideglusib

Recently published study demonstrated that tideglusib induces apoptosis in human neuroblastoma cells (54). Moreover, another recent study has demonstrated that disruption of the GSK-3 $\beta$ -USP22-KDM1A axis by tideglusib therapy suppresses glioma tumorigenesis and sensitizes intracranial GBM xenografts to TMZ leading to an improved mouse survival suggesting an inhibition of GSK-3 as a potential therapeutic approach for the treatment of human GBM (55).

**LY2090314**—LY2090314 is a potent and selective inhibitor of GSK-3 (56). Initial safety studies of LY2090314 (LY) were conducted in a first-in-human, phase I, dose-escalation study evaluating IV administration of LY in patients with advanced solid tumors (56). This study enrolled 41 patients with enrichment for patients with non-small cell lung cancer (24%) and mesothelioma (22%) (56). Forty-one patients received single-dose LY monotherapy lead-in and thirty seven patients received LY in combination with pemetrexed and carboplatin (56). The MTD of LY in combination with pemetrexed and carboplatin was 40 mg (56). Overall, LY monotherapy and combination therapy with pemetrexed and carboplatin were well-tolerated at the MTD with treatment related adverse events being similar across the dose cohorts (56). During the LY lead-in portion of the study, treatment-related adverse events were observed in 26 patients (63%), whereas 12% of patients had at least one drug-related grade 3–4 adverse with 7% of patients stopping treatment secondary to the side effects (56). In the LY combination with pemetrexed and carboplatin patients, 97% experienced treatment-related adverse events. 60% of patients had at least one drug-related grade 3–4 adverse event (mostly hematologic in nature), with 20% of patients stopping treatment secondary to side effects (56). For the entire study, there were 11 dose-limiting toxicities (DLTs) in 10 patients; in the LY monotherapy patients, the DLTs occurred at doses of 40 mg in 5 patients (56). Overall, 85% of patients who received combination treatment were evaluable for efficacy with 5 partial responses (3 patients with non-small cell lung cancer, one with mesothelioma, and one with breast cancer), 19 stable disease, and 11 progressive disease (56). Clinical benefit was durable in a subset of patients but the contribution of LY to the clinical benefit will require further evaluation.

In a recent open-label, phase II study, Rizzieri et al. evaluated the safety of LY in patients with acute myelogenous leukemia (AML) (57). Inhibition of GSK-3 in pre-clinical murine models of mixed lineage leukemia provided evidence for the antitumor activity of GSK-3 inhibitors in hematologic malignancies and a rationale for this phase II study (58). Twenty patients were enrolled in the phase II AML study with confirmed diagnosis of de novo (65%) or secondary AML (35%) that was refractory or relapsed or untreated AML for which the standard induction therapy would not be appropriate (57). LY was administered intravenously at a dose of 40 mg following pre-medication with ranitidine (57). The primary objective of this study was to determine the safety of LY in patients with AML and there were no DLTs reported in this study (57). The majority of the adverse events were of grade 1 severity, with only seven grade 3 and one grade 4 event (57). Twenty six percent of patients showed a reduced blast count, 32% had stable blast counts, and 42% had progressive disease (57). This study concluded that LY has an acceptable safety profile, but showed limited clinical benefits when administered as a monotherapy to patients with AML.

