Received: 29 March 2023 | Accepted: 3 July 2023

DOI: 10.1002/iub.2773

CRITICAL REVIEW

IUBMB LIFE WILEY

Therapeutic targeting of nuclear export and import receptors in cancer and their potential in combination chemotherapy

Stella Newell[1] | **Pauline J. van der Watt**[1,2] | **Virna D. Leaner**[1,3]

[1]Division of Medical Biochemistry and Structural Biology, Department of Integrative Biomedical Sciences, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

[2]Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, Cape Town, South Africa

[3]UCT/SAMRC Gynaecological Cancer Research Centre, University of Cape Town, Cape Town, South Africa

Abstract

Systemic modalities are crucial in the management of disseminated malignancies and liquid tumours. However, patient responses and tolerability to treatment are generally poor and those that enter remission often return with refractory disease. Combination therapies provide a methodology to overcome chemoresistance mechanisms and address dose-limiting toxicities. A deeper understanding of tumorigenic processes at the molecular level has brought a targeted therapy approach to the forefront of cancer research, and novel cancer biomarkers are being identified at a rapid rate, with some showing potential

Abbreviations: AKT, protein kinase B; AML, acute myeloid leukaemia; AP-1, activator protein 1; AR, androgen receptor; ARID1A, AT-rich interaction domain 1A; ATM, Ataxia-telangiectasia mutated; ATR, Ataxia telangiectasia and Rad3-related protein; Bax, Bcl-2 associated X protein; Bcl2, B-cell lymphoma 2; Bcl-XL, B-cell lymphoma extra-large; BCR-ABL, Abelson murine leukaemia viral oncogene homolog 1; BRCA1, breast cancer gene 1; CEBPD, CCAAT enhancer binding protein delta; Chk1, checkpoint kinase 1; c-MET, mesenchymal-epithelial transition factor; CML, chronic myeloid leukaemia; c-MYC, cellular myelocytomatosis oncogene; CSE1L, chromosome susceptibility like 1; DDAs, DNA-damage agents; DLBCL, diffuse large B-cell lymphoma; E2F1, E2F transcription factor 1; EGFR, epidermal growth factor receptor; elF4E, eukaryotic translation initiation factor 4E; EPE, importin 7 nuclear transport signal-derived phosphomimetic peptide; ErbB-2, receptor tyrosine-protein kinase erbb-2; ERK, extracellular signal-regulated kinase; FOXO, forkhead box protein O1; FRET, fluorescence resonance energy transfer; G3BP1, Ras-GTPase-activating protein SH3-binding protein 1; GATA6, GATA binding protein 6; GBM, glioblastoma multiform; GR, glucocorticoid receptor; HDAC, histone deacetylase; HEK293, human embryonic kidney 293 cells; HGBCL-DH, high-grade B-cell lymphoma with MYC and Bcl-2; HIF1a, hypoxia inducible factor 1-alpha; HR, homologous repair; HuR, human antigen R; HURP, hepatoma upregulate protein; IMPA2, importin-alpha 2; INI-43, inhibitor of nuclear import 43; INI-60, inhibitor of nuclear import 60; IPO11, importin 11; IPO7, importin 7; IPZ, importazole; IκB, I-kappa-B Kinase; K_d, dissociation constant; kDa, kilodalton; KPNA1, karyopherin-α1; KPNA2, karyopherin-alpha 2; KPNB1, karyopherin-beta 1; LMB, leptomycin B; LRPPRC, leucine rich pentatricopeptide repeat containing; MAPK, mitogen activated protein kinase; Mcl-1, myeloid cell leukaemia sequence 1; MDM2, mouse double minute 2; MEK, mitogen-activated protein kinase kinase; MM, multiple myeloma; mTor, mammalian target of rapamycin; NCOR2, nuclear receptor corepressor 2; NES, nuclear export signal; NFAT, nuclear factor of activated T cells; NFY, nuclear transcription factor Y; NF-κB, nuclear factor kappa B; NLS, nuclear localization signal; NPCs, nuclear pore complexes; NR1, glutamate receptor; NSCLC, non-small cell lung cancer; NTRs, nuclear transport receptors; NXF3, nuclear RNA export factor 3; P2RY2, purinergic receptor P2Y2; PARP, poly-ADP ribose polymerase; PDX, patient-derived xenograft; PERK, PRKR-like endoplasmic reticulum kinase; PI3K, phosphoinositide 3-kinase; PIM1, serine/threonine-protein kinase pim-1; PIs, proteosome inhibitors; PRKDC, protein kinase, DNA-activated, catalytic subunit; PTEN, phosphatase and tensin homolog deleted on chromosome 10; Puma, p53 upregulated modulator of apoptosis; Rad51, Rad51 recombinase; RAF, rapidly accelerating fibrosarcoma; Ran, Ras-related nuclear protein; RanGap1, RanGTPase activating protein 1; RAS, Rat sarcoma virus; RCC1, regulator of chromatin condensation 1; RRMM, relapsed/refractory multiple myeloma; SINE, selective inhibitor of nuclear export; Smad3, mothers against decapentaplegic homolog 3; Sp1, specific protein 1; STAT6, signal transducer and activator of transcription 6; TGF-ß, transformation growth factor beta; TNBC, triple negative breast cancer; TNPO1, transportin 1; TopIIα, topoisomerase IIα; TRIM59, tripartite motif containing 59; TSPs, tumour suppressor proteins; UPR, unfolded-protein response; XIAP, X-linked inhibitor of apoptosis protein; XPO1, exportin 1; y-TURC, gamma-tubulin ring complex.

Correspondence
Virna D. Leaner, Division of Medical Biochemistry and Structural Biology, Department of Integrative Biomedical Sciences, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa.
Email: virna.leaner@uct.ac.za

Funding information
Cancer Association of South Africa; National Research Foundation of South Africa; South African Medical Research Council; University of Cape Town Research Committee

therapeutic benefits. The Karyopherin superfamily of proteins is soluble receptors that mediate nucleocytoplasmic shuttling of proteins and RNAs, and recently, nuclear transport receptors have been recognized as novel anticancer targets. Inhibitors against nuclear export have been approved for clinical use against certain cancer types, whereas inhibitors against nuclear import are in preclinical stages of investigation. Mechanistically, targeting nucleocytoplasmic shuttling has shown to abrogate oncogenic signalling and restore tumour suppressor functions through nuclear sequestration of relevant proteins and mRNAs. Hence, nuclear transport inhibitors display broad spectrum anticancer activity and harbour potential to engage in synergistic interactions with a wide array of cytotoxic agents and other targeted agents. This review is focussed on the most researched nuclear transport receptors in the context of cancer, XPO1 and KPNB1, and highlights how inhibitors targeting these receptors can enhance the therapeutic efficacy of standard of care therapies and novel targeted agents in a combination therapy approach. Furthermore, an updated review on the therapeutic targeting of lesser characterized karyopherin proteins is provided and resistance to clinically approved nuclear export inhibitors is discussed.

KEYWORDS
cancer, combination therapy, KPNB1, nuclear transport proteins, targeted therapy, XPO1

1 | INTRODUCTION

The diverse nature of cancer pathogenesis has led to different treatment modalities with varying degrees of efficacy. Local modalities include surgery and radiation whereas systemic treatment options include conventional chemotherapies, targeted therapies, and immunotherapies. Conventional chemotherapies mediate their anticancer effects by disrupting fundamental cellular processes, such as DNA replication, and therefore frequently associate with side effects due to their inability to distinguish between cancer cells and naturally proliferative non-cancer cells. On the other hand, targeted agents inhibit specific cancer-sustaining pathways that healthy cells are less reliant on for survival, and thus are predicted to display an improved tolerability profile. However, dose-limiting toxicities as well as high instances of chemoresistance severely compromise the efficacy of systemic treatment methods in a clinical setting. As a result, combination regimens involving more than one therapeutic agent are commonly favoured over monotherapies; extensive research is looking into the joint effects of different conventional and targeted therapies that collectively disrupt multiple cancer-related pathways as a means of counteracting chemoresistance mechanisms that hinder the efficacy of standard if care therapies or novel targeted agents.[1] When a synergistic or additive relationship is observed, this allows for the lowering of dosage requirements while achieving the desired therapeutic outcome, thereby minimizing the severity and frequency of adverse events.[2]

Modern biotechnological advances have accelerated the biomarker discovery process and facilitated the expansion of the targeted therapy field.[3] In recent years, nuclear transport receptors have captured attention as novel therapeutic targets in a broad range of cancer types.[4] Spatiotemporal localization of proteins and RNAs is carefully regulated by the nuclear transport system to maintain a homeostatic balance and normal cellular function. The karyopherin superfamily of proteins orchestrate this process as import receptors (importins), export receptors (exportins) and bidirectional transport receptors (transportins). Dysregulation of nuclear transport receptors through modulations in their expression levels or subcellular localization alters the compartmentalization of tumour suppressors and key mediators of oncogenic signalling, often driving tumorigenesis.[5] While approximately 26 karyopherin proteins have been recorded to date, this review is centred around the most comprehensively researched nuclear transport receptors, namely exportin 1 (XPO1) and Karyopherin-β1 (KPNB1), for which inhibitors have already been developed.[4] This review will highlight both the success of targeting XPO1 and address the limitations of current clinically approved

inhibitors. Additionally, the preclinical anticancer efficacy of KPNB1 inhibition will be discussed. However, the main focus of the review will be directed towards the significant potentiating effects of nuclear export/import disruption when combined with existing chemotherapeutic drugs or experimental anti-neoplastic agents, thereby supporting the notion that combination regimens are typically favourable over single agent treatments.

