



Review

Survivin and YM155: How faithful is the liaison?



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ABSTRACT

Survivin belongs to the family of apoptosis inhibitors (IAPs), which antagonizes the induction of cell death. Dysregulated expression of IAPs is frequently observed in cancers, and the high levels of survivin in tumors compared to normal adult tissues make it an attractive target for pharmacological interventions. The small imidazolium-based compound YM155 has recently been reported to block the expression of survivin via inhibition of the *survivin* promoter. Recent data, however, question that this is the sole and main effect of this drug, which is already being tested in ongoing clinical studies. Here, we critically review the current data on YM155 and other new experimental agents supposed to antagonize survivin. We summarize how cells from various tumor entities and with differential expression of the tumor suppressor p53 respond to this agent in vitro and as murine xenografts. Additionally, we recapitulate clinical trials conducted with YM155. Our article further considers the potency of YM155 in combination with other anti-cancer agents and epigenetic modulators. We also assess state-of-the-art data on the sometimes very promiscuous molecular mechanisms affected by YM155 in cancer cells.

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1. Introduction

1.1. Survivin regulates cell survival and is appreciated as a drug target

The *BIRC5* gene encodes the protein baculoviral inhibitor of apoptosis repeat-containing-5, which is more commonly known as survivin. With 16.5 kDa this protein is the smallest member of the inhibitor of apoptosis (IAP) family [1,2]. While it is clear that survivin restricts the execution of apoptosis it has still not been fully resolved how survivin controls cell death [3]. Survivin has a critical impact on cell cycle control especially during cytokinesis and for the mitotic spindle checkpoint. Accordingly, survivin expression peaks in the G2/M-phase of the cell cycle and it might provide a link between cell cycle control, mitotic catastrophe, and apoptosis [4–6].

Expression of survivin correlates with metastatic spread, tumor invasiveness, and poor prognosis associated with chemoresistance [1,7]. Such cancer-relevant functions already make it an attractive anti-cancer target [8]. The facts that survivin (I) is required for fetal development but usually absent in adult tissues, (II) is again expressed in tumors, and (III) is an important survival factor for malignant cells, point out that survivin is a bona fide molecule for pharmacological intervention strategies. Thus, survivin represents an “Achilles’ heel” of several cancer types [3,4,9,10].

In this review, we summarize knowledge on agents tested as specific inhibitors of survivin expression. We particularly focus on the polyaromatic quinone YM155, which is used in several ongoing clinical trials. We try to shed light on the question whether the beneficial effects of YM155 and related experimental drugs might rely on inhibitory effects on survivin or rather on promiscuous, previously unanticipated effects.

1.2. Factors controlling the expression and stability of survivin

Survivin has no enzymatic activity that can be targeted. Hence, it is necessary to first characterize how the *BIRC5/survivin* gene and the survivin protein are regulated.

1.2.1. Alternative splicing of the survivin RNA

The *survivin* gene gives rise to an mRNA that can yield the four splice variants *survivin*, *survivin-2 α* , *survivin-2B*, *survivin- Δ -Ex-3*, and *survivin-3B*. These are translated into proteins with overlapping and individual functions [11–13]. Since the tumor suppressor p53 affects the splicing of the *survivin* mRNA in breast cancer and probably also in other transformed cells, the ratio of survivin variants may be relevant for tumorigenesis [14,15].

1.2.2. Regulation of survivin through direct posttranslational modifications

Apart from mRNA-based mechanisms the shuttling of survivin between the cytoplasm and the nucleus and posttranslational modifications determine its functions [3,4]. Examples are the phosphorylation

of survivin at threonine 34 by the cyclin dependent kinase 1 (CDK1) which promotes the progression of mitosis in a complex with cyclin B1 (the link between survivin and cell cycle progression is discussed more detailed in Section 1.2.5). This phosphorylation is necessary for the maintenance of a complex between survivin and the apical cell death inducer caspase 9 [16]. Furthermore, phosphorylation of survivin by CDK1 attenuates proliferation to promote cellular survival [17]. Via this mechanism and through the transcription factor p53 and caspase-2, survivin affects a cell death pathway termed mitotic catastrophe [1,4,6,18,19].

Posttranslational protein modification by acetylation also controls survivin. In estrogen receptor (ER)-positive luminal breast cancer cells survivin is controlled by acetylation of the lysine residue K129 [20]. The histone acetyltransferase (HAT) CREB binding protein (CBP) and histone deacetylase 6 (HDAC6) antagonistically regulate survivin acetylation and thereby its cellular localization [20,21].

With a 30 min half-life, the survivin protein is short lived and its degradation is regulated by the ubiquitin-dependent proteasomal machinery [22,23]. This mechanism involves the X-linked inhibitor of apoptosis (XIAP)-associated factor 1 (XAF1). XAF1 can activate the ubiquitin ligase activity of XIAP to target survivin for ubiquitinylation. This posttranslational modification of lysine residues within survivin promotes its proteasomal degradation.

1.2.3. Transcription factors controlling expression of the survivin gene

As for the multitude of functions of survivin, there are many upstream factors controlling its expression (Fig. 1). An example is p53 being a major player in the control of cell fate decisions including transformation and chemoresistance [24,25]. In resting cells, p53 directly and indirectly binds the *survivin* promoter to inhibit transcription of this gene lacking a TATA box [26–31]. For example, p53 antagonizes the activating effect of the transcription factors specificity protein-1 on the *survivin* promoter [26,32,33].

Of note, there is a stress-dependent reversal of the p53-dependent suppression of survivin expression in cells exposed to replication stress. Under such conditions p53 becomes necessary to induce survivin [28]. We interpret this as a fail-safe mechanism that facilitates cellular repair processes and regular mitosis in p53 proficient cells [25]. Moreover, if the damage is persistent or too severe such cells can still undergo the p53-dependent apoptosis program preventing transformation [25]. This p53- and replication stress-dependent mechanism controlling survivin involves the transcription factor NF- κ B [25]. Already in unstimulated cells NF- κ B activates the *survivin* gene [28,34], and this may be a reason for certain tumor promoting activities of NF- κ B. In p53 mutant cells, a p53/NF- κ B crosstalk enhances the expression of survivin, and such cells have increased chemoresistance [15,28].

Expression of a gene requires the concerted interplay of chromatin modifications, with acetylation of lysine residues in histones being often found in actively transcribed regions [35,36]. The epigenetic regulators DNA methyltransferase-1 (DNMT1), histone methyltransferase

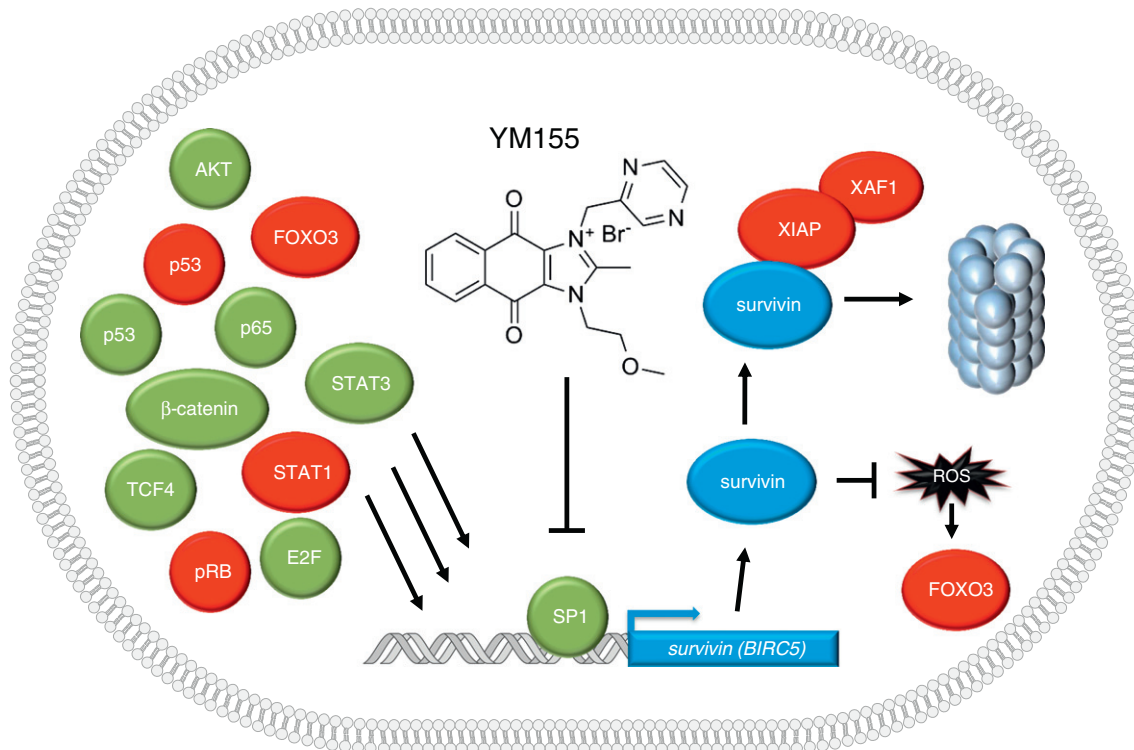


Fig. 1. Control of survivin expression at the mRNA and protein level. Several transcription factors control survivin. Additionally, the proteasome restricts the stability of survivin. YM155 blocks SP1 and this may reduce survivin expression. See text for further details.

G9a, heterochromatin protein-1 (HP1), and HDAC1 inhibit expression of the *survivin* gene in p53-positive colon carcinomas, glioblastomas, and other cancer cells [32,37–39]. Interestingly, the chemotherapeutic agent doxorubicin (Fig. 2), which integrates into DNA and inhibits topoisomerase relaxing DNA during replication represses survivin. A current model suggests that activated p53 recruits the above mentioned epigenetic regulators to condense and inactivate the *survivin* gene [32,37–39]. In contrast to this regulatory level, the suppressive effect of SIRT1 on survivin can equally occur in a p53 deleted setting [31].

Many additional factors control the expression of survivin at the mRNA and protein levels [40] (Fig. 1). For example, the inducible transcription factor signal transducer and activator of transcription-3 (STAT3) positively regulates survivin expression [41,42], and WNT-induced signaling involving nuclear β-catenin and T cell factor-4 (TCF4) induces survivin in colon cancer stem cells to avoid mitotic catastrophe and apoptosis [43,44]. The forkhead transcription factor FOXO3 is activated by high levels of intracellular reactive oxygen species (ROS), and this activation of FOXO3 directly suppresses the expression of *survivin* [45]. This process allows for a rapid loss of cytoplasmic survivin whereas mitochondrial survivin shows higher stability [45].