**9-ING-41**—The overexpression of GSK-3 $\beta$  correlates with poor prognosis in breast cancer patients (59). Patients with the highest GSK-3 $\beta$  expression had a 2.7 and 1.7-fold increased risk of distant relapse 5 and 10 years post-resection, respectively (59). We found aberrant nuclear accumulation of GSK-3 $\beta$  in five human breast cancer cell lines and in 89 of 128 (70%) human breast carcinomas, whereas no detectable expression of GSK-3 $\beta$  was found in benign breast tissue (AU and APM, unpublished results). These results suggest that detection of aberrant nuclear accumulation of GSK-3 $\beta$  in needle biopsy specimens may be a useful method for the pathological diagnosis of breast cancer and for patient identification for GSK-3-targeted therapy. To follow up on these observations in clinical breast cancer cases, a recent study showed that pharmacological inhibition of GSK-3 by two novel small molecule ATP-competitive GSK-3 inhibitors, 9-ING-41 and 9-ING-87 (60), suppressed the viability of breast cancer cells *in vitro* (61), consistent with the results of Shin et al. who demonstrated that the knockdown of GSK-3 $\beta$  expression significantly inhibited breast cancer cell proliferation (62). Compounds 9-ING-41 and 9-ING-87 are selective for GSK-3 over ~320 other related kinases by at least one order of magnitude, including closely related serine/threonine kinases such as CDKs, PDKs, PKA, Akt, and PKCs (60). *In vitro* results demonstrated that 9-ING-41 is a more potent inhibitor of breast cancer cell growth than other clinically tractable as well as toolkit GSK-3 inhibitors including LY2090314 (61). The treatment with 9-ING-41 enhanced the antitumor effect of CPT-11 (irinotecan) in breast cancer cells *in vitro* (61). Using breast patient-derived xenograft (PDX) tumor models established from metastatic pleural effusions obtained from patients with progressive, chemorefractory breast cancer, it has been demonstrated that 9-ING-41 potentiated the antitumor effect of CPT-11, leading to regression of established breast PDX tumors *in vivo* (61). These results support the hypothesis that targeting GSK-3 can overcome chemoresistance in human breast cancer, and credentialed 9-ING-41 as a novel GSK-3 targeted agent for the treatment of metastatic breast cancer. Consistent with the results in breast carcinoma models, 9-ING-41 antitumor activity has been demonstrated in ovarian, pancreatic and renal cancer models *in vitro* and *in vivo* and initial DMPK and toxicology studies support advancing this molecule into clinical translation (26, 60, 63).

It has been previously demonstrated that GSK-3 $\beta$  is a positive regulator of NF- $\kappa$ B-mediated survival in cancer cells, and that inhibition of GSK-3 decreases cancer cell survival via suppression of NF- $\kappa$ B-mediated Bcl-2 and XIAP expression, in leukemia, pancreatic and renal cancer cells (9, 10, 12). Constitutive activation of NF- $\kappa$ B has been reported in human GBM tumors and promotes GBM invasion and resistance to alkylating agents (64–66). It leads to a hypothesis that targeting NF- $\kappa$ B mediated expression by inhibiting GSK-3 $\beta$  represents a therapeutic strategy to overcome GBM chemoresistance and recent studies have independently credentialled GSK-3 $\beta$  as a therapeutic target for the treatment of human GBM (14–16). Using IVIS imaging of live mice, it has been shown that NF- $\kappa$ B is constitutively active in orthotopic GBM PDX tumors expressing an NF- $\kappa$ B luciferase reporter, and that a single intravenous injection of 9-ING-41 significantly reduced NF- $\kappa$ B transcriptional activity in intracranial GBM tumors (67). Then, it has been demonstrated that 9-ING-41 enhanced the antitumor effect of CCNU (lomustine) leading to complete regression of intracranial GBM PDX tumors (68). GSK-3 inhibitor 9-ING-41 significantly increased CCNU antitumor activity in two different orthotopic PDX models: GBM12, which is completely resistant to CCNU, and GBM6, which shows a partial response to CCNU (68). These *in vivo* studies are the first to our knowledge that demonstrate cures in orthotopic intracranial GBM PDX models with distinct chemoresistant phenotypes (68). Moreover, CCNU+9-ING-41 combination treatment also led to a complete recovery of mouse brain structures affected by intracranial GBM growth, as indicated by histopathological evaluation of serial H&E sections of mouse brain (68). Additional studies are now underway to test whether treatment with 9-ING-41 can also overcome radioresistance in orthotopic GBM PDX tumor models.

In fact, monotherapy with 9-ING-41 did not significantly affect GBM PDX tumor progression (68). These results are consistent with previously published studies showing that monotherapy with drugs having activity against GSK-3 are not effective in treating patients with GBM (69, 70). Enzastaurin, a small molecule inhibitor of GSK-3 (IC<sub>50</sub>~24 nM) and PKC $\beta$  (14, 71), failed to improve GBM patient survival despite some radiographic evidence of antitumor activity (69, 70). These results support a hypothesis that a GSK-3 inhibitor should be combined with chemotherapy for the potential curative treatment of GBM. However, the lack of activity in GBM PDX models observed when 9-ING-41 was combined with temozolomide suggests that it is not a universal enhancer of chemotherapy (68). Additional studies will be required with 9-ING-41 and other GSK-3 inhibitors to understand the molecular basis for combination treatments and to identify molecular profiles and biomarkers that can be used to identify and enrich clinical trials for patients most likely to benefit from combination treatments that include 9-ING-41 or other GSK-3 inhibitors.