2 | THE NUCLEAR TRANSPORT SYSTEM

The nuclear membrane distinguishes the nucleus from the cytoplasm and allows for controlled spatiotemporal localization of proteins and RNAs, which is critical to cellular function. Nucleocytoplasmic transport of such macromolecules is tightly regulated by the nuclear transport machinery. Nuclear pore complexes (NPCs), which perforate the nuclear membrane, function as gatekeepers that carefully mediate the active shuttling of substrates larger than 40 kDa between the nucleus and cytoplasm.[6] The karyopherin superfamily of proteins are the key orchestrators of the nuclear transport process and can be further divided into the karyopherin-β and karyopherin-α subfamilies. Karyopherin-β subtypes represent the soluble nuclear import receptors (importins), nuclear export receptors (exportins) and bidirectional transport receptors (transportins). Karyopherin-α subtypes function as adaptors that recognize and bind cytoplasmic cargoes bearing a nuclear localization signal (NLS) in the classical nuclear import pathway.[7] Once associated with its cognate cargo, the adaptors recruit karyopherin-β1 (KPNB1), which interacts with the phenylalanine-glycine repeats of nucleoporins, the functional components of the NPC, thereby facilitating nuclear membrane translocation.[8] Upon entry into the nucleus, RanGTP, a small Ras-like GTPase, displaces the adaptor, resulting in KPNβ1/KPNα dissociation and cargo release.[8] KPNB1/RanGTP is recycled back into the cytoplasm where RanGTP is hydrolyzed to RanGDP by RanGTPase Activating protein 1 (RanGAP1), thereby releasing KPNB1 for the next round of transport.[9] Alternatively, KPNB1 may import cytoplasmic cargoes independent of an adaptor in the nonclassical pathway. The nuclear export process transpires analogously; in association with RanGTP, an exportin cooperatively binds the NES of its cognate cargo and interactions with the NPC enable access into the cytoplasm where RanGTP hydrolysis by RanGAP1 stimulates cargo release. The regulator of chromatin condensation 1 (RCC1) is a chromatin-associated guanine exchange factor which, in concert with cytoplasmic RanGAP1, functions to maintain a RanGTP/GDP gradient across the nuclear membrane.[10] High levels of nuclear RanGTP in contrast to an enrichment of RanGDP in the cytoplasm provides directionality to the energy-dependent nuclear transport process.

Outside of interphase, karyopherin proteins take on alternate functions in coordinating mitotic division. KPNB1, in concert with NLS-binding adaptor proteins, acts as a global negative regulator of mitosis by preventing premature nuclear envelope breakdown and reformation, mitotic spindle-assembly, chromosomal segregation, and mitotic completion and exit.[11–13] For example, the spatio-temporal control of spindle assembly factors relies on their sequestration by KPNB1 in cytoplasmic regions; only upon interaction with RanGTP, which forms a concentrated cloud around mitotic chromosomes, will the factors be released to initiate spindle assembly.[14,15] Transportin 1 (TNPO1) functions analogously to KPNB1 and engage with various spindle assembly factors independent of Karyopherin-family members.[16,17] Contrastingly, XPO1 interacts with RanGTP and recruits protein complexes to the appropriate cellular structure; for instance, upon binding RanGTP, XPO1 localizes pericentrin and γ-TURC complexes to developing centrosomes and initiates the nucleation of spindle microtubules.[18–20] In mediating the opposing mitotic functions of KPNB1, an additional layer of nuclear receptor regulation is required. Phosphorylation of XPO1 serine 391 by CDK1-cyclin B promotes the transfer of sequestered RanBP1 from KPNB1 to XPO1, thereby facilitating RanBP1 localization to kinetochores, an event which is crucial for kinetochore function after chromosomal attachment.[21,22] Although the mitotic roles of XPO1 are poorly characterized relative to those of KPNB1, recent literature by Nord et al. illustrates that XPO1, in concert with Exportin 5 and Exportin T, has similar inhibitory effects on major mitotic events, such as membrane fusion and nuclear pore formation via interactions with various nucleoporins and RanGTP-dependent mechanism.[23]

Nuclear receptors globally control the distribution of proteins across the nuclear membrane. Given the extent to which spatiotemporal localization dictates the activity of both receptor and cargo, it is not surprising that aberrant functioning of the nuclear transport machinery is associated with pathogenic disease states, such as cancer.

3 | NUCLEAR TRANSPORT DYSREGULATION IN CANCER

Many of the karyopherin family members are dysregulated in cancer, most frequently in the form of elevated expression evident from studies comparing tumour samples to normal adjacent tissues.[4,24–28] Upregulation of

karyopherin proteins has been associated with enhanced transport efficiency, which is thought to benefit tumour cells via promoting oncogenic signalling and sustaining the high metabolic and proliferative demands of the cancer phenotype.[29] However, karyopherin proteins are also known to perform tumour suppressor functions; Importin 11 (IPO11) mediates the nuclear entry of monoubiquitinated PTEN, thereby protecting the tumour suppressor against proteasomal degradation in the cytoplasm.[30] The role of IPO11 in regulating PTEN function is further supported by genomics studies indicating frequent gene deletions of *Ipo11* across a broad range of solid tumours, which was shown to correlate with reduced PTEN expression levels in prostate cancer cell lines.[30] Conversely, elevated expression of IPO11 has also been observed in non-invasive bladder cancer and predicts poor prognosis.[31] Thus, the functional relevance of nuclear receptors in cancer may often be tumour-type specific.

Additionally, the mislocalization of nuclear receptors also comes with oncogenic consequences. In ovarian cancer, AKT-mediated phosphorylation of Chromosome susceptibility like 1 (CSE1L) stimulates its accumulation in the nucleus, where it performs its function as a KPNA recycler.[32] CSE1L depletion enhances cisplatin cytotoxicity in ovarian cancer cells, however, malignancies with predominantly cytoplasmic CSE1L show no evidence of sensitization.[32]

During mitosis, nuclear transport receptors (NTRs) take on alternate functions to collectively coordinate all stages of mitotic division.[16] Much like the nuclear transport pathway, NTRs rely on the RanGTP system to direct the spatial arrangement of NLS/NES-bearing cargoes, including mitotic spindle factors, components of the chromosomal passenger complex and nucleoporins, such that they are associated with the appropriate cellular structure at the correct stage of cell division. In the absence of this regulatory mechanism, the integrity of subsequent daughter cell genomes is under considerable threat. siRNA knockdown and synthetic overexpression of KPNB1 gives rise to mitotic defects, including chromosomal mis-segregation.[33] Verrico et al. corroborated this finding and demonstrated that KPNB1 overexpression in HeLa cells overrides the inhibitory effects of RanGTP and leads to prolonged sequestration of spindle assembly factors.[34] For example, HURP is typically released from KPNB1 to associate with the positive poles of growing (M +) microtubule polymers so as to stabilize microtubule-kinetochore interactions. However, KPNB1 overexpression destabilizes microtubule structures by instead targets HURP to M-regions where it remains in contact with dynein-bound-KPNB1. Furthermore, E517K/G point mutations in XPO1 that are enriched in specific blood cancers similarly gives rise to mitotic defects and G2/M arrest when introduced into transformed embryonic kidney cells (HEK293).[35,36] Therefore, nuclear receptor dysfunction favours the onset of genomic instability, an enabling hallmark of cancer.[37] This feature may prove to be a caveat that impacts the longevity of tumour responses to nuclear transport inhibitors. Nuclear import inhibitors have not shown to induce genotoxic stress. However, treatment with XPO1 inhibitor, Selinexor, has shown to elicit DNA damage and chromosomal defects; over time these may contribute towards elevated intratumoral heterogeneity and the selection of clones with inherited mutational aberrations that confer resistance to Selinexor.[38] This phenomenon is observed in tumours treated with conventional genotoxic chemotherapeutics.[39,40] As such, the impact of Selinexor on the tumour mutational burdens of treated tumours is warranted.

Current literature focuses more specifically on the tumorigenic contributions of karyopherin proteins in the context of their nuclear transport functions. Due to their altered expression in cancer, Karyopherin proteins show great promise as novel diagnostic and prognostic tools as well as candidate therapeutic targets. The remainder of the review focuses on the tumour promoting functions of XPO1 and KPNB1, the clinical and preclinical success of small inhibitors designed to target these receptors, and their potential in novel combination regimens. Additionally, recent literature implicating the lesser characterized NTRs in cancer will be highlighted and preliminary insights into their potential therapeutic benefits will be addressed.