The interplay between FOXO3 and survivin is mutual and a paradigm for a feed-back regulation involving mitochondrial survivin and one of its regulators (Fig. 1). Survivin plays a key role in protecting cells from FOXO3-induced mitochondrial apoptosis after stress [46]. Survivin interacts and recruits the dynamin-related protein 1 (DRP1) to mitochondria and thereby initiates their fission and fragmentation. In this case fragmentation is not causal for apoptosis but protects cells against FOXO-induced apoptosis. By a yet not entirely understood mechanism survivin is involved in lowering mitochondrial ROS levels. This pathway ties in with reduced protein levels of complexes I and IV from the mitochondrial electron transfer chain (ETC) and it depends on the interaction of survivin and DRP1 at mitochondria [46]. As a result of the decreased ETC complexes, the respiratory capacity, ROS production, and FOXO3-induced apoptosis are lowered. This may lead to a

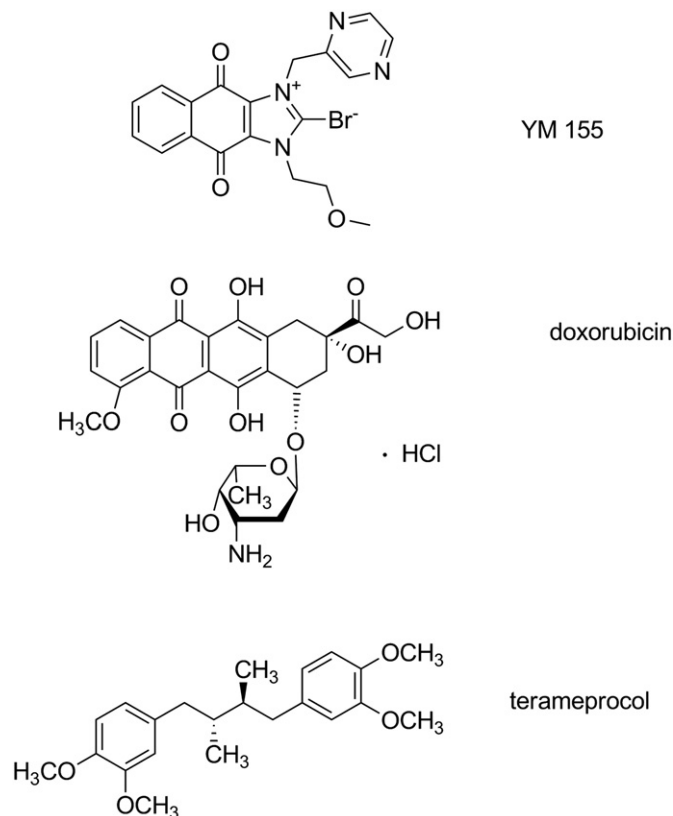


Fig. 2. Chemical structure of YM155, doxorubicin, terameprocol. Their functions and activities on survivin are summarized in the text.

higher resistance against chemotherapy [47]. Therefore, cells become more dependent on aerobic glycolysis. They increase glucose uptake and turnover and start to produce more lactate. Accordingly, the inhibition of glycolysis re-sensitizes cells for FOXO3-induced apoptosis although survivin is still present at mitochondria [46].

1.2.4. HDAC activity is necessary for the expression of survivin

Despite the above summarized negative control of the survivin promoter by deacetylases, histone deacetylase inhibitors (HDACi), agents evoking the (hyper) acetylation of histones and non-histone proteins, reduce survivin levels [29,34,48,49]. The details on this mechanism remain to be fully resolved and, based on the multitude of cellular and molecular effects altered by HDACi [50], likely involve more than one signaling pathway.

Data from melanoma cells reveal that acetylated STAT1 disrupts the NF- κ B-dependent induction of survivin [34,49]. Several other studies using various cancer-derived cells confirmed that HDACi reduce survivin expression. For example, the HDACi Trichostatin A (TSA) causes a cell cycle and cyclin B-dependent repression of survivin in prostate cancer cells [51]; both, the HDACi suberanilohydroxamic acid (SAHA) and TSA reduce the level of survivin in colon cancer cells [52,53]; the HDACi LBH589 (Panobinostat) antagonizes *survivin* mRNA expression and hepatocellular carcinoma growth [54]. The HDACi valproic acid (VPA) causes the proteasomal elimination of HDAC2 in transformed and primary cells [55] and also reduces the expression of survivin in cells derived from melanomas and colon carcinomas [29,34,49]. Since the depletion of HDAC2 after treatment with VPA or RNAi eliminated survivin and reduced the migration of human MDA-MB-231 breast adenocarcinoma cells [56], it is conceivable that the effect of VPA on HDAC2 can be sufficient to eliminate survivin in certain cancer cells. A possible mechanism underlying this regulation could be the control of p53 acetylation by HDAC2. HDAC2 specifically deacetylates the lysine residue K320 in the tetramerization domain of p53 and this correlates with an inability of p53 to suppress the *survivin* promoter [29].

In agreement with the above findings, HDACs and survivin are often overexpressed together. For example, there is a statistically significant correlation between the mRNA levels of *survivin* and of *HDAC1*, -2, and -11 in primary human liver carcinomas [54]. Experiments with mice showed that in p53-deficient mammary tumors the *survivin* gene is negatively controlled by deacetylation of histone H3 through the class III HDAC SIRT1 [31,57]. This NAD⁺-dependent sirtuin is not inhibited by HDACi used in ongoing clinical trials [50].

While these findings do not support the combined usage of HDACi and sirtuin inhibitors against survivin-dependent tumors, HDACi may combine well with the cytokine beta-interferon (IFN β) to reduce survivin levels. IFN β attenuates survivin expression via stabilization of a XIAP-XAF1-survivin complex [22,23] and this may complement the suppression of survivin through HDACi. Since IFNs are used as drugs against cancer [58] the reduction of survivin by IFNs may aid in tumor elimination. Novel IFN formulations [59,60] may increase such beneficial effects.

A recent report shows a further link between HDACi and survivin, with the underlying mechanism being linked to STAT3 phosphorylation [61]. A sodium-coupled monocarboxylate transporter termed solute carrier gene family 5A member 8 (SLC5A8) localizes to the plasma membrane and DNA methylation-dependent, p53-independent mechanisms frequently silence the *SLC5A8* gene in breast tumors [62]. Of note, *SLC5A8* can negatively regulate survivin in p53- and estrogen receptor-positive and negative cells (MCF-7/MDA-MB-231 cells) [61]. *SLC5A8* facilitates the transport of endogenous HDACi (such as butyrate or pyruvate) and causes the delocalization of survivin to the plasma membrane together with a decreased transcription of the *survivin* gene [61,62]. Since overexpression of *SLC5A8* does not evoke acetylation of histone H4 at lysine residue K16 (H4K16), it was concluded that this transporter decreases survivin expression independent of its function to import endogenous HDACi. Furthermore, the negative effect of *SLC5A8* on

survivin was not amplified in the presence of added pyruvate. *SLC5A8* rather seems to reduce survivin via inhibition of STAT3 phosphorylation [61].

In a study testing human colon cancer-derived cells it was also found that overexpression of *SLC5A8* does not evoke acetylation of histone H4 [63]. This work further reveals that pyruvate, but not its degradation product lactate, specifically inhibits the class I HDACs HDAC1 and HDAC3 [63]. Thus, the high conversion of pyruvate to lactate in cancer cells, the so-called Warburg effect [64,65], may serve to decrease the levels of an endogenous HDACi. It is though surprising that butyrate should only induce histone acetylation in breast cancer-derived MCF-7 cells ectopically expressing *SLC5A8* [62] and that butyrate does not inhibit recombinant and immunoprecipitated cellular HDAC2 [63]. It is well established that butyrate inhibits the enzymatic activity of HDAC2 [66] and being a fatty acid butyrate can also traverse the cellular lipid bilayer via passive diffusion [67]. Moreover, in cells butyrate leads to the depletion of HDAC2 via proteasomal mechanisms [68,69]. Nevertheless, it is remarkable that a link exists between metabolic intermediates, histone acetylation, and survivin expression in cancer cells.

1.2.5. Association of the cell cycle with the expression of survivin

Cell cycle progression affects the expression of survivin [4]. The levels of survivin peak during M-phase and the lowest survivin levels are found in cells arrested in the G1-phase of the cell cycle [70,71]. The CDK4-cyclin D kinase complex phosphorylates pRB and thereby promotes E2F-dependent gene expression accelerating cell cycle entry [72] (Fig. 1). In pancreatic cancer cells the inhibition of CDK4 results in G1 arrest, decreased survivin expression, and increased sensitivity toward TRAIL-induced apoptosis [73]. Luciferase reporter assays of the proximal *survivin* promoter region harboring the *E2F* cis element show a significant loss of the promoter activity after CDK4 knockdown [73]. Interestingly, in normal human melanocytes, lacking the *E2F* isoforms *E2F1* and *E2F3*, the binding of *E2F2* to the proximal *E2F* site seems to repress *survivin* gene expression [74]. However, *E2F1*, *E2F2*, and *E2F3* can induce *survivin* in the human lung embryo cell line WI-38 [75]. Despite these contrary results both studies show that the retinoblastoma protein (pRB), which recruits transcriptional corepressors to DNA-bound *E2F*, represses the *survivin* gene [74,75].

An influence of survivin on cell cycle progression has equally been discovered. In hepatoma cells survivin affects pRB phosphorylation and the activation of the CDK2/cyclin E kinase complex promoting G1-S-phase transition through inactivation of the cyclinD/CDK4 inhibitor p27 (KIP1) [76–78]. Like p27, p21 represses several cyclin-dependent kinases promoting cell cycle entry and/or progression [79], and a link between p21 (WAF/CIP1) and survivin was also identified. It was shown that only p21-proficient murine hematopoietic progenitor cells and not cells lacking the *p21* gene can be protected by survivin against apoptosis [80]. These data add further credit to the complex interplay between survivin and its regulators.

2. Discovery of YM155

The discovery of YM155 (1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d]imidazolium bromide; sepantronium bromide, Fig. 2) took place in the laboratories of the pharmaceutical company *Astellas Pharma* in 2007 [81]. Nakahara and colleagues used an artificial reporter system encompassing the *survivin* promoter to screen for compounds inhibiting its activity. They reported that YM155 specifically repressed *survivin* promoter activity with an IC₅₀ of 0.54 nM. The initial screen identifying YM155 was done with human cervical epithelial carcinoma cells (HeLa) and with Chinese hamster ovary cells. Ensuing analyses with p53 null or mutant prostate cancer derived cells demonstrated that YM155 time- and dose-dependently suppressed survivin expression [81].

How might YM155 target expression of survivin? Researchers from *Astellas* subsequently found that the RNA binding proteins interleukin enhancer-binding factor-3 (ILF3/NF110) and p54/nrb associate with the *survivin* promoter. YM155 directly binds and disrupts the ILF3–p54/nrb complex [82]. The unique C-terminal region of ILF3/NF110 is important for both, promoting survivin expression and for a high affinity binding to YM155 [83]. Thus, ILF3/NF110 might be a physiological target through which YM155 suppresses survivin. An additive global effect of ILF3/NF110 inhibition on cellular survival signaling is plausible.

YM155 may also suppress survivin expression through the disruption of SP1 binding to the *survivin* core promoter [84] (Fig. 1). SP1 is a zinc finger transcription factor that preferentially binds GC-rich DNA sequences. Remarkably, YM155 alters the localization of SP1 and overexpression of SP1 could block the negative effect of YM155 on the *survivin* promoter [84]. Whether or not this mechanism may play a role in cancer cells, i.e. if different levels of SP1 loaded to the *survivin* promoter may confer resistance to YM155 should be investigated. Additionally, wider effects of YM155 on global SP1 binding to GC-rich promoter regions have to be considered. Moreover, the SP1 transcription factor has a general impact on cell cycle progression. Blocking SP1 leads to cell cycle arrest due to inhibition of cyclin D1 and induction of p27 (KIP1) [85]. Therefore, SP1 inhibition by YM155 may exert general anti-proliferative effects against tumor cells.

Moreover, it is surprising that it is yet unclear whether YM155 and other drugs targeting survivin also alter posttranslational modifications and thereby stability of the survivin protein.

3. Cellular uptake of YM155

There are several factors determining the uptake of a drug into the body and ultimately into cells. The same holds true for the excretion phase and both parameters determine the duration of desired and undesired effects of the drug.

Tolcher and colleagues found in a phase I clinical study that the excretion of YM155 can occur via the renal route [86]. Nonrenal elimination via the hepatic route linked to excretion via the bile is considered as an additional way to eliminate intravenously administered YM155; metabolism of YM155 plays a minor role [87]. A study involving 33 Japanese patients with advanced solid tumors showed that no dose adjustment is required for patients with mild renal impairment [88].

In another study enrolling 96 patients with non-small-cell lung cancers (NSCLCs), hormone refractory prostate cancer, or unresectable melanoma were treated continuously with intravenous YM155. Cardiac and renal adverse events occurred in eight patients and the data collected in this trial suggest that renal functions have to be monitored to prevent excess accumulation of YM155 [89].