### Conclusions and future direction

A number of small molecule GSK-3 inhibitors (CHIR-99021&98014, SB216763 & 415286, AR-A011418, CG701338 & CG202796, other compounds described in the patent literature) have been used in cell and animal models to study the role of GSK-3 in cancer. However, the majority of these compounds are considered to be toolkit compounds and do not have the necessary ADMET properties to be advanced as drug candidates into the clinic. Of the GSK-3 inhibitors studied in humans to date, tideglusib and LY2090314 were well-tolerated

suggesting that some of the concerns in the field that targeting GSK-3 would lead to broad acute metabolic toxicities were unfounded although the pharmacokinetic profile of LY2090314 is poor so the lack of toxicity could be due to insufficient systemic exposures (72). Nevertheless, the clinical data suggests that toxicities of GSK-3 targeted drugs will be manageable and the hypothesis that targeting GSK-3 will sensitize resistant tumors to radiation and chemotherapy remains to be tested in patients. However, off target effects due to differences in kinase selectivity may lead to toxicities and other pharmacological effects that go beyond GSK-3 inhibition. There is now sufficient evidence to show that targeting GSK-3 $\beta$  is a rational clinical strategy for the treatment of cancer and that the role of GSK-3 $\beta$  in tumor progression is contextual and dependent on the clinical setting. The evaluation of GSK-3 expression profile and localization in subcellular compartments remains to be conducted in many types of cancer. This data combined with molecular profiling data of signatures associated with radiation and chemoresistance may help select cancer patients likely to benefit from treatment with novel GSK-3 inhibitors.

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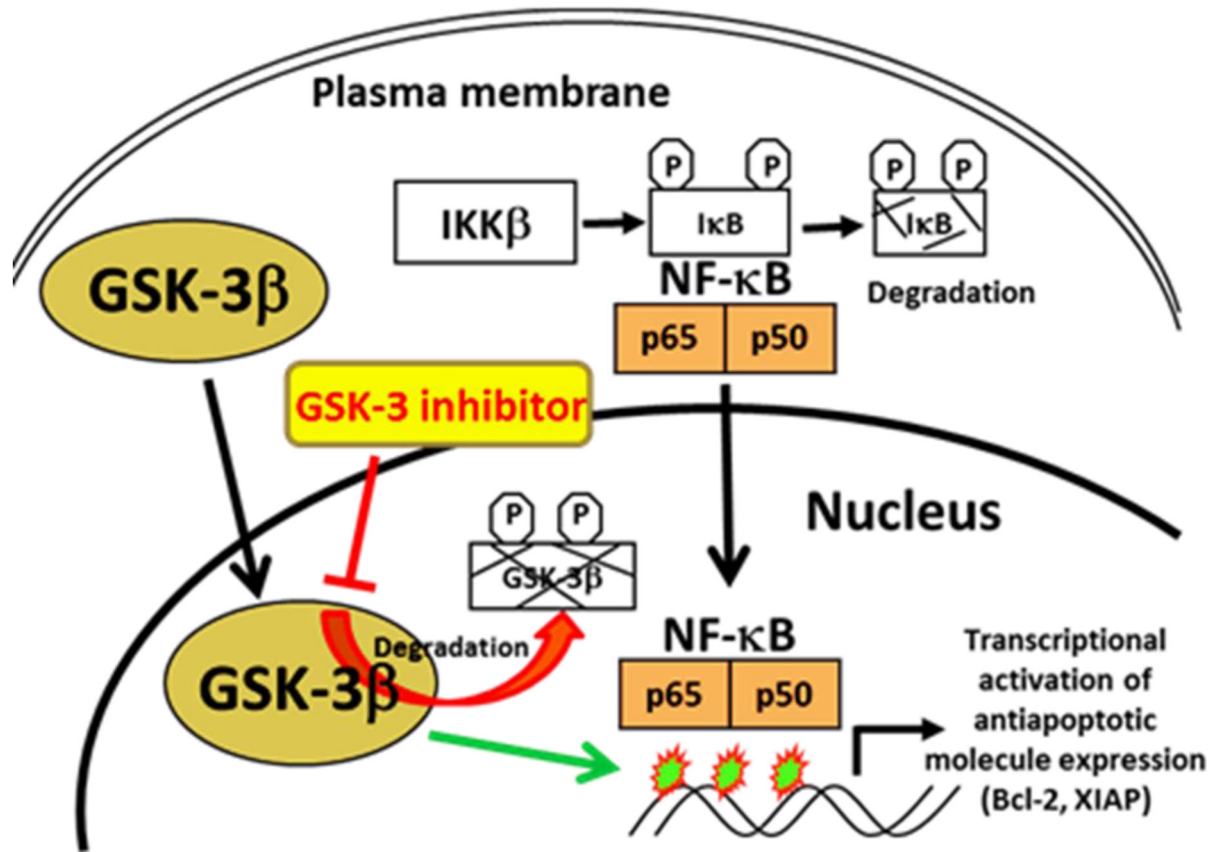