4 | EXPORTIN 1/CHROMATIN MAINTENANCE PROTEIN 1 (XPO1/CRM1) AND ITS ROLE IN CANCER

The substantial exportome of XPO1 comprises >1,000 human cargo proteins involved in a broad spectrum of biological functions, including, autophagy, peroxisome biogenesis, ribosome maturation, translation, and mRNA degradation.[41,42] From a tumorigenic perspective, XPO1 shuttles the vast majority of major tumour suppressor proteins (p53, p21, p27, pRb, FOXO proteins, BRCA1 and IκB), several drug targets (topoisomerase IIα and BCR-ABL), and various oncogenic signal transducers, (STAT6 and Survivin).[18,43–50] As such, enhanced nuclear exclusion of these proteins by upregulated XPO1 promotes cancer development. In addition to protein export, XPO1 transports various RNA species to the cytoplasm, and in doing so regulates ribosome assembly, mRNA splicing and cytoplasmic access of tRNAs and microRNAs.[51–53] Interestingly, XPO1 interacts with various mRNA-

binding chaperones, including HuR, LRPPRC, NXF3, and elF4E, and in doing so facilitates the nuclear exit and translation of subsets of mRNA encoding oncogenes.[54] For example, c-MYC, Cyclin B1, Cyclin D1, MCl-1, Bc-l2, and PIM1 are shuttled by elF4E in concert with XPO1 and the accumulation of cytoplasmic elF4E-associated mRNAs stimulates the synthesis of proteins that promote tumour cell proliferation, survival and chemoresistance.[55–57]

XPO1 is overexpressed in a broad range of cancer subtypes, where high mRNA or protein levels are observed in more advanced disease states and predict poor patient prognosis.[58–69] Upregulation of XPO1 at the mRNA level is partially attributed to enhanced activity of the transcription factors, Sp1 and NFY, in both transformed human fibroblasts and cervical cancer.[70] Additionally, p53 interactions with NFY result in *Xpo1* promoter repression in response to DNA damage; a substantial proportion of tumours are p53-deficient and abrogation of this negative regulatory mechanism likely contributes towards enhanced XPO1 levels in cancer cells under genotoxic stress.[70] In the context of colorectal cancer, Golomb et al. 2012 corroborate this notion and further suggest that c-Myc engages in positive regulatory functions that give rise to upregulated XPO1 and Importin 7 levels so as to facilitate the nucleo-cytoplasmic shuttling of proteins and RNAs involved in ribosomal biogenesis.[71] At the genomic level, genetic aberrations are reported at very low frequencies in XPO1 upregulated tumours,[72] with the exception of subsets of chronic lymphoid leukaemia patients harbouring specific chromosomal translocations (*2p+*) and subsequent amplification of *Xpo1*.[73] However, further investigation is warranted to uncover mechanism behind ubiquitous XPO1 upregulation in cancer and delineate tumour specific mechanisms, which are evident in select blood cancer subtypes.[36,72]

In addition to XPO1 upregulation, recurrent patterns of somatic missense mutations have also been observed across various tumours types, largely of haematopoietic origin.[74–77] However, the functional relevance of these mutations in cancer has only recently been elucidated. Glu517 is positioned within the hydrophobic NES-binding pocket of XPO1; its alteration from a negatively-charged glutamic acid residue to a positively charged lysine or glycine residue (E517K/G) is positively selected for in various cancers, most notably in B-cell malignancies. E517K/G modifies the affinity of XPO1 for its cargoes in a sequence-specific manner; cargoes harbouring negatively charged residues C-terminal to their NES sequences are preferentially exported over those with positively charged C-termini.[36] Taylor et al., 2019 demonstrated that the genetic knock-in of E517K in haematopoietic lineages of transgenic mice is sufficient to initiate malignant transformation and drive the development of disease which accurately recapitulates human chronic lymphoid leukaemia.[36] This phenomenon presents a novel model of tumorigenesis whereby the global disruption of protein localization across the nuclear membrane constitutes a primary driver of cancer development.

5 | KARYOPHERIN B1 (KPNB1) AND ITS ROLE IN CANCER

While XPO1 is a nuclear exporter of cargoes, Karyopherin-β1 (KPNB1) is a universal vector of nuclear import that interacts with various adaptors formerly bound to cognate cargoes harbouring classical NLS sequences. Alternatively, nuclear entry occurs via direct interactions with KPNB1 in the non-classical pathway. Many KPNB1 cargoes are central mediators of oncogenesis which, in part, is a consequence of their abnormal cellular localization. Such cargoes include signal transducers (STAT3, Smad3), growth factor receptors (EGFR, Erb2, c-MET, GR), and transcription factors (Snail, NFκB, AP-1, NFAT, c-MYC).[78–83] Collectively, KPNB1 cargoes have the potential to promote malignant transformation and the acquisition of many proposed cancer hallmarks.[37]

Van der Watt et al., 2011 demonstrated that KPNA2 and KPNB1 overexpression is predominantly mediated by E2F1, which is commonly dysregulated almost all human cancers owing to defects in the CDK-RB-E2F axis.[84–86] Thus, it is not surprising that KPNB1 upregulation is observed across a multiplicity of cancer types and is associated with progressive disease and poor prognosis.[87–92] Interestingly, E2F1 is a KPNB1 cargo that sustains proliferative behaviour in chronic myeloid leukaemia; siRNA depletion of KPNB1 reduced the nuclear localization and transcriptional activity of E2F1, thereby impairing cancer cell proliferation and viability.[93] In addition to enhanced nuclear activity of cognate cargoes, alternate functions of KPNB1 in cancer were uncovered by siRNA knockdown studies in various tumour model systems. Zhu et al., 2018 showed that KPNB1 is essential in balancing the nucleocytoplasmic distribution of proteins at an amenable level. KPNB1 depletion disrupted proteostasis and triggered the unfolded protein stress response which resulted in significant apoptotic death in glioblastoma multiforme cell culture models.[94]

Due to their altered expression in cancer, karyopherin proteins show great promise both as novel diagnostic and prognostic tools, and in some instances represent novel candidate anti-cancer targets. XPO1 and KPNB1 will be discussed in more detail below and recent evidence that

has uncovered oncogenic roles of the lesser characterized nuclear transport receptors will be highlighted.

6 | THERAPEUTIC TARGETING OF NUCLEAR TRANSPORT RECEPTORS

The frequent overexpression of XPO1 and KPNB1 in cancer implicates these proteins as potential therapeutic targets; this notion is further substantiated by siRNA knockdown studies that demonstrate the essentiality of heightened XPO1 and KPNB1 expression on tumour cell survival. Importantly, siRNA depletion of both of these receptors has limited effects on normal cell viability.[59]

An advantage of the therapeutic targeting of proteins like XPO1 and KPNB1 is that, unlike in the case of many other targeted therapies, a mutated target is not required, hence the spectrum of cancers that can be treated is much broader. Furthermore, disrupting nucleocytoplasmic transport mediated by these receptors has the potential to interfere with a plethora of tumorigenic signalling pathways. Thus, the pleiotropic effects of this anticancer approach confer a major advantage when compared to agents that are designed to target a single pathway. Intriguingly, it is important to note that there are instances where targeting both the import or export of carcinogenic cargoes has similar anti-cancer effects, explaining anti-tumour activity of XPO1 and KPNB1 inhibitors in the same cancer subtypes.[92,93,95–98] For example, the NFκB group of transcription factors are involved in transmitting pro-survival, anti-apoptotic, and inflammatory signal that contribute towards tumour development and chemoresistance.[99,100] Nuclear import impediment of KPNB1 prevents the nuclear accumulation of active p60/p65 heterodimers, resulting chemosensitizing effects that were attributed to reduced NFκB transcriptional activity in cervical cancer.[101] Furthermore, nuclear export impediment of XPO1 permits the nuclear accumulation of tumour suppressor, IκB, which sequesters and inhibits the transcriptional activity of NFκB in various solid and liquid tumours.[45,96,102,103]

7 | THERAPEUTIC TARGETING OF XPO1

Therapeutic targeting of XPO1 is an attractive, broad spectrum anticancer approach.

Mechanistically, XPO1 inhibition promotes the nuclear retention and restores the functional activity of tumour suppressor proteins or targets tumorigenic signalling events by blocking oncoprotein synthesis. For nearly two decades, a wealth of XPO1-targeted inhibitors have been developed and a select few are currently under clinical investigation.[104]

Leptomycin B (LMB), a natural anti-fungal compound, was the first XPO1 inhibitor to be discovered and clinically tested. However, high levels of toxicity and limited therapeutic efficacy instead directed LMB towards an investigatory role in uncovering the biological function of XPO1 in eukaryotic cells.[105] LMB irreversibly binds and modifies the Cysteine 528 (Cys528) residue which is positioned within the NES-binding pocket of XPO1, thereby prohibiting XPO1-cargo interactions and hindering nuclear export.[106] A plethora of natural and synthetic small molecules, which adopt a similar mode of inhibition as LMB, have since been tested for preclinical anticancer activity.[107] In 2012, Karyopharm Therapeutics adopted a novel computation-based molecular modelling strategy to develop the SINE (selective inhibitors of nuclear export) series of highly potent and orally bioavailable small molecules that modify Cys528 in a slowly reversible manner, resulting in transient XPO1 inhibition in normal cells and a significantly improved therapeutic window.[108] A summary of current XPO1 inhibitors is shown in Table 1.