In primary human hepatocytes the radioactively labeled hydrophilic cation [¹⁴C]-YM155 is taken up by the human organic cation transporter 1 (OCT1/SLC22A1) and this process can be blocked with the physiological metabolite corticosteron [87]. Curiously, cancer cells and hepatocytes use divergent uptake mechanisms for YM155. While overexpressed OCT1 and OCT2 accelerated the uptake of [¹⁴C]YM155 into human epithelial kidney cells, OCT1, -2 and -3 are not mediating the uptake of YM155 into human solid tumor-derived cells and lymphoma cells [90,91]. Further investigations are necessary to identify the details on the cancer cell-specific uptake of YM155 and whether these can be exploited to augment the accumulation of YM155 in tumor tissue.

It is equally important to understand how YM155 is carried out of the cellular milieu. Inhibiting this pathway in tumors may increase the efficacy of YM155. In cells from the kidney and colon, the transport of YM155 out of cells may occur via the efflux transporter multidrug resistance protein-1/permeability glycoprotein/ATP-binding cassette, subfamily B, member 1 (MDR1/P-glycoprotein/ABCB1) [92]. Further data suggesting that specifically MDR1 affects the pharmacokinetics of YM155 were found in a study using neuroblastoma (NB) cells [93].

4. Efficacy of YM155 against cultured cancer cells and in experimental animals

Tables 1 and 2 list in vitro and murine xenograft studies conducted with YM155 alone and in combination schedules.

4.1. Prostate cancer

Prostate cancer is a common cause of death in men [94,95]. Prostate cancers are initially dependent on androgens and therefore sensitive to hormone antagonists and castration. During therapy such cancers become increasingly independent of androgens and/or express androgen receptor (AR) mutants hypersensitive to low hormone levels [96].

Nakahara and colleagues, who initially discovered YM155 [81], tested YM155's effects on the p53-deficient human hormone-refractory prostate cancer (HRPC) cell lines PC3 and PPC1 using doses ranging from 10–100 nmol/L. While these researchers noted an inhibitory effect on survivin they found that up to 100 nmol/L YM155 did not alter the levels of other IAPs or BCL2 family members (c-IAP2, XIAP, BAD, BCL2, or BCL-XL) in these cancer-derived cells [81]. PC3, PPC1, and several further prostate cancer cells have a functional deletion of the p53 gene [97]. In addition, PC3 cells lack AR, STAT3, and STAT5. The fact that YM155 halts the growth of such cells even when they lack p53 [25] appears very promising.

The activity of YM155 against HRPCs was also tested in murine subcutaneous and orthotopic PC3 xenograft tumor models. In this model, YM155 prevented tumor growth and suppressed survivin protein expression without YM155 causing body weight loss or hematopoietic disturbances. Curiously, ~20-fold higher doses of YM155 were detectable in tumors compared to plasma [81]. Accumulation of YM155 in prostate tumor cells in vivo was also shown in another study [98]. Nude mice xenografted with human prostate cancer-derived cells developed tumors that could be infiltrated with subcutaneously administered radioactively labeled YM155 [81]. The distribution of YM155 was also analyzed with [¹¹C]-YM155 applied to mice bearing human prostate tumor xenografts. High levels of radioactivity were found in the animals' tumors, kidneys, livers, and ceca [98]. These pharmacokinetic data and the anti-cancer effects of YM155 support its usage in further tests. Several clinical trials encompassing prostate cancer patients were conducted (see below).

It was further shown that YM155 evokes both, apoptotic cell death and autophagy of prostate cancer cells. Remarkably, the levels of survivin translate into autophagy-dependent apoptosis, with autophagy preceding apoptosis [99].

4.2. Leukemia and lymphoma

4.2.1. Leukemia

4.2.1.1. YM155 as single treatment. YM155 proved effective against various pediatric acute lymphoblastic leukemia (ALL) cells. Unexpectedly, while the levels of survivin in four ALL cell lines were comparable and peaked in the G2/M phase of the cell cycle, the half-maximal inhibitory concentration (IC₅₀) of YM155 ranged between 17 nM to 560 nM [100]. In primary ALL cells, survivin levels varied more than in the cell lines and IC₅₀ values from 10 nM to 1 mM were observed. The authors therefore concluded that factors other than survivin also determine the cellular sensitivity towards YM155 [100]. Perhaps MDR1 is such a factor as it is frequently overexpressed in E2A-HLF-positive ALL cells [101] (see also Section 4.5). The study further illustrates that ALL cells are sensitive to the inhibition of survivin by RNAi and that ALL cell death caused by these strategies is caspase-independent [100]. Tyner and colleagues further show that the silencing of p53 abrogates the anti-leukemic effects of YM155 and of RNAi against survivin in ALL cells. In addition, the phosphorylation of p53 was found to be activated by YM155 [100]. These

Table 1

Evidence for the efficacy of YM155 alone and in combinatorial application against cultured cancer cells.

Tumor tissue origin	Cell line(s)	Molecular effect(s) and combination treatments	Ref.
Prostate: HRPC	PC3, PPC1	YM155 (10 nM) suppressed expression of survivin and induced apoptosis	[81]
Leukemia: pediatric ALL cells	RCH-ACV (E2A–PBX1), REH (ETV6–RUNX1), SUPB15 (BCR–ABL), HAL01 (E2A–HLF) and primary ALL patient samples	Inhibition of cellular viability, inhibition of survivin expression and induction of apoptosis	[100]
Adult T-cell leukemia (ATL)	ATL-43b/ATL-55	10 nM or 100 nM YM155 significantly suppressed survivin expression, dose-dependent inhibition of cell proliferation, induced apoptosis	[109]
Lymphoma: GCB-DLBCL (NHL)	WSU-DLCL2, SU-DHL-4	Cell death induced by 1 nM YM155 <i>combination with STAT3 inhibitors:</i> synergistic induction of apoptosis	[119]
Leukemia and lymphoma	HL-60, U937	Inhibition of cell growth with IC ₅₀ of 0.3 nM–0.8 nM, inhibition of survivin and MCL1 expression using 1 μM YM155, induction of apoptosis	[201]
Breast: TNBC	MRK-nu-1, MDA-MB-231-Luc-D3H2-LN	YM155 (1–100 nM) shows anti-proliferative activity and triggered the induction of apoptosis,	[126]
Skin: melanoma	MRK-nu-1 A375, SK-MEL-5	Suppresses survivin expression in a dose-dependent manner (1–10 nM) YM155 in the range of 1 to 100 nM decreases survivin mRNA, results in growth inhibition and apoptosis <i>combination with docetaxel:</i> YM155 abrogates docetaxel induced upregulation of survivin, higher rate of apoptosis than sum of single treatments	[131]
Skin: MCC	MCV-positive: MKL-1, MKL-2, MS-1, WaGa MCV-negative: MCC13, BJ, BJhTERT M059K and M059J	YM155 is highly active and selective for inhibiting MCV-positive MCC cell growth [(EC ₅₀), 1.34–12.2 nM]. MKL-1 cells: YM155 induces non-apoptotic cell death with autophagy but no mitotic catastrophe Growth inhibition at YM155 concentrations one- to two-fold higher than needed for MCV-positive cells	[134]
Glioblastoma	CAL27 and cisplatin resistant derivative TW01	Inhibition of cell growth with IC ₅₀ 30–35 nM, suppression of survivin and securin expression, induction of cell death Decrease of survivin protein and cell proliferation in a dose-dependent manner <i>combination with cisplatin:</i> YM155 restored sensitivity to cisplatin, reduced levels of survivin 500 nM YM155 abrogated the protective effect of TGFβ ₃ -induced survivin ex- pression	[138] [145] [146]
Sarcoma: ES	RD-ES, SK-ES-1, TC-32, TC-71	YM155 reduced cell growth and viability, EC ₅₀ ranging from 2.8–6.2 nM	[152]
Pancreas: PDAC	MiaPaCa2 PANC1	<i>Combination with gemcitabine:</i> antagonizes the induction of survivin upon treatment with gemcitabine Reduction of EGFR, survivin, XIAP and PI3K protein, less phosphorylation of ERK and STAT3	[157] [202]
Wilms tumor	SK-NEP-1	Alteration of transcriptome, cell cycle arrest and apoptosis	[163]
Lung: NSCLC	NCI-H460 (H460) and Calu6 HCC827 (EGFR mutation positive), PC9, H1650, erlotinib-resistant PC9 derivatives	Downregulation of survivin expression in a concentration- and time- dependent manner; <i>combination with γ-radiation and platinum compounds:</i> synergistic induction of apoptosis; YM155 delayed the repair of double-strand breaks induced by γ-radiation, cisplatin and carboplatin 10 nM YM155 induce apoptosis and phosphorylation of AKT and ERK in PC9 cells <i>combination with erlotinib:</i> abrogation of erlotinib resistance due to suppression of survivin protein level Growth inhibition and apoptosis	[171] [176] [127]
Including NHL, HRPC, ovarian cancer, sarcoma, NSCLC, breast cancer, leukemia and melanoma	Panel of 119 cancer cell lines	YM155 inhibits survivin and MCL1 expression	[200]
Mesothelioma	H28	<i>combination with ABT-263:</i> downregulation of MCL1 by YM155 enhances ABT-263-triggered cell death	
Prostate cancer	PC3		
Glioblastoma	D37, U251		
Lung cancer	H661, H157		
Colorectal cancer	DLD-1		
HNSCC	SCC9		
Liver: HCC	HepG2, Hep3B, Huh7 LH86, Huh7, HB01, normal human hepatocytes	<i>Combined YM-155 and TRAIL treatment results in decreased cell viability and an increase of cells in subG1 (apoptotic cells)</i> <i>In combination with YM-155, very low dose of ABT-263 induces significant apoptosis in HCC cells only in 6 h but shows no apoptotic toxicity to normal human hepatocytes</i>	[159] [160]

data indicate that the depletion of survivin provokes a p53-dependent apoptosis pathway in ALL cells.

The above summarized study discovered that pro-B cell ALL samples positive for the oncogenic E2A–HLF fusion protein (trans-activation domain of E2A fused to the basic region and leucine zipper domain of HLF; translocation t(17;19)(q22;p13)) had the highest IC₅₀ values for YM155 [100]. Another work found that E2A–HLF induces survivin at the transcriptional level [102]. Accordingly, survivin could be inhibited through

expression of survivin-T34A and by a dominant-negative E2A–HLF. Induction of a caspase-independent and apoptosis-inducing factor (AIF)-dependent cell death upon inhibition of survivin was also found in this study [102].

YM155 is also active against B cells transformed through Epstein–Barr virus (EBV). These malignant cells are characterized by an upregulation of the mitotic progression factors CDK1 and cyclin B as well as an increased expression of survivin. Both, the CDK inhibitor flavopiridol

Table 2

Evidence for the efficiency of YM155 in mouse models.
See text for abbreviations.