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A



B

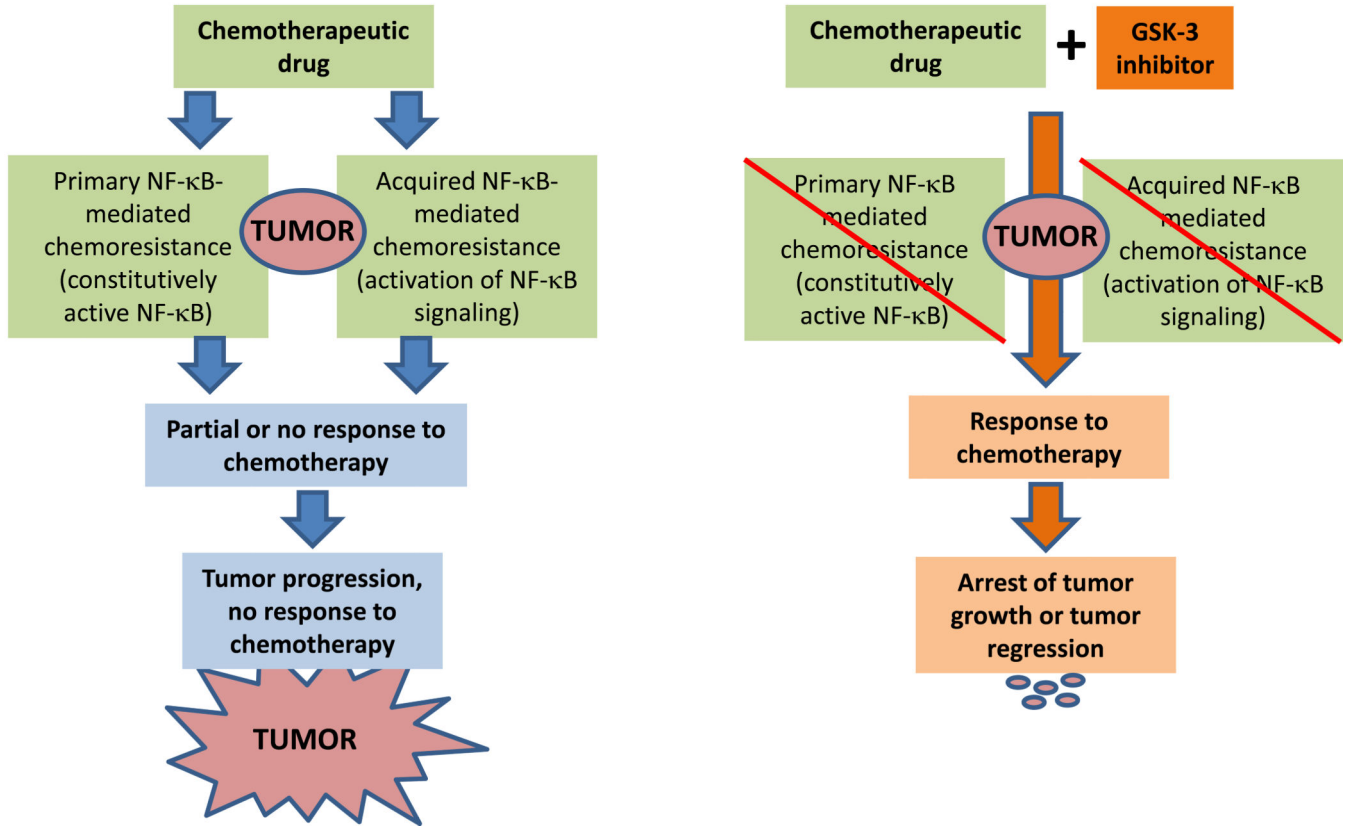


Figure 1.