KPT-330 (Selinexor) is the most clinically advanced SINE compound and first nuclear export inhibitor to reach FDA approval for several applications, including combined treatment with dexamethasone in relapsed or refractory multiple myeloma and diffuse B cell lymphoma patients who have received at least one prior therapy.[109] Selinexor is being investigated in a plethora of clinical trials involving both solid tumours and haematological malignancies as a single agent or in combination with other anticancer agents.[110] Early clinical studies involving Selinexor as a single agent have demonstrated promising response rates in patients with liposarcoma, endometrial carcinoma, and glioma.[111] However, the prospect of improving therapeutic efficacy, especially in solid tumours, and avoiding future instances of chemoresistance has warranted the combinatorial testing of Selinexor with both standard of care backbone therapies and targeted chemotherapeutic drugs in order to realize the full benefit of this first-in-class inhibitor in a clinical setting.[112] Importantly, while the broad spectrum potentiating effects of Selinexor with other anti-cancer agents is promising, resistance to SINE compounds and dose-limiting toxicities also exist as major limitations in the clinic.

8 | XPO1 INHIBITORS IN COMBINATION REGIMENS

Apart from its obvious use in combination with inhibitors of independent upregulated or mutated gene targets, such

TABLE 1 Inhibitors of XPO1-dependent nuclear export.

Inhibitor	Preclinical/clinical anticancer activity	Compound type
Leptomycin B (irreversibly binds XPO1 Cys528)	Phase I clinical trial in advanced solid tumours (terminated early due to dose-limiting toxicities)[105]	Natural compound (antibiotic, antifungal agent)
KPT-330 (Selinexor) (reversibly binds XPO1 Cys528)	>100 ongoing phase I/II/III clinical trials (monotherapy or combination therapy) in solid and haematological malignancies[110] FDA-approved for the following applications[219]: • Combination treatment with dexamethasone in relapsed/refractory multiple myeloma (RRMM) patients who have received at least one prior therapy • Combination treatment with dexamethasone and bortezomib in RRMM patients who have received at least one prior therapy • As a single agent in relapsed or refractory diffuse B cell lymphoma patients	Small molecule
KPT-8602 (Eltanexor) (reversibly binds XPO1 Cys528)	Ongoing phase I/II clinical trials (monotherapy or combination therapy) for patients with relapsed or refractory multiple myeloma, metastatic colorectal cancer, metastatic castration resistant prostate cancer and high-risk myelodysplastic syndrome[220]	Second generation SINE (poor blood brain barrier penetrability; predicted improvement in tolerability profile)[162]
CBS9106 (Felezonexor) (reversibly binds XPO1 Cys528; targets XPO1 for proteasomal degradation[221])	Ongoing phase I trial (single agent) for patients with advanced solid tumours[222]	Small molecule

as the PI3K/mTOR inhibitor GSK2126458 (omipalisib), the main reason for its success in pre-clinical co-treatment protocols is its ability to overcome drug resistance mechanisms.[113] Resistance to mainstay anti-cancer agents often arises as a result of: (i) the activation of alternate growth or survival pathways; (ii) the mis-localization of drug targets; and (iii) in the context of DNA-damage agents (DDAs), the upregulation of DNA repair enzymes and mending of drug-induced lesions. XPO1 inhibition has been shown to overcome these various resistance mechanisms in both preclinical and clinical settings. Of course, XPO1 inhibitors are not agnostic to the development of drug resistance, and current literature is focusing on delineating these mechanisms to rationalise the initiation of clinical studies involving XPO1 inhibition as a combination therapy approach.

8.1 | Attenuating growth and survival pathways

Upregulation of anti-apoptotic Bc-l2 family members is one mechanism tumour cells may adopt to evade apoptotic cell death.[114] ABT199 (Venetoclax) is a small molecule inhibitor of Bc-l2 that is active in various haematological malignancies. However, the therapeutic efficacy of Venetoclax is often limited by enhanced expression of alternate Bc-l2 members, such as Mcl-1.[115] Additionally, tumour heterogeneity further limits Venetoclax sensitivity as subsets of malignant cells instead rely on elevated Bcl-XL or Mcl-1 levels for apoptotic tolerance.[116] Selinexor transcriptionally downregulates and inhibits the activity of Mcl-1, thereby synergistically enhancing Venetoclax cytotoxicity in preclinical and patient-derived xenograft models of AML and DLBCL.[117] This combination regimen is being tested in ongoing phase I clinical trials in MM, AML, DLBCL and non-Hodgkin Lymphoma patients.[118] Recently, another mechanism of enhanced Venetoclax activity by XPO1 inhibitor, Eltanexor, a second generation SINE, relates to the nuclear accumulation of Bcl-2. Nuclear Bcl-2 has shown to block DNA repair; this effect was synergistically enhanced by SINE-induced DNA damage and downregulation of DNA repair genes, which will be discussed later on.[38,119] Similarly, Selinexor synergizes with ABT263 (Navitoclax), a dual Bcl-2/Bcl-XL inhibitor, in pre-clinical glioblastoma models. However, this novel treatment strategy is yet to be investigated in a clinical setting.[120]

The ERK MAPK pathway is constitutively active in a broad range of cancer types, including KRAS-mutated

non-small cell lung cancer (NSCLC). Recently, sotorasib was granted FDA-approval for the treatment of NSCLC tumour harbouring the KRAS G12C mutation; however, patients frequently develop resistance to the KRAS inhibitor over time.[121] Co-targeting of XPO1 and KRAS G12C resulted in synergistically enhanced growth impediment in sotorasib resistant and sensitive cell lines and enhanced the survival of murine xenografted mice models when compared to monotherapy. Mechanistically, the observed synergism was explained by Selinexor-mediated nuclear accumulation of pRB and attenuated NFκB signalling in vitro and in vivo.[122] Another related survival pathway in NSCLC, that is often upregulated after chemotherapy treatment and responsible for driving drug resistance, is the PI3K/Akt/mTOR pathway.[123] The authors validated that the attenuation of hyperactive Akt signalling was a core mechanism behind Selinexor-induced sensitization to cisplatin and irinotecan across several independent patient-derived xenograft tumours and cell culture models with diverse genetic backgrounds.[124]

IκB is a tumour suppressive XPO1 cargo that sequesters the classical NFκB complex (p60/p65 heterodimer) in the cytoplasm, thereby blocking its transcriptional activity.[125,126] Signals that activate the NFκB pathway do so via phosphorylation and subsequent proteasomal degradation of IκB, an event which can be blocked by proteosome inhibitors (PIs).[127] Nuclear retention of IκB similarly inhibits NFκB transcriptional activity; however, tumour cells with high basal NFκB activity and diminished accumulation of nuclear IκB following XPO1 inhibition demonstrated reduced sensitivity to Selinexor.[128] Hence, the combination of Selinexor with PIs such as bortezomib, carfilzomib or ixazombin, results in a synergistic cytotoxic effects in sarcoma cell lines refractory to PIs, SINEs or both drug classes. Combination treatment associates with the enrichment of p65 subunits in nuclear fractions of IκB immunoprecipitants and a significant reduction in DNA-binding and transcriptional activity of NFκB.[102] Enhanced cytotoxicity was also observed in vivo with a 15%, 50% and 76% reduction in tumour growth compared to vehicle control when Selinexor-resistant fibrosarcoma murine xenograft tumours were treated with bortezomib, Selinexor and combination therapy respectively.[102] Synergistic drug interactions were similarly observed in multiple myeloma patient samples and cell lines with acquired PI resistance as well as Selinexor resistant diffuse large B-cell lymphoma models.[96,129] The BOSTON clinical trial compared a mainstay multiple myeloma regimen, bortezomib with low dose dexamethasone, in the presence or absence of Selinexor. Results from the trial led to FDA-approval of the combination in relapsed or refractory multiple myeloma patients who have received at least one prior therapy.[130] The addition of Selinexor allowed for a 40% and 25% reduction in bortezomib and dexamethasone dosing respectively while maintaining the desired therapeutic effect. This translated to a notable reduction in the frequency and severity of adverse events, including peripheral neuropathy, a dose-limiting toxicity of bortezomib.

8.2 | Drug target re-localization

The relocalization of drug targets or drug effector proteins is another leading cause of drug resistance. For example, acquired resistance to cytotoxic agents that target Topoisomerase IIα (TopIIα) arises from its nuclear exclusion by overactive XPO1.[131] Concomitant treatment of Selinexor with anthracyclines, such as idarubicin and doxorubicin, overcomes this de novo resistance mechanism in acute myeloid leukaemia and multiple myeloma respectively.[48,131,132] XPO1 inhibition promotes nuclear localization of TopIIα such that it can interact with tumour cell DNA, leading to the generation of double-stranded breaks and apoptotic cell death in the presence of TopIIα inhibitors. In the context of AML, drug potentiation by Selinexor is also attributed to the downregulation of DNA repair enzymes, Chk1 and Rad51, which resulted in impaired homologous repair capabilities and increased double-stranded DNA damage.[131] Combined treatment with Selinexor, dexamethasone, and liposomal doxorubicin was investigated in a Phase I/II clinical trial in relapsed and refractory multiple myeloma patients; unfortunately, the regimen did not improve overall response rates or provide survival benefits when compared so Selinexor and dexamethasone alone. Phase 1b clinical investigation of Selinexor and doxorubicin in advanced sarcoma patients has been completed and results are currently pending (NCT03042819).