Tumor type	Cell line(s)	YM155 dose and combination treatment	Refs.
HRPC	PC3 Subcutaneous xenograft	3-day continuous infusions (weekly for 2 weeks): 3 and 10 mg/kg YM155 completely inhibited the tumor growth and induced massive tumor regression; continuous YM155 infusions: no sign of tumor regrowth or systemic toxicity; 3-day continuous infusions: 10 mg/kg decrease intratumoral survivin levels on days 3 and 7; daily intravenous bolus injections of YM155: at ≥ 6 mg/kg mice did not survive, 2 mg/kg showed a 64% tumor growth inhibition of only 3-day continuous infusion (weekly for 2 weeks): 1 and 5 mg/kg showed 47% and 80% inhibition of tumor growth	[81]
NHL	PC3 orthotopic xenograft BALB/c nude mice bearing s.c. aggressive NHL xenografts (tumors reached >300 mm ³) WSU-DLCL-2	7-day continuous infusion, 1 and 3 mg/kg/d resulted in regression in large, established lymphomas (WSU-DLCL-2 and Ramos xenograft models), 0.3 mg/kg/d induced significant tumor growth inhibition in WSU-DLCL-2 models, no evidence of toxicity was observed;	[116]
DLBCL Burkitt's lymphoma	Ramos RL (tumors reached 500–2000 mm ³)	7-day infusion once every 3 weeks for 4 cycles at 1 mg/kg/d RL tumors underwent regression in response to each cycle followed by regrowth, after the 4th cycle of YM155, tumor regrowth was observed in 2 of 8 animals, with 2 CRs, 3 mg/kg/d also resulted in RL tumor regression, response was durable with no regrowth observed, CRs were achieved in 8 of 12 animals, with partial responses in the remaining 4 animals; RL xenografts treated with YM155 at 3 mg/kg (as a 7-day continuous subcutaneous infusion), reduced RL tumor volume, induced by YM155 is accompanied by down-regulation of intratumoral survivin, cell growth inhibition and induction of apoptosis; SCID/Ramos lymphoma model (lymphoma affecting the spinal cord and indicative of the terminal phase of the disease), on day 7 after intravenous cell injection YM155 (3 mg/kg 7-day continuous infusions) was administered once every 3 weeks for 4 courses;	
TNBC (human mammary gland adenocarcinoma)	MRK-nu-1 subcutaneously xenografted mice	YM155 extended the median survival 7-day continuous infusion once every 3 weeks at 5 mg/kg/d, completely inhibited tumor growth and induced marked regression of established tumors, no signs of tumor regrowth, 2 out of 5 mice experienced complete tumor regression, and no evidence for systemic toxicity	[126]
NSCLC melanoma breast cancer bladder cancer	MDA-MB-231-luc mouse metastasis model from orthotopically implanted mammary gland tumors Xenografts: Calu 6, NCI-H358 A375 MDA-MB-231 UM-UC-3	7-day infusion at 2 mg/kg/d (day 0–7), YM155 reduced the frequency of metastasis and prolonged survival	
HNSCC	SCID mouse xenograft model UM-SCC-74A	3- or 7-day continuous infusions of 1–10 mg/kg YM155, effective tumor growth inhibition in a wide variety of human cancer xenograft models without systemic toxicity	[127]
	CAL27, CAL27-CisR	YM155 at 10 mg/kg showed 65% tumor growth inhibition, whereas YM155 at 3 and 1 mg/kg showed 31% and 10%, at day 36; YM155 treatment at 1 mg/kg did not significantly decrease survivin levels, 3 and 10 mg/kg doses significantly decreased survivin expression YM155 and cisplatin treatment alone showed 40% and 24% inhibition of tumor angiogenesis, combination treatment showed 86%; YM155 (3 mg/kg) in combination with cisplatin was most effective in inhibiting tumor growth of CAL27-CisR (66% inhibition at day 36); YM155 and cisplatin treatment alone showed 38% and 29% inhibition of tumor angiogenesis in CAL27-CisR; combination treatment showed 83%	[145]
MCC	MKL-1 (subcutaneous) xenograft mouse model (NSG), MS-1 (MCV-positive) and UIISO (MCV-negative) tumor cell xenografts	YM155 (2 mg/kg, subcutaneous, five times weekly); delayed MKL-1 xenograft growth and significantly prolonged survival, but all tumors resumed growth once YM155 was stopped; YM155 rather cytostatic for MKL-1 xenografts	[134]
PDAC	MiaPaCa2 xenografts	YM155 increases sensitivity to gemcitabine YM155 (50 mg/kg) 3 day continuous infusion per week for 2 weeks; tumor growth was inhibited by 77.1% on day 31 after treatment	[157] [202]
Wilms tumor	SK-NEP-1 xenografts	YM155 5–10 mg/kg; YM155 treatment significantly decreased tumor weight	[163]
B-NHL; DLBCL/mantle cell lymphoma/ follicular lymphoma	DB, WSU-DLCL-2, Mino subcutaneous human cancer xenografts	0.5–2 g/kg; tumors were allowed to establish or disseminate before treatment; significantly prolonged survival of the mice; combination with rituximab: resulted in significant tumor regression ($>100\%$ inhibition)	[117]
	WSU-FSCCL and Jeko aggressive xenografts	YM155 (2 mg/kg/d) alone extended the median survival; combination with rituximab: significantly prolonged survival compared with the respective monotherapies	
ATL, cells were established from a patient with acute ATL	MET-1; NOD/SCID mice	2 mg/kg per day as a 7 day continuous infusion through a micro-osmotic pump; experiments were performed when serum sIL-2Ra levels were > 1000 pg/mL YM155 reduce serum levels of sIL-2Ra, prolonged the survival of leukemia-bearing mice, median survival of 86 ; combination with alemtuzumab: sIL-2Ra levels were Undetectable 8 weeks, as well as at 6 months posttherapy, median survival >6 months	[109]
MPNST			[151]

Table 2 (continued)

Tumor type	Cell line(s)	YM155 dose and combination treatment	Refs.
Melanoma	SCID mice, STS26T and MPNST724 subcutaneous xenografts and experimental lung metastasis model injection of STS26T cells into tail vein A375 and SK-MEL-5 xenograft model	Continuous YM155 (6 mg/kg/d) via micro-osmotic pump for a total of six treatment days; no complete remissions 0.3–10 mg/kg YM155, 3-day continuous infusion every week for 2 weeks (SK-MEL-5), tumor regression but regrowth during post-observation period; <i>combination with docetaxel:</i> YM155 2 mg/kg/d with 7 days infusion, 20 mg/kg bolus day 0 (SK-MEL-5), or day 0, 4 and 8 (A375), significantly inhibited tumor growth compared with each single-compound group	[131]
NSCLC	Calu 6 subcutaneous xenograft model	0.3–10 mg/kg YM155, 7-day continuous infusion, completely inhibited tumor growth and induced tumor regression, but recurrence after treatment, YM155 suppresses intra-tumoral survivin expression; <i>combination with docetaxel (intravenous bolus at 20 mg/kg):</i> significantly inhibited tumor growth compared with either compound given alone, resulted in a complete regression, YM155 enhances docetaxel-induced apoptosis	[127]
NSCLC	H460 or Calu6 cells in nude mice	YM155 (5 mg/kg over 7 consecutive days) inhibited Calu6 tumor growth <i>combination:</i> YM155 (5 mg/kg) continuous infusion over 7 d: and/or mice were subjected to local irradiation with a single dose of 10 Gy on day 3 of YM155 treatment or: combined treatment with radiation and YM155 inhibited H460 or Calu6 tumor growth to a markedly greater extent than did either modality alone; and/or γ -irradiation with a daily dose of 2 Gy on days 3 to 7 of YM155 treatment: combined treatment with radiation and YM155 inhibited H460 tumor growth to a greater extent than did either modality alone combined treatment with CDDP or CBDCA (CDDP 3 mg/kg or CBDCA 60 mg/kg intravenously on each of days 0–3 and days 7–11) and YM155 inhibited Calu6 tumor growth to a markedly greater extent than did treatment with either drug alone	[171]
NSCLC	Calu 6 subcutaneous xenograft model	<i>Combination:</i> continuous infusion of 2 mg/kg YM155 for one week and three bolus doses of 20 mg/kg docetaxel. Tumor regression in 15 out of 16 mice only when YM155 was co-administered with docetaxel and when YM155 was given before docetaxel	[172]
NSCLC	H1650 subcutaneous xenograft model	5 mg/kg YM155 over 7 consecutive days via an implanted micro-osmotic pump, YM155 inhibited tumor growth, but recurrence after treatment	[176]

and drugs reducing the expression of survivin, YM155 and tetra-O-methyl nordihydroguaiaretic acid (terameprocol; Fig. 2), were effective against EBV-transformed B cells [103]. Like YM155, terameprocol can repress the expression of survivin. Terameprocol is a semi-synthetic tetra-methylated derivative of nordihydroguaiaretic acid that blocks the binding of SP1 to the *survivin* and *CDK1* promoters [104–106].

Despite these positive effects of YM155 against leukemic cells, animal experiments indicate that it may be necessary to monitor normal hematopoiesis. In adult mice the inducible genetic depletion of survivin caused a loss of hematopoietic progenitors and rapid death [107].

4.2.1.2. YM155 as combinatorial treatment. Survivin is overexpressed in adult T cell leukemia (ATL) being characterized by a clonal expansion of T cells positive for CD4 and CD25 and immunodeficiency. This difficult to treat blood cancer is caused by infection with the human retrovirus T cell lymphotropic virus type 1 (HTLV-1) [108]. Survivin is also overexpressed in HTLV-1 infected cell lines [109], and RNAi against survivin attenuates the growth of an ATL cell line [110].

The combination of YM155 and the anti-CD52 monoclonal antibody alemtuzumab was tested in a mouse model of a human ATL (MET-1) [109]. CD52 is expressed on ATL cells [111]. While alemtuzumab can increase β -catenin and TCF4 promoting the expression of survivin and chemoresistance in peripheral blood mononuclear cells (PBMCs) of ATL patients, YM155 antagonizes survivin in ATL cell lines and causes apoptosis. Although SPC3042 as well as terameprocol (Fig. 2) failed to induce apoptosis and a loss of survivin, YM155 was effective in the MET-1 ATL mouse model and increased the survival of the mice. The tumor surrogate marker serum soluble IL-2R α (sIL-2R α) levels became lowered and the survival of tumor-bearing mice was significantly prolonged in comparison with the monotherapies. Even more than six months after combination treatment the mice remained tumor-free.

These findings encouraged a clinical trial of the combination of YM155 with alemtuzumab in ATL [109].

The HTLV-1 protein Tax activates the survivin promoter via activation of NF- κ B [112]. A recent work reveals an unexpected effect when CD34-positive human hematopoietic progenitor cells (HPCs) are infected with HTLV-1. Contrary to the hyperproliferation of CD4(+) T lymphocytes, HTLV-1 infected HPCs arrest in the G0/G1 phase of the cell cycle and the expression of survivin becomes attenuated dependent on the CDK inhibitor p21 (CIP1/WAF1) [113]. Further experimental data are necessary to understand such differences and how HTLV-1 regulates survivin and tumorigenesis. It seems possible that the complex formation between p53 and NF- κ B induced through Tax affects the NF- κ B-dependent regulation of survivin [114]. Such a molecular pathway would be reminiscent of the control of survivin through p53 and NF- κ B in cells infected with EBV encoding the oncoprotein LMP1 [115].

4.2.2. Lymphoma

So far all reported studies assessing the efficacy of YM155 against lymphomas were done in combination with other agents. A recent study by *Astellas Pharma* suggests that YM155 may represent an effective treatment for aggressive non-Hodgkin lymphoma (NHL). They compared YM155 and rituximab against aggressive non-Hodgkin lymphomas. Rituximab is a monoclonal antibody directed against the surface protein molecule cluster of differentiation-20 (CD20), which is mainly expressed on B lymphocytes. The IC₅₀ for growth inhibition of diffuse large B cell lymphoma (DLBCL) cell lines by YM155 ranged between 0.2–3.9 nM YM155. In mice, already established subcutaneous WSU-DLCL-2 diffuse large cell lymphomas and Ramos Burkitt lymphomas could be eliminated with continuous infusion of four cycles of YM155. Those tumors showed reduced survivin mRNA and protein levels which in turn resulted in a decreased mitotic index and less proliferation. YM155 increased survival significantly versus rituximab in a

disseminated Ramos model [116]. Interestingly, in this trial even high doses of YM155 did not affect hematopoiesis.