Chronic myeloid leukaemia (CML), is characterized by aberrant BCR-ABL signalling which requires its cytoplasmic localization. Interestingly, combinatorial treatment of Selinexor with Imatinib, a BCR-ABL-targeted tyrosine kinase inhibitor, resulted in enhanced cytotoxicity in CML cell lines and imatinib-resistant murine xenograft models.[97] Interestingly, the nuclear localization of BCR-ABL is impaired under normal condition, however treatment with Imatinib stimulates nuclear entry of the oncogenic protein, which has shown to have pro-apoptotic effects.[47] Hence, the chemosensitizing impact of Selinexor combination treatment may in part be explained by sustained nuclear activity of BCR-ABL upon XPO1 inhibition.[133]

8.3 | DNA damage repair responses

Finally, a commonly observed acquired chemoresistance mechanism in response to chemotherapies that mediate their cytotoxicity through DNA damage is the upregulation of DNA damage repair (DDR) pathways, and increased efficiency in the mending of drug-induced lesions.[134] Selinexor downregulates critical DDR genes, thereby sensitizing cancer cell lines and murine xenograft tumours to DNA damage-based chemotherapy.[135,136]

Various classes of DNA-damaging agents (DDAs) induce different types of lesions which rely on distinct repair mechanisms for rectification. Selinexor targets all five major DDR pathways at mRNA and protein levels; this explains the observation that DDA potentiation is apparent across different drug classes.[46,48,131,137,138] For example, Turner et al., 2020 showed synergistic interaction between Selinexor and Melphalan, an alkylating agent.[139] The authors proposed that this was attributed to reduced DNA repair mediated by Selinexor. Additionally, gemcitabine (Gem), a frontline pancreatic cancer drug and nucleoside analogue which generates double-stranded DNA breaks (repaired by the HR pathway), displays enhanced activity in combination with Selinexor.[135] In a pancreatic cancer model system, Gem treatment alone upregulated Chk1, a critical component of HR repair and surveyor of DNA damage. However, in combination with Selinexor, HR components, including Chk1, were attenuated, resulting in increased Gem-induced DNA damage and enhanced levels of apoptotic cell death in vitro and in vivo.[137] It was also recently shown that the synergistic anti-leukaemic interaction between Bcl-2 inhibitor Venetoclax and Selinexor, discussed earlier, is not only mediated by Mcl-1, but also due to inhibition of DNA repair, as the authors showed downregulation of various DNA repair proteins including c-Myc, Chk1, Wee1, Rad51 and RRM2.[38] Interestingly, Selinexor treatment in combination with AZD-6738, an inhibitor of the ATM/ATR-Chk1/2 axis itself, results in enhanced antitumour effects in p53-mutant models of colorectal cancer.[140]

Importantly, pairing the appropriate DDA with tumours of a specific molecular background, such as BRCA-deficient triple negative breast cancers (TNBC) with PARP inhibitors (PARPi), results in cytotoxic effects by means of synthetic lethality. Preclinical data suggested that TNBC cell lines were responsive to PARP inhibition irrespective of BRCA status.[141] However, it is unclear whether this finding translates in a clinical setting. In order to expand PARPi-targeted therapy beyond BRCA-deficient tumours, researchers are exploring combination therapies involving PARPi with other targeted therapies that disrupt overlapping DNA repair pathways. Marijon et al., 2021 showed that Selinexor interacts synergistically with Olaparib in TNBC cell lines and murine xenograft models with wildtype or mutated BRCA1.[142] Currently, Selinexor and second generation PARP inhibitor, Talazorib, are being investigated in a phase I/II clinical trial as a combination therapy for TNBC patients independent of BRCA status.[143] It is important to note that inhibition of DDR is not the only mechanism behind such drug synergy. Nuclear retention of tumour suppressor proteins, such as FOXO3a, and depletion of oncogenic survivin play comparable roles in the potentiation of Selinexor in DNA-damage based chemotherapy. Several ongoing phase I/II clinical trials are investigating the combined effects of Selinexor with various DDAs in both solid tumours and haematopoietic malignancies.[110]

Given that c-MYC is known to regulate various DDR components, including Rad51, Chk1, BRCA1/2 and, it Is likely that the broad spectrum sensitizing effects of Selinexor with genotoxic chemotherapy are in part due to the Selinexor-induced downregulation of c-MYC at the mRNA and protein level.[131,139,144–146] Interestingly, HDAC inhibitor, azacitidine, has also shown to reduce c-MYC mRNA expression in AML cell lines; combined treatment with Selinexor resulted in synergistically attenuated c-MYC expression levels and enhanced anti-leukaemic effects that were partially dependent on the elF4E/c-MYC/XPO1 axis.[147]

9 | SINE RESISTANCE

Nuclear export inhibitor are not impervious to the conventional limitations of targeted therapies; hence, to improve their overall clinical benefit, biomarkers that confer resistance to Selinexor specifically are being actively investigated.

Upon generating a SINE resistant fibrosarcoma cell line, Crochiere et al. illustrated that compromised drug-target interaction, increased drug efflux, and mutational changes in XPO1 were not mediators of SINE compound resistance.[128] Rather, an impaired nuclear accumulation of TSPs has shown to be a prominent feature in resistant cell models.[96,128,148] Additionally, the transcriptional response elicited by drug treatment was similar across resistant and sensitive cell lines, however the magnitude of the expressional changes were attenuated in the resistor phenotype. Microarray analysis revealed that alterations in several key pathways downstream of XPO1 inhibition relating to apoptosis, inflammation, and cell adhesion, collectively associate with SINE resistance.[128] As such, targeted modulation of nodes within these pathways represents a plausible resolution for tumour cell resensitization.

In searching for intrinsic resistance mechanisms, Emdal et al. conducted an extensive phosphoproteomics study that sought to uncover differential dependencies of AML ex vivo blasts and cell lines on signalling cascades that may influence Selinexor sensitivity. Results indicated that cytoplasmic FOXO3a sequestration due to hyperactive PI3K/Akt/mTOR signalling was a prominent resistance marker.[148] Combined treatment with Akt inhibitor, MK-2206, enhanced Selinexor efficacy in ex vivo non-responder blasts as well as resistant AML cell lines.[148] Interestingly, Lin et al. corroborate this finding and show that enhanced Akt activity was due to Selinexor-mediated upregulation of the purinergic receptor, P2RY2.[149] Akt inhibitor, ipatasetib, potentiates the anti-leuekmic activity of Selinexor in syngeneic murine xenografts and AML cell lines.[149]

The second flagged phosphorylation event identified by Emdal et al. implicated p53 functionality as a critical mediator of Selinexor sensitivity. Phosphorylation events that are known to enhance p53 activity, such as phospho-S315 in p53 or phospho-S116 in MDM2, were enriched in responder patient samples and sensitive cell lines.[148] Furthermore, combined treatment with MDM2 inhibitor, nutlin-2, resulted in synergistic anti-leukaemic effects in responsive blasts and cell lines.[148] Similarly, MDM2 inhibition enhanced Selinexor cytotoxicity in both neuroblastoma and ovarian cancer cell models and upregulated expression of p53 by HDAC inhibitor, tuconidostat, improved responsiveness of TNBC cell lines to Selinexor in a p53-dependent manner.[150–152] Conflicting evidence implicating p53 as a predictor of Selinexor sensitivity suggests that differential in vitro and in vivo responsiveness is likely dependent on cancer subtype. For example, in DLBCL, AML, dedifferentiated liposarcoma, cholangiocarcinoma, and gastric cancer, p53 status has been associated with more pronounced Selinexor cytotoxicity.[148,153–157] Whereas, in other tumours, such as NSCLC, ovarian, and mesothelioma, Selinexor is active independent of p53 mutational status.[98,158,159] Importantly, restored Selinexor sensitivity can be achieved in p53-defective tumours; for example, combined treatment with pan-RAF inhibitor, TAK-580, elevated FOXO3a levels in the nucleus while accumulating phospho-FOX3a in the cytoplasm.[155] As a result, cytoplasmic Bim expression synergistically enhanced the levels of apoptotic cell death in MM cell models and relapsed and refractory MM patient samples that all harboured p53 deficiencies.[155] Additionally, an analysis of 30 DBCLC cell lines revealed that Selinexor's mean IC50 value (concentration at which 50% cell kill is achieved) was significantly higher in heterozygous mutant p53 cell lines relative to wildtype.[154] However, combined treatment with c-MYC inhibitor, BET (INCB057643) overcame Selinexor resistance; this effect was only observed in cell lines with the mutant p53 HGBCL-DH phenotype which is characterized by high Bcl-2 and c-MYC expression.[154]