The potency of YM155 in combination with rituximab was also addressed with human B cell NHL xenograft models. Two different murine models were used. DB, WSU-DLCL-2, and Mino cells were injected into the animals' flanks, and WSU-FSCCL and Jeko cells were injected intravenously to mimic the disseminated state of NHL. Indeed, the continuous infusion of YM155 via a micro-osmotic pump plus rituximab given on two days reduced cancer cell proliferation and glucose metabolism. Moreover, such combinatorial treatment prolonged the survival of the mice [117]. Since the YM155/rituximab combination was effective against disseminated WSU-FSCCL and Jeko cells in NOD/SCID mice, the rituximab effect is likely linked to the inhibition of yet unidentified cellular survival pathways and not to CD20 antibody/cell or to complement system-mediated toxicity.

DLBCL patients with an activated B cell (ABC) gene expression profile represent a subgroup with high STAT3 activity [118]. Since STAT3 also induces the survivin gene [41,42], survivin may be the biological target downstream of STAT3 inhibition (Fig. 1). The analysis of the interplay between YM155 and the STAT3 inhibitors AG490 and STA-21 showed that such drug combinations synergistically induce apoptosis of DLBCL cells [119]. While the typhostin AG490 blocks Janus kinase-2 acting upstream of the phosphorylation-dependent STAT3 signaling cascade [120], STA-21 interacts with the Src-homology domain-2 of STAT3 and thereby blocks its interaction with the phosphorylated tyrosine residue of another STAT3 molecule. The formation of such dimers is important as they mediate the strong induction of STAT3 target genes [121]. Perhaps YM155 will also combine favorably with HDACi, as acetylation dampens STAT3 phosphorylation in DLBCLs [118]. This finding is reminiscent of the STAT1 phosphorylation–acetylation switch [58,122].

4.3. Breast cancer

Metastatic triple negative breast cancers (TNBCs) lack expression of estrogen and progesterone receptors and of the HER2/neu/ERBB2 growth factor receptor [123,124]. Due to these molecular features, this type of breast cancer does not offer therapeutically amenable targets. Accordingly, it remains a major therapeutic challenge with an often poor overall patient prognosis [123,124]. Survivin promotes breast cancer cell growth, apoptosis resistance, metastases and patient mortality [125].

Astellas Pharma tested YM155 against these cancers. In a murine pre-clinical model of spontaneous metastatic human TNBCs, continuous infusion of YM155 decreased expression of *survivin* and its splice variants *survivin-2B*, $\Delta Ex3$ and $-3B$ in tumors. This tied in with increased apoptosis and a reduced mitotic index of subcutaneously established tumors. Tumor regression and reduced metastases significantly prolonged the survival of animals. As the MDA-MB-231-Luc-D3H2-LN orthotopic model used in this work stems from an aggressive estrogen receptor negative breast cancer, the collected data propose that YM155 may represent a novel therapeutic option for patients with metastatic TNBCs [126].

The potency of YM155 against MDA-MB-231 xenografts was also demonstrated in another study [127]. Furthermore, mutations in the tumor suppressor breast cancer associated gene-1 (BRCA1) predispose women to breast and ovarian cancers [128], and the analysis of a BRCA1-deficient mouse model illustrated that survivin is associated with mammary cancers [31]. These data suggest that YM155 and other agents directed against survivin might be an option for breast cancer patients.

4.4. Skin cancers

4.4.1. Melanoma

Normal keratinocyte stem cells, melanocytes, and fibroblasts belong to the few tissues expressing survivin in adult life [129]. While survivin

protects these cells from apoptosis, melanoma and skin squamous cell carcinoma are frequently characterized by an overexpression of survivin associated with tumor grading and a notorious resistance to apoptosis [129,130].

To investigate YM155's potency in melanoma treatment, malignant p53 wild-type human melanoma cells (A375 and SK-MEL-5) were challenged in vitro and in vivo with YM155 and the anti-mitotic drug docetaxel. Both cell lines were equally sensitive to YM155 applied in the nanomolar range and YM155 was well tolerated and effective against established melanoma xenografts. Docetaxel arrested cells in the G2/M phase of the cell cycle and this led to the accumulation of survivin [131]. This observation is consistent with the cell cycle-dependent control of survivin allowing high survivin levels at G2/M [4]. While this increase in survivin did not prevent melanoma cell death, cotreatment with YM155 repressed survivin mRNA and protein expression below its levels in resting and docetaxel-treated cells. Moreover, such treatment pronouncedly and synergistically killed A375 and SK-Mel-5 melanoma cells [131].

Another study also found that melanoma cells (KUL58-MEL, RPMI-7951, SK-MEL-5, A375, SK-MEL-28, SK-MEL-2, A375-SM, Hs 294 T, G361, UZG4-MEL, Malme-3 M, BB74-MEL) are sensitive to YM155 [127]. These data encourage the use of YM155 alone and in combinatorial treatment schedules against melanoma. Since HDACi antagonize the NF- κ B-induced expression of survivin in melanoma [34,49], YM155 may combine favorably with such epigenetic modulators as well.

4.4.2. Merkel cell carcinoma

In the early 19th century Friedrich Sigmund Merkel discovered cells that are associated with nerve terminals and form mechanoreceptors. "Merkel-cell-like" cells belong to the neuroendocrine system, do not function as mechanoreceptors, and likely are the origin of Merkel cell carcinoma (MCC) as described by Cyril Toker in 1972 [132]. MCC is an aggressive skin cancer characterized by the diagnostic marker cytokeratin-20. The Merkel cell polyomavirus (MCV) causes ~80% of primary and metastatic MCCs [133]. Metastatic MCC represents a serious unresolved clinical problem and there is a disease-specific mortality of approximately 40% [132,133]. A recent study revealed a significant upregulation of survivin in MCV-positive compared to virus-negative MCCs. The MCV large T antigen encoded in the MCV dsDNA genome induces the expression of survivin in primary cells. Cell cycle regulatory proteins including E2F1 and cyclin E are required for this effect [134], which is in accordance with cell cycle progression promoting survivin expression [4]. Nonetheless, it should not be concluded that the T antigen only mediates its transforming effects through survivin. This protein rather mediates various oncogenic processes independent of survivin [135].

Studies with YM155 suggest that survivin is important for the survival of MCV-positive MCC cells [134]. YM155 was effective against MCV-positive MCC xenograft tumors in mice. While this success was reached without gross cytotoxicity, YM155's effect was cytostatic and could not stop tumor recurrence when treatment with YM155 was stopped [134]. Both, preventing infection with MCV and continuously targeting survivin expression might be a valid treatment options for MCC.

The study by Arora and colleagues [134] not only revealed that YM155 might be a novel treatment option for Merkel cell carcinomas. It further disclosed that YM155 may induce biological effects beyond the inhibition of survivin expression (see also Section 6).

4.5. Neuro- and glioblastoma

The expression of survivin correlates with a poor prognosis for NB patients, and the elimination of survivin with anti-sense molecules triggers NB cell death through mitotic catastrophe [136]. Such findings encourage the testing of YM155 against NBs. Interestingly, nine out of 23 cell lines were relatively resistant to YM155 ($IC_{50} > 200$ nM in a cell

survival assay), but sensitive to shRNAs targeting survivin [93]. The remaining cells, including four early passage tumor-initiating cells, were killed by YM155 and showed the cleavage of the DNA repair enzyme PARP, a sign of apoptosis. Furthermore, sensitive NB cells became more resistant to YM155 when survivin was overexpressed indicating that survivin is a major target of this agent. Transcriptome analyses with the five most and five least YM155-sensitive NB cell lines revealed that the expression of MDR1 (encoded by *MDR1* aka *ABCB1*) predicts the resistance of NB cells to YM155. Furthermore, a non-cytotoxic inhibition of MDR1 with the fungal peptide cyclosporine or via genetic elimination with siRNAs increased tumor cell sensitivity to YM155. Hence, YM155 appears as a promising agent for the treatment of NB when MDR1 is compromised [93].

Glial cells are the most abundant cell type in the human brain. Glioblastomas (GBMs) are aggressive, incurable, and mostly fatal cancers [137]. The expression of nuclear survivin indicates a particularly poor prognosis [138]. Treatment of human U87 glioblastoma cells (p53 wild-type, isolated from a 44 year old patient) with YM155 increased their sensitivity to irradiation. This effect was linked to an increase in giant multinucleated cells and centrosomal overduplication [139]. YM155 was also tested in other human glioblastoma cell lines, the radiation-resistant M059K and their radiation-sensitive M059J counterpart [138]. These cell lines were derived from the same tumor of a 33 year old patient and harbor mutant p53 [140]. The sensitivity of these cells toward irradiation is due to the wild-type or mutant expression of the catalytic subunit of the nuclear serine/threonine protein kinase DNA-PK [141]. DNA-PK, together with KU70/80, Artemis, XRCC4, and DNA ligase IV, is an essential part of the non-homologous end-joining (NHEJ) pathway combating DNA double strand breaks [142,143]. Whereas DNA damaging drugs like the anthracycline doxorubicin are more effective against M059J cells lacking wild-type DNA-PK [144], YM155 caused equal levels of apoptotic cell death in M059K and M059J cells. This was associated with a drug-induced reduction of survivin but not with the basal expression levels of survivin [138].

4.6. Head and neck squamous cell carcinoma

4.6.1. YM155 as single treatment

Activities of YM155 were also assessed in head and neck squamous cell carcinoma (HNSCC), a tumor type frequently overexpressing survivin, in vivo and in permanently cultured HNSCC cell lines [145]. In the human nasopharyngeal carcinoma cell line TW01 the cytokine TGF- β induced the overexpression of survivin and this process is sensitive to YM155 [146]. Nasopharyngeal carcinoma is often associated with the infection with EBV. EBV encodes an oncoprotein termed latent membrane protein 1 (LMP1) and LMP1 positively regulates the transcription and expression of survivin via p53. Moreover, p53 not only promotes the expression of survivin in such cells, but also fails to cause apoptosis and cell cycle arrest [147]. Although this unexpected finding contrasts the repression of survivin by p53 [26–30,100], it is in line with the ambivalent role of p53 on the *survivin* promoter in resting or stressed cells [25].

In extranodal nasal-type natural killer/T cell lymphoma LMP1 enhances the expression of survivin through p53, NF- κ B, and MYC. Such cells are sensitive to terameprocol which indicates that survivin is a valid target for the treatment of such cancers [115].

4.6.2. YM155 as combinatorial treatment

HNSCCs are commonly treated with cisplatin, but resistance against this drug occurs recurrently and there is often no clear benefit for the patients [148]. Of note, reducing survivin restores cellular sensitivity against cisplatin and rapidly counteracts cytoplasmic survivin in HNSCCs. Also in SCID mice bearing HNSCC xenografts, YM155 favorably combined with cisplatin at no cost of toxicity to the mice. Both, anti-tumor and anti-angiogenic effects of cisplatin became augmented suggesting anti-tumor effects as well as effects of the YM155/cisplatin

combination on the tumor environment. Hence, such drug regimen may be a useful new therapeutic strategy [145].

4.7. Sarcomas

YM155 is also effective against human malignant peripheral nerve sheath tumors (MPNSTs). This cancer arises from cells forming the connective tissue surrounding nerves and accounts for up to 10% of all soft tissue sarcomas. MPNSTs, malignant schwannoma, neurofibrosarcoma, or neurosarcoma, are associated with the autosomal-dominant disorder neurofibromatosis type 1 or develop sporadically [149]. As for most cancers, novel treatment options are also required for MPNST [150]. Cytoplasmic and nuclear survivin is overexpressed in primary human MPNST specimen and in MPNST cell lines. Inhibiting survivin caused cell cycle arrest of MPNST cells in the G2 phase and apoptosis [151]. Furthermore, YM155 was effective against locally and metastasis-like spreading of human MPNST cells in xenografted immunodeficient mice. However, no complete regressions were observed [151]. As with many other drugs targeting tumor cells, combinatorial regimens appear necessary to combat MPNSTs.