Nuclear export inhibition has emerged as a promising novel drug class, with Selinexor already shifting the treatment paradigm for advanced haematological malignancies.[160,161] However, there is still an unmet clinical need for rational drug combinations that can improve patient response rates and lessen the frequency of severe adverse events associated with Selinexor treatment. Alternatively, new generation SINE compounds with more favourable therapeutic indices will be beneficial. Eltanexor is undergoing phase I clinical investigation as a single agent in several advanced solid tumours and haematological malignancies after demonstrating reduced blood brain barrier penetrability and in vivo toxicity.[162,163] Improved therapeutic efficacy may also be achieved through a precision oncology approach. According to ClinicalTrials.gov, >100 Selinexor-based trials are underway or have been completed; future patient stratification relies on the systematic analysis of retrospective trial data and the identification of predictive molecular signatures that might accurately select for single- and combination treatment responsive tumours. Researchers are already identifying such biomarkers; recently, Restrepo et al. uncovered and validated a three-gene signature that predicts the depth and duration of multiple myeloma patient responses to Selinexor-based therapy.[164] Interestingly, analysis of clinical trial data from the STORM study identified heightened E2F1 expression and its impaired nuclear sequestration as a biomarker or Selinexor resistance.[165] Investigation into other biomarkers, such as p53 functionality, and further validation of these signatures in solid tumours and haematopoietic malignancies is merited.

10 | THERAPEUTIC TARGETING OF KPNB1

The pre-clinical and clinical success of targeting XPO1, as well as the broad-spectrum overexpression of KPNB1 in cancer, prompted the development of inhibitors against nuclear import. Although in its infancy, therapeutic targeting of KPNB1 shows great promise, with several small molecule inhibitors already demonstrating preclinical anticancer activity.[166]

KPNB1 overexpression is observed in progressive disease and validates the concept of tumour cell 'addiction', whereby cancer cells display an enhanced reliance on the nuclear receptor relative to normal counterparts.[59] Specific inhibition of KPNB1 has the potential to globally disrupt oncogenic nuclear import as both classical and

TABLE 2 Inhibitors of KPNB1/KPNA- and KPNB1-dependent nuclear import.

Inhibitor	Target	Preclinical/clinical anticancer activity	Compound type
Karyostatin 1A[168]	Disrupts RanGTP binding to KPNB1	Blocked nuclear entry of NFAT cancer cell lines	Small molecule
Importazole[169]	Disrupts RanGTP binding to KPNB1	Blocked nuclear entry of NFKB p65 and c-MYC in cancer cell lines Antiproliferative and pro-apoptotic effects in multiple myeloma, chronic myeloid leukaemia, prostate, melanoma, ovarian, glioblastoma, and breast cancer cell lines Slowed tumour growth in mouse xenograft models of melanoma, prostate cancer, and ovarian cancer[87,92–95,195]	Small molecule
Ivermectin[170]	Binds IMPA and inhibits KPNB1-KPNA-mediated import	KPNB1-dependent anti-proliferative and pro-apoptotic effects in epithelial ovarian carcinoma and chronic myeloid leukaemia cell lines[95,223] Blocked nuclear import of HIF-1a, thereby downregulating hypoxia-induced tumorigenic transcriptional responses[224]	Natural compound (antibiotic, antiparasitic agent)
2-Aminothiazole derivative compound 1	Potently binds KPNB1 (K_d: ~20 nM); inhibits classical and non-classical import pathways[172]	Blocks nuclear entry of Erb2, EGFR and STAT3 in several cancer cell lines Anti-proliferative (G2/M arrest) and pro-apoptotic effects in cancer cell lines[171]	Small molecule
2-Aminothiazole derivative compound 6		Blocked tumour growth in murine xenograft model of pancreatic cancer[173]	Small molecule
INI-43[174]	Designed to target overlapping RanGTP and IMPA2 binding site of KPNB1	Blocked nuclear entry of AP-1, NFAT and NFKB in cancer cell lines Anti-proliferative (G2/M arrest) and pro-apoptotic effects in breast, cervical and oesophageal cancer cell lines; inhibits motility and invasive potential of cervical cancer cells[5] Slowed tumour growth in cervical and oesophageal murine xenograft models	Small molecule
INI-60[175]	Designed to target the overlapping RanGTP and KPNA2 binding site of KPNB1	Anti-proliferative (G1/S arrest) and pro-apoptotic effects in cervical and oesophageal cancer cell lines. Slowed tumour growth in oesophageal murine xenograft model	Small molecule

nonclassical pathways may be targeted. Furthermore, nuclear impediment can lead to the inhibition of lineage-defining transcription factors in small cell lung cancer, highlighting its potential as an anti-cancer approach.[167]

Hintersteiner et al., 2010 described the first inhibitor of KPNB1-mediated import, Karyostatin 1A, which is thought to disrupt interactions between RanGTP and KPNB1, thereby prohibiting KPNB1/KPNA/cargo dissociation in the nucleus and blocking classical nuclear import.[168] A FRET-based high throughput screen for compounds that similarly interrupt RanGTP binding to KPNB1 identified Importazole, a small molecule with antiproliferative and pro-apoptotic activity in chronic myeloid leukaemia, multiple myeloma, prostate, and breast cancer cell lines, but not normal counterparts.[87,89,93,169] Mechanistically, this was explained by the nuclear exclusion of nuclear factor-κB (NFκB) and c-MYC, consequent of KPNB1 inhibition. Additionally, intravenous administration of Importazole significantly slowed the growth of xenografted tumours in a murine prostate cancer model.[87] Ivermectin is an FDA-approved antiparasitic, but also displays anti-tumour properties with broad mechanisms of action that appear to be cancer-type dependent.[170] Ivermectin blocked proliferation and induced apoptosis in epithelial ovarian cancer cell lines; the anti-cancer effects were KPNB-1 dependent and associated with enhanced expression of p27, p21 and pro-apoptotic Bax.[95] Compound 1 is a 2-aminothiazole derivative that was discovered in a phenotypic screen for small molecules with potent anticancer activity; a proteomics-based target deconvolution study revealed compound 1 as an inhibitor of KPNB1.[95] Although demonstrating anticancer effects in vitro, the chemotherapeutic efficacy of compound 1 was negligible in vivo and prompted lead optimization studies, from which compound 6 was developed and shown to notably block prostate tumour growth in murine xenograft models.[171,172] An in silico, structure-based screen identified a series of small molecule inhibitors that target the overlapping RanGTP/KPNA2-binding site of KPNB1.[173] INI-43 most potently inhibited nuclear entry and transcriptional activity of NFAT, AP-1 and NFκB, all representative cargoes of KPNB1/KPNA-mediated import. INI-43 induced G2/M cell cycle arrest and apoptotic death in breast, cervical and oesophageal cancer cell lines; importantly, these effects were absent in normal counterparts treated with the same dose as that for the cancer cell lines and counteracted by ectopic KPNB1.[174] In vivo antitumour activity of INI-43 was confirmed in murine xenograft models for oesophageal and cervical cancer, as well as in specific small cell lung cancer PDX models; hence this small molecule shows therapeutic potential as a novel inhibitor of nuclear import.[167] Interestingly, INI-43 targets other aspects of cancer cell biology; the migratory and invasive capacity of cervical cancer cell lines is impeded by INI-43 treatment, which can be explained by downregulation of NFκB and AP-1 target genes.[5] INI-60 was identified in the same virtual screen and demonstrated similar anti-cancer activity in cervical and oesophageal cell lines, however oesophageal and not cervical xenograft tumours were responsive to INI-60 treatment.[175] A summary of current KPNB1 inhibitors is shown in Table 2.

11 | KPNB1 INHIBITORS IN COMBINATION REGIMENS

While the development of nuclear import inhibitors is in its infancy and investigations into their anticancer effects are limited to preclinical models, preliminary evidence suggests that the efficacy of available chemotherapeutic agents can be augmented when concomitantly targeting relevant nuclear import pathways.

INI-43 pre-treatment sensitized HPV-positive cervical cancer cell lines to cisplatin through the stabilization of functional p53 and attenuation of NFκB signalling via its cytoplasmic retention.[101] p53 stabilization led to the upregulation of cell cycle inhibitor, p21, and downregulation of potent anti-apoptotic factor, Mcl-1. NFκB is a key mediator of cisplatin resistance; subsequent to cisplatin treatment, several transcriptional targets, including Cyclin D1 and c-MYC, are upregulated to participate in DNA repair and survival responses respectively. XIAP directly inhibits caspase-3 and -7, thus prohibiting apoptotic induction and promoting tumour cell survival.[99,176–179] INI-43 pre-treatment reduced the expression of the aforementioned NFκB targets, thereby enhancing platinum-induced DNA damage and shifting the balance towards a pro-apoptotic cellular state, resulting in synergistic apoptotic tumour cell death.[101]

Additionally, upregulation of the PI3K/AKT/mTOR pathway promotes NSCLC cell survival in response to cisplatin challenge; siRNA knockdown of KPNB1 sensitized NSCLC cell lines to cisplatin treatment, partially via downregulation of PI3K/AKT signalling.[180,181] Given that KPNB1 was shown to interact directly with PI3K, the authors speculate that a positive feedback mechanism constitutively stimulates the PI3K/AKT/E2F1/KPNB1 axis to promote NSLC proliferation and resistance to chemotherapy. However, further investigation into this mechanism and other potential contributors is warranted.