While survivin expression does not appear to correlate with the prognosis of MPNST patients [151], survivin correlates with a poor prognosis for patients suffering from Ewing sarcoma (ES) [152]. These cancers are the second most common pediatric primary bone sarcoma and one of the most fatal malignancies in children and young adults. The remission rate is approximately 50% for patients with localized ES, but curative options for patients with metastatic ES are not available [153,154]. Testing YM155 against various ES cell lines revealed that YM155 reduced ES cell viability by 50% [152]. One ES cell line was also tested with RNAi against survivin. Depletion of survivin reduced cell proliferation and induced caspase activation. Of 24 patients with ES, 10 had low and 14 had high expression of survivin in their tumors. Remarkably, of these 10 patients only one died of ES and 9 of the 14 patients with high survivin expression died of disease progression [152]. Blocking survivin with YM155, anti-sense nucleotides, or immunotherapy therefore appears as a plausible strategy to treat ES.

Further approaches may combine YM155 with other drugs to eliminate survivin or other transforming pathways in ES cells. Since ES are childhood cancers usually positive for wild-type p53, activation of p53-dependent cell death programs [155], which can be linked to the suppression of survivin [26–30] may represent a valid combination for the treatment of ES.

4.8. Pancreatic cancer

Pancreatic cancer is a dismal disease with poor prognosis [156]. So far all reported studies assessing the efficacy of YM155 against pancreatic cancer cells were done in combination with other agents. YM155 favorably combined with gemcitabine against human pancreatic cancer cells [157]. Gemcitabine is a nucleoside analog of deoxycytidine that blocks DNA replication and the enzyme ribonucleotide reductase (RNR). Gemcitabine is currently the standard chemotherapy for such cancers [156]. Like another agent evoking cell cycle arrest by RNR inhibition, hydroxyurea [28], gemcitabine induces survivin accumulation in cancer cells [157]. It is plausible that both agents promote survivin expression through a p53/NF- κ B crosstalk and that the mutant p53 protein expressed in MiaPaCa2 cells activates the survivin gene [25]. Moreover, a reduction of survivin might enhance the efficacy of gemcitabine. Indeed, YM155 as well as the knockdown of endogenous survivin by RNA interference increased the growth inhibitory effects of gemcitabine against MiaPaCa2 cells in vitro and as xenograft tumors.

4.9. Hepatocellular carcinoma

Approximately 50% of human hepatocellular carcinomas (HCCs) stain positive for survivin [158]. Furthermore, survivin-positive HCCs

show a significantly higher recurrence rate after hepatectomy and are linked to poor prognosis [158]. Consistent with the idea of survivin as a therapeutic target in HCC, YM155 was shown to sensitize HCC cells to the pro-apoptotic effects of TRAIL [159] and of the BCL2 family inhibitor ABT-263 [160].

Recent work also demonstrates the critical role of survivin in the initiation stage of HCC in a diethylnitrosamine (DEN)-induced mouse model, which mimics the human disease [161]. In an effort to uncover the molecular determinates of the c-JUN-dependent survival pathway, a complex and interesting genetic network was uncovered. c-JUN represses the promoter of a further AP1 family member, c-FOS. Reduced expression of c-FOS limits the expression of the class III deacetylase SIRT6, which is able to shut-off the transcription of the *survivin* gene by deacetylation of lysine 9 of histone H3 and by controlling binding of NF- κ B to the *survivin* gene promoter [161]. Importantly, this pathway seems to be operative in human dysplastic liver nodules. Moreover, temporary interference with survivin function during the initiation stage significantly reduces tumorigenesis in the DEN-induced mouse model [161].

These data suggest that survivin plays an important role during the carcinogenesis and maintenance of HCC, and modulating this complex genetic network might offer a preventive strategy.

4.10. Wilms tumor

Wilms tumor is a childhood cancer of the kidney. While the Wilms tumor 1 protein (WT1) is a transcription factor that is lost or mutated in such tumors, WT1 serves as a marker for leukemia and indicates minimal residual disease [162].

Human SK-NP-1 kidney cells are derived from Wilms tumor [163]. In response to YM155 these cells underwent growth arrest and apoptosis, and YM155 reduced SK-NP-1 xenograft growth. Microarray analyses conducted with these cells revealed that YM155 affects mRNA expression beyond the *survivin* promoter [163] (see also Section 6).

4.11. Bladder cancer

The absence of survivin expression in the normal urothelium and its expression in bladder cancer tissue make survivin a marker for this tumor and position survivin as a potential target for cancer-specific intervention [164]. In the UM-UC-3 bladder cancer subcutaneous xenograft model, YM155 evoked tumor regression associated with the repression of survivin and the execution of apoptosis [127]. These results propose further testing of YM155 and other anti-survivin agents against bladder cancer.

4.12. Non-small cell lung cancer

NSCLCs fall into the categories squamous cell carcinoma, large cell carcinoma, and adenocarcinoma. NSCLCs represent more than 80% of all lung cancers. This tumor type is the leading cause of cancer-related deaths worldwide and there are over one million newly diagnosed cases per year. Squamous cell carcinomas are usually found in smokers [165–169]. Hopefully, further research will reveal which combinations might be useful against NSCLC [165–167]. So far, all reported studies assessing the efficacy of YM155 against NSCLC were done in combination with other agents.

Data collected with in vitro cultured human NSCLC cell lines (H460 [p53 wild-type] and CaLu6 [p53 null]) and murine xenografts suggest that YM155 could be combined with γ -radiation [170]. Furthermore, YM155 and γ -radiation synergistically induced apoptosis and favorably combined against NSCLC xenografts growing in immunocompromised nude mice. It became clear that YM155 delays the repair of radiation-induced double strand DNA (dsDNA) breaks (measurable through the presence of the phosphorylated form of histone H2A.X (γ H2A.X)). Inclusion of YM155 in radiation protocols may hence be a valid strategy

[170]. YM155 also combines well with platinum-based drugs [171] which are commonly used as a first-line strategy to treat NSCLCs [165–167]. The efficacy of YM155 against CaLu6 xenografts was also found in another study [127].

Additional studies assessed the YM155-induced cell death of human NSCLC lines (H460, CaLu6, H358 [p53 null], PC14 [p53^{Arg248Gln} mutation]) in vitro and as tumor xenografts in vivo. These researchers found that YM155 delays the repair of DNA strand breaks induced by the chemotherapeutics cisplatin and carboplatin. Consequently, YM155 promotes caspase-dependent apoptosis of NSCLC cells [171].

In combination with docetaxel YM155 was well tolerated and active against CaLu6 xenografts. Remarkably, the schedule of administration of these drugs was relevant for anti-cancer effects [172]. This mechanism was discovered when xenografted mice were treated with YM155 and docetaxel. In 15 out of 16 mice an impressive, nearly complete tumor regression could be achieved when YM155 was co-administered with docetaxel and when YM155 was given before docetaxel. This success was associated with tumor growth inhibition and apoptosis. Curiously, tumor inhibition was seen in only three out of eight animals that were first exposed to docetaxel and then to YM155. These differences were not related to side effects, i.e. how the animals coped with the treatment schemes [172].

Non-smokers suffering from lung adenocarcinomas frequently express mutants of the tyrosine kinase epidermal growth factor receptor (EGFR) [173]. The small molecule tyrosine kinase inhibitors (TKi) gefitinib and erlotinib are the first line therapy for NSCLC patients carrying tumors with mutations in the EGFR [174]. Patients lacking such mutations do not benefit from these TKi and there are other mechanisms conferring NSCLC resistance to these drugs [175]. Interestingly, YM155 favorably combines with erlotinib, an agent inhibiting EGFR signaling [176]. This study further shows that a lack of the protein phosphatase and tensin homolog (PTEN) causes erlotinib resistance in lung cancer cells expressing hyperactive EGFR mutant molecules. Two independent cellular systems were used to collect these data. PTEN-positive (HCC827; PC9) and PTEN-negative cells as well as PC9 with acquired resistance to erlotinib (PC9/GEF cells). At the molecular level, erlotinib resistance correlates with a persistent expression of survivin and phosphorylation of the protein kinase B (PKB/AKT) [176]. These data agree with the regulation of survivin expression through the phosphoinositide 3-kinase (PI3K)/AKT signaling node [177]. Drugs targeting the EGFR block the PI3K–AKT signaling node and thereby reduce survivin and the survival of NSCLC cells [177]. Remarkably, erlotinib resistance does not correlate with a lack of drug efficacy at the level of the EGFR being the primary target of erlotinib. Elimination of survivin, either by RNAi or with YM155, antagonizes erlotinib resistance of PC9/GEF cells in vitro as well as the erlotinib resistance of H1650 cells in vitro and in xenografted nude mice [176]. Hence, survivin appears as the biologically relevant target to break the erlotinib resistance of NSCLCs.

It may be possible that YM155 also combines favorably with other drugs used to treat lung cancer. An example is crizotinib which is an orally available inhibitor of the anaplastic lymphoma kinase (ALK) and of the hepatocyte growth factor receptor (HGFR/c-MET) TKs. In around 4% of lung adenocarcinomas the *echinoderm microtubule-associated protein-like 4* (*EML4*; located on chromosome 2 p21) gene is fused to the *ALK4* gene (located on chromosome 2p23) giving rise to the EML4–ALK fusion protein. Crizotinib shows promising effects in these often younger light or never-smokers and combining this compound with other agents may prevent chemoresistance [175,178].

Another survivin antagonist, terameprocol is also effective against HCC2429 and H460 lung cancer cell lines [106]. H460 cells are large cell lung cancer cells that express wild-type p53 and depend on survivin [179]. HCC2429 cells are derived from an aggressive, metastatic lung cancer from a never-smoker [180]. These cells express the fusion protein double bromodomain protein/nuclear protein in testis, BRD4–NUT (translocation t(15;19)(q13;p13)). BRD4–NUT was found

to sequester the HAT p300 and to thereby inactivate p53 [181]. While terameprocol reduced survivin expression in both cell lines, there was no correlation between apoptosis and the reduction of survivin. Anyhow, the increased sensitivity of some terameprocol-treated lung cancer cells might be a treatment option to potentiate YM155 efficacy and warrants further investigation [106].

The fact that YM155 showed activities against p53 wild-type, null, and mutant tumor cells is promising as p53 signaling is very frequently disturbed in lung cancers [182,183]. YM155 was also found to act in a p53-independent fashion in several human tumor-derived cell lines in vitro and as murine xenografts. In a panel of 119 cancer cell lines, cells from NHL, HRPc, ovarian cancer, sarcoma, NSCLC, breast cancer, leukemia, and melanoma were among the most sensitive specimen [127]. The activity of YM155 against human cancer-derived cells was confirmed in murine xenografts studies. YM155 caused the depletion of survivin and caused tumor cell apoptosis in the absence of systemic toxicity. On average, cells with mutant or no p53 were about 1.4-fold more sensitive to YM155 than cells with wild-type p53 [127]. It is plausible that the increased expression of survivin in p53 mutant cells [25] renders them oncogene-addicted to survivin and thereby more sensitive to the depletion of survivin.

4.13. YM155 as a modulator of stem cell fate

A further application of YM155 may repose on its ability to beneficially modify human pluripotent stem cells (hPSCs). Such embryonic stem cells and induced pluripotent stem cells may bring therapeutic opportunities of unappreciable value. However, they can equally bear the risk of undifferentiated stem cells contributing to teratoma formation, and this process is linked to the expression of survivin [184].

A recent study found that undifferentiated human embryonic stem cells (hESCs) are characterized by high expression levels of *survivin* and *BCL10* [185]. Moreover, a single pulse of YM155 or quercetin, both of which can target the expression of survivin, may render the therapeutic use of hESCs more safely. Whereas undifferentiated hESCs were eliminated by such treatment, cells differentiated from hESCs were not affected and could differentiate properly [185]. While quercetin may block survivin by virtue of its ability to inhibit the β -catenin/TCF-dependent activation of the *survivin* promoter in colon cancer cells [186], others found that quercetin increases survivin protein levels in lung cancer cells [187]. The interesting study provided by Lee and colleagues reveals that 2.5 nM YM155 can eliminate undifferentiated hESCs, which are sensitive to the depletion of survivin through RNAi, and 10 nM YM155 can decrease the mRNA levels of *survivin* [185]. Unfortunately, it was not analyzed whether the treatment with YM155 depleted survivin on the protein level.

It is conceivable that not every cell controls survivin expression at every time by the same mechanisms. Therefore, YM155 may differentially affect survivin in various cell populations. This may equally apply to tumor tissues which contain different types of malignant cells [188]. Further studies will reveal whether drugs specifically targeting survivin may eliminate the indefinitely proliferating cancerous stem cells (Table 2).

5. Clinical studies

Table 3 summarizes clinical studies conducted with YM155 alone and in combination schedules.

5.1. Blood cancers

YM155 and the *survivin* anti-sense molecule LY2181308 have entered clinical trials as agents targeting survivin [189,190]. While they show acceptable toxicity and certain therapeutic efficacy, combination treatment schedules appear necessary to demonstrate the full therapeutic potential of survivin inhibitors [7,40,191].

In 2008, a phase I study reported on the maximum-tolerated dose (MTD), safety, pharmacokinetics, and preliminary evidence of antitumor activity of YM155 [86]. This study enrolled 41 patients suffering from chemotherapy-refractory cancers or from tumors for which no standard therapy protocols exist. Within the cohort, grade 1–2 toxicities (stomatitis, pyrexia, and nausea) occurred, but grade 3–4 toxicities were seldom. The MTD was 4.8 mg/m²/d YM155, allowing an approximate steady-state plasma concentration of 7.7 ng/mL and a chemical half-life of YM155 lasting 26 h. Minor responses were seen in two of nine prostate cancer cases and in one out of two NSCLC patients. However, of five patients with recurrent and refractory NHLs, three experienced partial to complete responses [86]. At the 4.8 mg/m²/d dose level, one of two heavily pretreated patients with DLBCLs had a partial response after two cycles of YM155. The patient experienced a complete response by cycle 6. Subsequent high-dose chemotherapy and peripheral stem-cell transplantation kept the patient disease-free without further treatment for more than four years. Another patient with recurrent DLBCL achieved a partial response after 16 cycles and this success lasted eight months. A patient with a follicular large B cell lymphoma resisting various chemotherapeutics and peripheral stem-cell transplantation had a partial response from cycle 8 on and has been on further treatment for over two years [86]. Of note, expression of the NF- κ B target genes *survivin* and *BCL2* is seen in about 43% and 25% of all B cell malignancies, respectively [192].

DLBCL is the most common NHL in the US and no cure options can be offered to the 30% of patients with chemoresistant NHL [193]. The study by Tolcher and colleagues, which was supported by *Astellas Pharma*, concluded that YM155 alone and in combination with chemotherapy might prove efficacy against various tumors. Moreover, the data above particularly suggests the usage of YM155 in NHL [86]. Unfortunately, the study does not inform on whether the success rates in the NHL patients were linked to the level of survivin reduction upon treatment with YM155.

A multicenter phase II study testing YM155 in 41 refractory DLBCL cases appears less positive [193]. These patients were treated with 5 mg/m² YM155 per day, as a continuous infusion for one week every 21 days (median of patient age was 66 years and a median of three prior regimen had been conducted). The median number of completed cycles was three and a maximum of 15 cycles could be reached. While one patient had a complete remission, two further patients achieved a median progression-free survival of 58 days. This study still shows that YM155 is a well-tolerated agent, apart from some serious cases of anemia and fatigue (14.6% and 12.2% percent of patients, respectively). Cheson and colleagues also suggested using YM155 in combination with other agents, such as rituximab [193].

5.2. Solid tumors

The safety profile, plasma concentrations achieved, and antitumor activity observed with YM155 merit further studies with this agent, alone and in combination regimens. Patients with advanced refractory solid tumors were treated with escalating doses of YM155 administered by continuous intravenous infusion for one week in 21-day cycles. The patients suffered from NSCLC (21.2%), esophageal cancer (18.2%), colorectal cancer (12.1%), and thymic cancer (9.1%). 33 patients with a median age of 59 years received at least one dose of YM155. The MTD was determined to be 8.0 mg/m²/d. Dose-limiting toxicity of increased blood creatinine was observed in two patients treated with 10.6 mg/m²/d [194]. Stable disease was achieved in nine patients.

A multicenter phase II trial assessed YM155 in 37 patients (five never-smokers, 28 male patients) suffering from NSCLCs [195]. This cohort comprised 17 adenocarcinoma, 15 squamous cell carcinoma, and five large cell carcinoma cases. The patients had treatment failures during platinum-based chemotherapy and their tumors were of advanced stages IIb and IV. Treatment with YM155 was well tolerated, with fatigue, pyrexia, chills, and nausea being the most common adverse

Table 3

Clinical studies involving YM155; SD, stable disease; PR, partial response; CR, complete response.

Tumor(s)	Trial phase	Patients (#)	Success SD PR CR	YM155 dosing schedule	Refs.
Advanced solid malignancies or lymphoma chemotherapy-refractory cancers or tumors lacking standard therapy protocol: prostate, colorectal, non-Hodgkin's lymphoma, head and neck, sarcoma, breast, liver, NSCLC, melanoma, ovarian, small-cell lung cancer	I	41	CR: 1 patient (8 months) PR: 2 patients (24 +, 48 + months) 1 PSA response	1.8 to 6.0 mg/m ² /d, continuous intravenous injection of escalating doses for 7 days every 3 weeks, 2 to 98 cycles	[86]
Advanced refractory solid tumors	I	33: NSCLC (7), esophageal (6), colorectal (4), thymic (3), thyroid (2), MFH (2), pleural mesothelioma (2), others (1 each)	SD: 9 patients (42–438 days)	1.8 to 10.6 mg/m ² /d, continuous intravenous injection for 7 days every 3 weeks, 1 to 19 cycles	[194]
CRPC (Prostate cancer, resistant to standard hormone therapy (taxane-containing chemotherapy) and to castration therapy)	II	35: Previous castration therapies, chemotherapy and partly radiation therapy (13 patients with diagnostic response to prostate specific antigen test)	YM155 had modest activity in taxane-pretreated prostate cancer with 25% of patients having prolonged stable disease	4.8 mg/m ² /d, continuous intravenous injection for 7 every 3 weeks, end point disease dependent	[198]
Refractory DLBCL stages I–IV	II	41	CR: 1 patient (5 + months) PR: 2 patients SD: 11 patients	5 mg/m ² /d, continuous intravenous injection for 7 days every 3 weeks, 2 to 12 cycles	[193]
Melanoma (unresectable, no prior chemotherapy) stages III–IV	II	34	PR: 1 patient (24 weeks)	4.8 mg/m ² /d, continuous intravenous injection for 7 days every 3 weeks, 1 to 6 cycles	[197]
NSCLC stages IIIb/IV	II	37: Adenocarcinoma (17), squamous cell carcinoma (15), large cell carcinoma (5)	PR: 2 patients after 6 and 8 cycles SD: 14 patients after second cycle Dead: 12 patients died during trial due to disease progression or non-drug related, 20 patients died after the trial	4.8 mg/m ² /d, continuous intravenous infusion for 7 days every 3 weeks, up to 6 cycles	[195]
CRPC	II	35	PR: 1 patient 2 confirmed PSA responses	4.8 mg/m ² /d, continuous intravenous injection for 7 days every 3 weeks	[198]
NSCLC	I	22 (Refractory)	SD: 10 patients	3.6 to 12 mg/m ² , escalating doses YM155 combined with carboplatin and paclitaxel	[196]
	II	19 (Untreated stage IV)	PR: 2 patients SD: 14 patients	10 mg/m ² intravenous over 72 h every 3 weeks, 6 cycles	

events. 31 patients could complete at least one treatment cycle and 12 patients completed six to ten cycles. Of the 37 patients two experienced a partial response (4.4%) and 14 other patients (37.8%) achieved stable disease. There was no obvious correlation between the number of cycles and the success rates. The median progression-free survival was 1.7 months and survival rates were between four to 12.2 months. A one year survival rate of 35.1% was observed. These data show that YM155 exhibits modest single-agent activity against NSCLCs, but with a disease control rate of 43.2% YM155 performed similar as other second-line agents for advanced NSCLC (e.g., erlotinib and gefitinib, docetaxel, and the anti-metabolite pemetrexed) [195]. The pharmacodynamics of YM155 was also tested in this study. Peripheral blood mononuclear cells (PBMCs) could be collected from eleven patients to assess *survivin* expressions at multiple data points. An initial repression of *survivin* mRNA was clearly observed within the first but not after the second treatment cycle. Moreover, the suppression of *survivin*, which was seen in the majority of patients, did not correlate with response [195]. Obviously, the number of patients was too low to make a definite conclusion and further assessment of YM155 in larger studies is warranted.

A recent phase I/II study assessed the safety and efficacy of YM155 in combination with paclitaxel and carboplatin in 41 NSCLC patients [196]. 19 patients with untreated stage IV non-small-cell lung cancer (NSCLC) were treated for six cycles with the maximum tolerated dose of

intravenously administered YM155 (10 mg/m²) over 72 h every three weeks. YM155 was well tolerated and two partial responses not clearly correlated with pharmacodynamic parameters were observed. Hence, the combination tested did not confer an improvement for the treatment of NSCLC.

A multi-center phase II study of YM155 monotherapy was carried out with patients suffering from unresectable stage III or IV melanoma. Chemotherapy-naïve patients were treated with YM155 [197]. While YM155 was overtly well tolerated, out of 29 patients there was only one partial responder experiencing this success for eight cycles (1.3 months median progression-free survival and 9.9 months median survival) [197].

Continuous YM155 doses were given to 35 heavily pretreated patients suffering from advanced prostate cancers. These tumors were independent of androgens and could not be restricted by castration [198]. While animal experiments assessing YM155's activity against prostate cancers were promising [81,98], YM155 was modestly effective [198]. Elevated levels of the serine protease prostate-specific antigen (PSA) are a marker for tumor burden and their reduction indicates therapeutic success. The high levels of PSA and its precursor stem from the inability of prostate tumors to cleave PSA (into its inactive form, "free PSA") and pro-PSA. Hence, these tumors have elevated PSA and pro-PSA [199]. Two out of 32 eligible patients had reduced PSA levels and a partial response was only seen in one patient. Since 25% of the patients had

disease stabilization for 18 weeks and more and because YM155 was tolerated well, combining YM155 and docetaxel may be a promising approach [198].

6. Is YM155 a survivin-specific inhibitor?

Efficiently hitting one relevant target can be a valid strategy against cancer at the cost of lower side effects. Although there is evidence supporting that YM155 can antagonize survivin expression, recent data argue that YM155 induces biological effects apart from the inhibition of survivin (Fig. 3 and Table 1).

For example, YM155 reduces transcription of the *MCL1* gene and expression of the anti-apoptotic BCL2-family protein MCL1 in various tumor-derived cells [200] (Fig. 3). A further study showed that YM155 targets both, survivin and MCL1 in human histiocytic lymphoma and promyelocytic leukemia cells [201]. YM155 was found to induce apoptosis in these cells, and the underlying cytotoxic mechanism is tightly linked to caspase-8 activation [201].

In the pancreatic adenocarcinoma cell line PANC1, YM155 was found to evoke the ubiquitinylation and proteasomal degradation of EGFR, survivin, and XIAP, but not of BCL-XL or MCL1 [202]. Na and colleagues additionally report that YM155 reduces several other cancer-relevant molecules such as PI3K, extracellular signal-regulated kinase (ERK) and STAT3 in PANC1 cells (Fig. 3). The fact that YM155 also inhibited the growth of MiaPaCa2 cells in vivo suggests that YM155 may be a valid therapeutic agent in the treatment of PDAC [202].