Imatinib is a frontline targeted therapy tailored for the treatment of chronic myeloid leukaemia, however responses to the tyrosine kinase inhibitor are highly variable and often short-lived.[182] KPNB1 and KPNA2 are

overexpressed in CML and contribute towards malignant progression; Importazole (IPZ) treatment lowered expression levels of c-MYC, NFκB, E2F1 and BCR-ABL, resulting in G2/M arrest and enhanced induction of apoptosis in CML cell lines.[93] IPZ sensitized resistant CML cells to imatinib, resulting in significant anti-proliferative and pro-apoptotic effects. The mechanisms behind the observed sensitization were not fully explored, however, they are likely related to the inhibitory effects of IPZ on BCR-ABL expression. Interestingly, the imatinib resistant cell line responded more favourably to both KPNB1 inhibition and combined treatment. A recent study by Rodriquez-Bravo et al., illustrated that heightened expression of nucleoporin, POM121, enhanced the nuclear import efficiency of KPNB1 in prostate cancer cell models.[183] Targeted KPNB1 inhibition with IPZ demonstrated significant anti-cancer effects in vitro and single-agent IPZ or combined treatment with docetaxel or mitoxantrone synergistically blocked tumour growth and improved survival outcomes in patient-derived murine xenograft models.[183] Immunohistological staining revealed that single agent IPZ and combined treatment with standard of care chemotherapies reduced the nuclear accumulation of tumorigenic KPNB1 cargoes, namely the androgen receptor (AR), GATA6, NFκB and c-Myc. Interestingly, POM12 upregulation is evident in other tumour types, including oral squamous cell carcinoma, laryngeal cancer, gastric cancer, and colorectal cancer and heightened expression is predictive of poor prognosis.[184–187] Thus, it is possible that facilitated KPNB1 translocation by POM12 partially contributes to the imatinib resistant phenotype in CML, making the POM121/KPNB1 axis a promising novel therapeutic approach in CML and prostate cancer.[93]

Zhu et al. illustrated a novel mechanism by which KPNB1 inhibition exerts its anti-tumour effects.[94] In glioblastoma multiform models (GBM), shRNA knockdown of KPNB1 and treatment with Importazole (IPZ) results in the accumulation KPNB1 cargoes and subsequent cytosolic protein overload which in turn triggers the unfolded-protein response (UPR) and several cellular mechanisms to mediate the clearance of abnormally localized proteins.[188] Alternatively, sustained ER stress by 'protein overload' results in apoptotic cell death in GBM cell lines.[94] Resistance against this cell death mechanism involves the activation the of autophagy-lysosomal- and the ubiquitin-proteasomal-degradation pathways.[189,190] Combined treatment of IPZ and proteasome inhibitor, MG132, or lysosome inhibitors, Bafilomycin A1 and chloroquine, results in enhanced apoptosis in GBM models.[94] Another pathway triggered by perturbed proteostasis is the PERK branch, which regulates the balance of anti- and pro-apoptotic factors; sustained activation of this pathway results Mcl-1 inactivation by Puma and Noxa and subsequent apoptotic cell death.[188,191,192] ABT-263 is a dual Bcl-2/Bcl-XL inhibitor that displays strong dose-limiting toxicities in treated GBM patients, mainly due to the reliance of platelet survival on Bcl-XL.[193] However, monotherapy with Bcl-2 inhibition is ineffective due to the development of heterogenous tumour populations that show differential dependencies on Bcl-2 and Bcl-XL for apoptotic evasion.[194] Combined treatment of the dual inhibitor, ABT-263, with IPZ resulted in synergistically enhanced apoptotic effects in GBM cellular models. Hence, this approach permits the lowering of individual drug doses and holds potential as a novel GBM treatment regimen given the synthetic lethal effects of UPR-mediated Mc-l1 inactivation by KPNB1 inhibition and dual targeting of Bcl-2/Bcl-XL with ABT-263.

KPNB1 overexpression is closely linked with disease progression and worsened prognostic outcomes in melanoma patients.[195] Cellular melanoma models display reduced tumorgenicity and metastatic potential following KPNB1 knockdown, whereas synthetic overexpression amplifies the carcinogenic function of KPNB1 and enhances the aggressive melanoma phenotype.[195] Ras-GTPase-activating protein SH3-binding protein 1 (G3BP1) is a multifunctional RNA-binding protein that is involved in stress granule formation.[196] G3BP1 is upregulated in various cancers, including melanoma, and is known to contribute towards disease progression and chemoresistance.[197] Interestingly, KPNB1 was shown to interact with G3BP1 and stabilize its protein levels via reduced ubiquitination; however, genetic knockdown of KPNB1 had opposing effects. Furthermore, genetic inhibition of G3BP1 demonstrates anti-melanoma effects in vitro and negates the survival advantage incurred by KPNB1 overexpression.[195] It is possible that the nuclear exclusion of cargoes following KPNB1 genetic inhibition initiates the ubiquitination and subsequent degradation of various cytoplasmic proteins, including G3BP1, to retain a proteostatic balance; in melanoma this gives rise to anti-cancer activity as well as enhanced cell line and murine xenograft sensitivity to cisplatin.[94,195] However, further investigation into the oncogenic functions of both G3BP1 and KPNB1 in melanoma is warranted.

12 | OTHER NUCLEAR TRANSPORT RECEPTORS IN CANCER

Over the past decade, almost all members of Karyopherin superfamily have been implicated in cancer

development.[4,25,43] While previous reviews by van der Watt et al. (2016) and Catagay and Chook (2018) cover the carcinogenic roles of the majority of the nuclear receptors, this review will give insights into more recent literature that implicates the remaining receptors in tumour development and will report on instances of enhanced chemosensitivity upon their inhibition.

Karyohperin-ß2, otherwise known as Transportin 1 (TNPO1) predominantly facilitates RNA processing and gene transcription via the nuclear import of various RNA-binding proteins.[198] TNPO1 is overexpressed and associates with poor prognosis in cervical and ovarian cancer and genetic silencing significantly reduced cancer cell line viability in ARID1A-deficient cell lines.[24] ARID1A is a critical component of the SWI/SNF complex and its homologue, ARID1B, represents a vulnerability point for synthetic lethal targeting in ARID1A-deficient cancers.[199] Further investigation uncovered ARID1B as a cargo of TNPO1; hence, genetic knockdown of the transport receptor blocked nuclear entry of ARIDB1 resulting in anti-proliferative and pro-apoptotic effects that were functionally related to reduced chromatin accessibility of AP-1 transcription factors to the promoters of genes involved in PI3K and TGF-ß signalling pathways.[24]

Both Transportin 2 and Importin 4 are highly expressed and predict poor long-term survival in gastric cancer. SiRNA knockdown reduced tumour cell proliferation, colony formation and migration abilities and increased apoptosis in vitro.[26,27]

Karyopherin-α1 (KPNA1) expression is elevated in colon cancer and tracks with disease progression. Colon cancer cell proliferation, migration, colony formation and in vivo xenograft tumour growth was impaired by KPNA1 genetic knockout and enhanced by overexpression of the import protein. Further functional analysis revealed that the observed tumorigenic properties were due to enhanced nuclear import of NF-KB by KPNA1.[200] Interestingly, pro-tumorigenic roles of KPNA1 have also been reported in and glioblastoma.[201] TRIM59 is a ubiquitination ligase that targets tumour suppressive macroH2A1 for proteasomal degradation in the nucleus, leading to increased STAT3 signalling. However, nuclear entry of TRIM59 is mediated by KPNA1 in response to aberrant EGFR activity and genetic knockdown of KPNA1 abrogated the tumorigenic effects of this signalling axis. Conversely, cervical cancer tumours expressing low KPNA1 associates with progressive disease and synthetic upregulation of KPNA1 led to reduced proliferation of HeLa cells due to the nuclear accumulation of IRF3.[202] Further investigation into the tumour suppressive effects of both IRF3 and KPNA1 are warranted.

13 | COMBINATORIAL TARGETING OF OTHER NUCLEAR TRANSPORT RECEPTORS

Although in its infancy, there is an emergence of literature that supports the notion of targeting lesser characterized NTRs as a mechanism of overcoming resistance to standard of care chemotherapies or novel targeted agents. Current studies employ siRNA knockdown techniques as well as the use of mimetic peptides that competitively bind the NLS/NES sites of specific NTRs.