Data illustrating that YM155 targets molecules and pathways beyond survivin were also collected when Tao and colleagues analyzed SK-NEP-1 Wilms tumor cells [163]. Transcriptome analyses showed that treating SK-NEP-1 cells with YM155 significantly induced 32 and reduced 54 genes. Although *survivin* was decreased after treatment with YM155, several other factors were also reduced (e.g., the apoptosis

inhibitor *BCL2*). Furthermore, many mRNAs encoding tumor-relevant molecules were altered after treatment with YM155. For example, YM155 evoked an upregulation of *caspase-7*, *-9* and *DIABLO* (Fig. 3), which control apoptosis emanating from mitochondria. Moreover, YM155 induced the transcripts coding for the cell cycle regulator *p21^{WAF/CIP1}* and of *FOXO3*. These alterations in the cellular transcriptome may well be reasons for the anti-proliferative effects of YM155. Nevertheless, also some members of the Inhibitor of the apoptosis-gene family, namely *BIRC3* and *BIRC8* were induced at the same time [163]. This leads to the suggestion, that YM155 to some extent also provokes survival signals in SK-NEP-1 cells. The role of these factors on the efficacy of YM155 remains unclear. Surely, these RNA-based data need verification at the protein level and such analyses should be carried out in several cell types. Such data will further elucidate the molecular mechanisms induced by YM155. Perhaps, they also allow identifying of optimal combination approaches involving YM155. The inhibition of *DIABLO* by survivin for example is important for apoptosis induced by the microtubuli-targeting drug taxol, and *DIABLO* and XIAP in conjunction with survivin can block caspases [203]. On the other hand, p21 can antagonize cell death through cell cycle arrest, repair, and caspase inhibition [48,204].

In glioblastoma, YM155 was found to decrease the expression of survivin and securin, both control mitosis, apoptosis, and tumorigenesis [138] (Fig. 3). Securin is also known as pituitary tumor-transforming gene and can act as an oncogene in various cell types. The expression of securin is higher in glioblastoma than in normal brain tissue [205]. Hence, an effect of YM155 on securin can be a favorable additional effect of this drug. Moreover, the reduction of securin after treatment with YM155 is not necessarily a side effect. Data collected in A549 lung cancer cells with RNAi-reduced expression of survivin suggest that securin is a downstream target of survivin and that a combined inhibition of these cell cycle regulatory proteins favorably promotes cancer cell death [206].

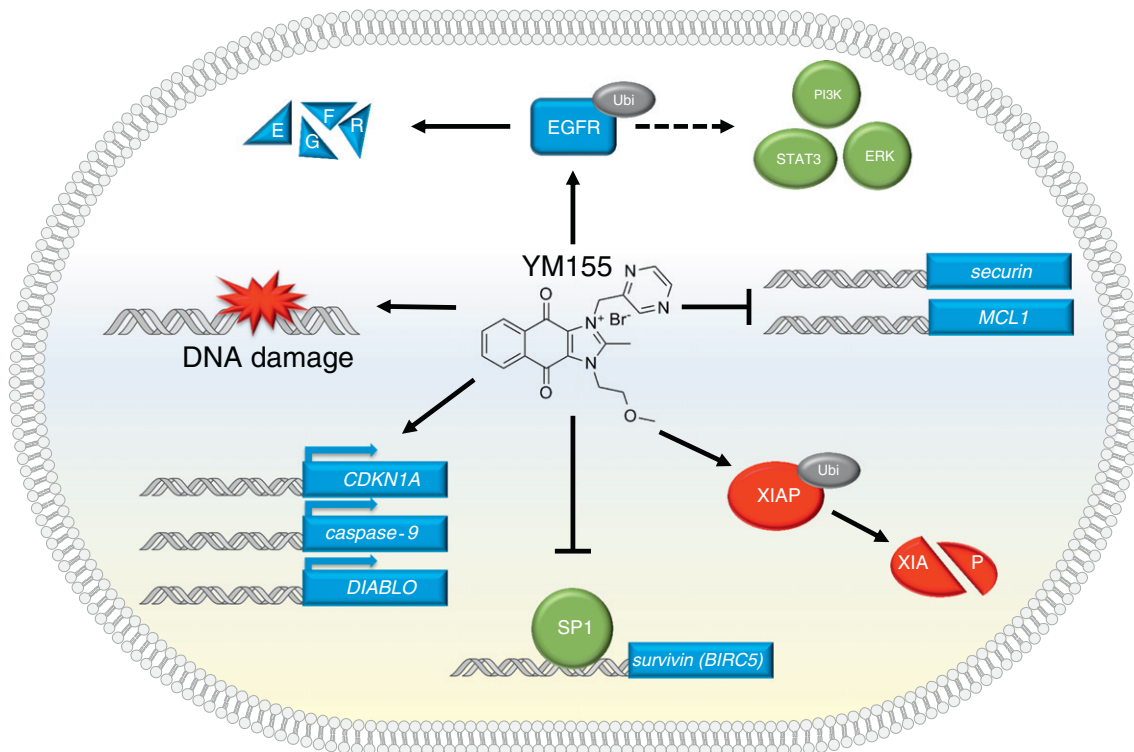


Fig. 3. YM155 affects various cellular factors and signaling. YM155 affects survivin through transcriptional repression and protein stability. Furthermore, YM155 leads to the onset of DNA damage responses and to the induction of cell cycle regulatory (e.g., *CDKN1A*) and pro-apoptotic genes (e.g., *caspase-9* and *DIABLO*). Inhibition of survivin further occurs via suppression of EGFR signaling and downstream factors. Additionally, repression of tumor-promoting gene expression (e.g., *MCL-1* and *securin*) by YM155 accelerates cell death in a wide range of tumor tissues.

NSC80467 is a novel fused naphthquinone imidazolium in-house compound of the US National Cancer Institute's Developmental Therapeutics Program in Bethesda, Maryland. Glaros and colleagues [207] compared NSC80467 and YM155 in the NCI-60 screen, which is a human tumor cell bank [208]. NSC80467 and YM155 were confirmed to block survivin protein levels. However, the researchers also found that YM155 as well as NSC80467 induce one of the most basic cellular mechanisms, the DNA damage response [207] (Fig. 3). This pathway is engaged by most chemotherapeutic drugs [142,209]. The ability of NSC80467 and YM155 to preferentially inhibit DNA, over RNA and protein synthesis prompted to analyze DNA damage responses in a panel of cell lines. Indeed, NSC80467 and YM155 evoke a dose-dependent induction of γ H2A.X and of the corepressor KAP1. Phosphorylation of these proteins by the stressor kinases Ataxia telangiectasia mutated (ATM) and Ataxia telangiectasia mutated related (ATR) marks DNA damage events and the initiation of the cell's attempt to repair them [142,210]. Importantly, low concentrations of NSC80467 and YM155 are able to induce the accumulation of γ H2A.X but do not inhibit survivin expression [207]. Noteworthy, studies using glioma and rectal cancer cell lines revealed an additional effect of nuclear survivin on enhanced double strand break repair activity after radiation-induced DNA damage [211]. Survivin was shown to interact with key proteins of primary DNA damage response, like γ H2A.X and KU70 [212]. Therefore, it can be expected that YM155 is not only able to inhibit survivin expression but also to compromise the DNA repair ability of cells. This may lead to increased sensitivity of tumor cells for therapy-induced cell death. From these results it was concluded that NSC80467 and YM155 primarily are DNA damaging agents and that the repression of survivin is a secondary event [207,213]. It should also be noted that YM155 and doxorubicin share some structural features (Fig. 2).

The drugs may share this mechanism with terameprocol which can also stall transcription and the SP1-dependent expression of survivin [104–106]. Yet, the observation that the pro-apoptotic effects of doxorubicin depend on the expression of DNA-PK in glioblastoma cells [144], but that YM155 causes apoptosis independent of DNA-PK expression [138], suggests that YM155 may well be more than just a DNA damaging drug. It may also be relevant that survivin promotes the activity of DNA-PK to allow DNA damage recognition and subsequent signaling cascades for DNA repair in cells derived from glioblastoma and colon cancer [212,214]. Data on which moieties of YM155 confer certain pharmacological effects can also permit the creation of hybrid molecules. However, since it is unclear which parts of YM155 mediate its biological effects, this is a challenging task at present.

Remarkably, a recent study identified another agent targeting survivin by a high throughput strategy with 4080 bp of the human *survivin* promoter [215]. This agent termed FL118 is able to stall the proliferation of various p53-proficient and p53-deficient cancer cell lines in vitro and in mouse xenografts. FL118 has structural similarities to the topoisomerase I inhibitor irinotecan. Of note, despite its discovery in a *survivin*-based screen FL118 inhibits the expression of the anti-apoptotic factors MCL1, XIAP, and cIAP2 [215].

The work by Arora and colleagues assessing the efficiency of YM155 against Merkel carcinomas also indicates that instead of mitotic catastrophe, YM155 disrupted DNA synthesis [134]. DNA damaging agents promote DNA damage signaling activating p53. The observation that YM155 activates p53 in ALL cells [100], may also be caused by YM155 inducing DNA damage.

If YM155 primarily causes DNA damage, it also has to be considered whether combinations of YM155 and other chemotherapeutics can accentuate undesired effects. Currently, ES is treated with local surgery, radiotherapy, and high dose chemotherapy [153,154], and survivin appears as a promising target in ES [152]. Anthracyclines (doxorubicin, daunorubicin, epirubicin, etc.; Fig. 2) can be included in such regimen and a retrospective analysis of patients below 17 years revealed a high dose-dependent incidence of cardiac toxicity [216]. One cannot exclude that

YM155 and anthracycline co-administration aggregates symptoms of cardiotoxicity.

The studies summarized above also illustrate that virally transformed tumor cells appear particularly sensitive to drugs acting against survivin [103,109,134]. It though remains unclear whether this is really due to the repression of survivin or linked to the genotoxic stress evoked by such agents. Perhaps, the viral transforming principle encoded in the host genome is more sensitive to transcription inhibition upon DNA damage, or such cells are more susceptible to drugs intercalating into DNA.

Another general difficulty in the comparison of drug effects on cells may be that the stress-inducible expression of survivin can depend on p53 [25].

In the light of the various effects of YM155, it will also be interesting to see whether YM155's effects on undifferentiated hESCs [185] rely solely on the impaired expression of survivin. For example, despite their similar effects on survivin, YM155 and quercetin had a differential effect on p53 target genes [185], and p53 and its posttranslational modifications are differentially regulated in undifferentiated and differentiated hESCs [217]. Furthermore, hESCs are highly sensitive against genotoxic stress and DNA damaging drugs were suggested as a strategy to eliminate teratoma forming populations [218]. Thus, the DNA damaging effects caused by YM155 [207] may also be responsible for the elimination of undifferentiated hPSCs.

Modern chemotherapies consist of drug cocktails. Therapeutic success rates often correlate directly with the possibility of surgical removal as well as with the availability of precise pharmacological approaches eliminating tumors. Examples for targeted approaches are the elimination of the oncogenic leukemia fusion protein PML-RAR α in promyelocytic leukemia [219], the abrogation of male/female sex hormone-dependent growth and survival signals [96,220], and the inhibition of dysregulated growth factor signaling [221,222]. Anti-sense nucleotides targeting the *survivin* mRNA are a more specific method to eliminate oncogenic survivin. LY2181308 currently undergoes clinical testing [189,190] and SPC3042 is a novel potent anti-sense molecule against *survivin* [223]. The clinical validity of such agents remains to be verified.

7. Conclusions

This review focuses on YM155. We discuss potential molecular mechanisms exerted by this drug in different settings and how YM155 affects transcription factors and regulators controlling survivin and other factors. Due to the limitations in the specificity of YM155 and NSC 80467 for survivin [207], these agents may have to be considered as drugs primarily causing DNA damage. However, even if YM155 is not a highly specific survivin inhibitor, it may well be a valuable drug in the fight against cancer.

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