Importin 4 is upregulated in cervical cancer and associates with progressive disease and poor prognosis. However, genetic knockdown of the importin provides little therapeutic benefit as a single agent approach. On the other hand, Cisplatin-induced upregulation of DNA repair protein, PRKDC, is dependent on the nuclear import of transcription factor, CEBPD, by importin 4. Hence, shRNA knockdown of importin 4 led to cytoplasmic retention of CEPBD, downregulation of PRKDC and enhanced sensitivity of SiHa cells to cisplatin in vitro and in vivo.[203]

BQ is a splice variant of NCOR2 that interacts with the transcription factor to form a co-repressor complex in ER-positive breast cancer; high nuclear BQ expression is associated with Tamoxifen resistance partially due to the transcriptional upregulation of HIF1a.[204–206] Recent evidence implicates HIF-1a-mediated metabolic reprogramming of breast cancer tumours as a critical contributor to Tamoxifen resistance.[207,208] Knockout of KPNA1 abrogates the nuclear accumulation of BQ, resulting in reduced HIF1a activity and sensitization of Tamoxifen-resistant xenograft tumours to the standard of care therapy.[205]

The RAF/RAS/MEK/ERK pathway is frequently hyperactive in many cancers, including melanoma, due to constitutive mutations in different components of the cascade.[209] As such, inhibitors targeting various nodes in this pathway have been developed; Vemurafenib (BRAF inhibitor) and Trametinib (MEK1 inhibitor) demonstrate improved overall survival in BRAF V600E mutant melanoma patients.[210,211] However, these patients frequently return with recurrent and refractory disease.[211,212] The onset of acquired chemoresistance and generally poor pan-cancer efficacy of these inhibitors has been linked to the drug-induced inactivation of negative feedback loops that are mediated by cytosolic ERK1/2.[213–215] Abrogating this intrinsic feedback mechanism results in an elevated signalling flux that overwhelms pharmacological BRAF/MEK inhibition during single agent treatment.[212,213] In some cases, tumour cells can develop a reliance on alternative MAPK pathways, such as PI3K/AKT, which then

renders the treatment ineffective.[214] Interestingly, the ERK cascade culminates at the shuttling of ERK1/2 into the nucleus by Importin 7 (IPO7) where it activates proliferative transcription factors; inhibition with the IPO7 NTS-derived phosphomimetic peptide (EPE) blocks the nuclear entry and activity of ERK1/2 and results in potent anti-cancer effects in NRAS, BRAF, and NR1 melanoma cell lines, including those that are resistant to clinically approved BRAF and MEK1 inhibitors.[216–218] Intriguingly, EPE treatment was shown to outperform Vemurafenib in vivo; the phosphomimetic peptide eradicated murine BRAF V006E xenograft tumours, hindered the development of recurrent disease, and demonstrated a more favourable therapeutic index.[216,217] Although resistance to EPE was observed across various mutational backgrounds, combined treatment of EPE with Trametinib results in synergistic anti-proliferative and pro-apoptotic effects in NRAS mutant melanoma cell lines that are both responsive or resistant to each single agent.[217,218] This was in part due to restored nuclear exclusion of ERK1/2 in combination treated cells versus single agents as well as retained cytosolic ERK1/2 activity, evident by increased phosphorylation of cytosolic targets in combination treated cells versus monotherapy.[218] Further investigation into the combined effects of EPE with MEK1 inhibition in ERK-addicted melanoma cell lines harbouring other mutational defects is warranted.

14 | CONCLUSION

Cancer is a global health crisis that calls for novel interventions, including anti-neoplastic agents that disrupt tumour-addictive molecular pathways. Recent studies identified the Karyopherin superfamily of proteins as novel cancer biomarkers, which led to the concept of nuclear transport inhibition as an anticancer approach. However, the clinical benefits of such targeted agents are threatened by the current limitations associated with cancer chemotherapy, namely chemoresistance and dose-limiting toxicities. Therefore, nuclear transport inhibitors should be considered in the context of combination therapies. Furthermore, the notion of utilizing nuclear transport inhibitors to improve the therapeutic efficacy of other cancer agents, namely standard of care chemotherapies or novel targeted drugs, is supported by the existing body of literature, particularly with regards to XPO1 inhibition. Results from ongoing clinical trials investigating Selinexor in combination regimens will shed light on tumour-specific chemoresistance mechanisms associated with SINE compounds and potentially broaden their application to patient tumours of various solid tissue and haematological origin. Of similar importance is the identification of molecular signatures that stratify responsive and non-responsive tumours, thereby improving the clinical benefits of Selinexor, both in combination and as a single agent treatment. On the other hand, the development of potent nuclear import inhibitors is proving more challenging. Importantly, rigorous investigation into potential chemoresistance mechanisms associated with KPNB1 inhibition or instances of synergistic interactions with other anti-cancer agents will be hugely beneficial in promoting existing nuclear import inhibitors towards clinical approval. Overall, a wealth of possibility lies ahead for the targeting of nuclear transport receptors as an anti-neoplastic therapeutic intervention and potent inhibitors against the remaining karyopherin family members will be beneficial in uncovering their biological functions in both healthy cells and various pathologies, including cancer. As normal cells rely on nuclear transport for normal function, which explains the unfavourable toxicity profile of Selinexor in select patients, combination therapies will be a useful in lowering drug doses to a point where normal cells are less affected and adverse events are hopefully mitigated. Disrupting receptors that transport a smaller spectrum of cargoes, relative to XPO1 and KPNB1, or designing inhibitors such that specific cargoes are targeted may also pave the way towards novel anti-cancer regimens with more favourable tolerability and improved clinical outcomes.

FUNDING INFORMATION

This work was supported by grants obtained by VL from the South African Medical Research Council (SAMRC), the National Research Foundation, the Cancer Association of South Africa (CANSA), and the University of Cape Town's Research Committee (URC).

ORCID

Virna D. Leaner https://orcid.org/0000-0002-0417-8610

REFERENCES

1. Kwak EL, Clark JW, Chabner B. Targeted agents: The rules of combination: Table 1. Clin Cancer Res. 2007;13(18):5232–7.
2. Bayat Mokhtari R, Homayouni TS, Baluch N, et al. Combination therapy in combating cancer. Oncotarget. 2017;8(23):38022–43.
3. Sun J, Wei Q, Zhou Y, Wang J, Liu Q, Xu H. A systematic analysis of FDA-approved anticancer drugs. BMC Syst Biol. 2017;11(S5):27–42.
4. Çağatay T, Chook YM. Karyopherins in cancer. Curr Opin Cell Biol. 2018;52:30–42.
5. Stelma T, Leaner VD. KPNB1-mediated nuclear import is required for motility and inflammatory transcription factor activity in cervical cancer cells. Oncotarget. 2017;8(20):32833–47.

6. Keminer O, Peters R. Permeability of single nuclear pores. Biophys J. 1999;77(1):217–28.
7. Hodel M, Corbett A, Hodel A. Dissection of a nuclear localization signal. J Biol Chem. 2001;276:1317–25.
8. Stewart M. Molecular mechanism of the nuclear protein import cycle. Nat Rev Mol Cell Biol. 2007;8(3):195–208.
9. Bischoff FR, Görlich D. RanBP1 is crucial for the release of RanGTP from importin β-related nuclear transport factors. FEBS Lett. 1997;419(2):249–54.
10. Kim YH, Han M-E, Oh S-O. The molecular mechanism for nuclear transport and its application. Anat Cell Biol. 2017; 50(2):77–85.
11. Ciciarello M, Roscioli E, Di Fiore B, et al. Nuclear reformation after mitosis requires downregulation of the Ran GTPase effector RanBP1 in mammalian cells. Chromosoma. 2010; 119(6):651–68.
12. Ciciarello M, Mangiacasale R, Thibier C, et al. Importin β is transported to spindle poles during mitosis and regulates Ran-dependent spindle assembly factors in mammalian cells. J Cell Sci. 2004;117(26):6511–22.
13. Roscioli E, Di Francesco L, Bolognesi A, et al. Importin-β negatively regulates multiple aspects of mitosis including RANGAP1 recruitment to kinetochores. J Cell Biol. 2012;196(4): 435–50.
14. Kalab P, Heald R. The RanGTP gradient—a GPS for the mitotic spindle. J Cell Sci. 2008;121(10):1577–86.
15. Harel A, Forbes DJ. Importin beta: Conducting a much larger cellular symphony. Mol Cell. 2004;16(3):319–30.
16. Forbes DJ, Travesa A, Nord MS, Bernis C. Nuclear transport factors: Global regulation of mitosis. Curr Opin Cell Biol. 2015;35:78–90.
17. Bernis C, Swift-Taylor B, Nord M, Carmona S, Chook YM, Forbes DJ. Transportin acts to regulate mitotic assembly events by target binding rather than Ran sequestration. Mol Biol Cell. 2014;25(7):992–1009.
18. Knauer SK, Bier C, Habtemichael N, Stauber RH. The Survivin–Crm1 interaction is essential for chromosomal passenger complex localization and function. EMBO Rep. 2006; 7(12):1259–65.
19. Vagnarelli P, Earnshaw WC. Chromosomal passengers: The four-dimensional regulation of mitotic events. Chromosoma. 2004;113(5):211–22.
20. Liu Q, Jiang Q, Zhang C. A fraction of Crm1 locates at centrosomes by its CRIME domain and regulates the centrosomal localization of pericentrin. Biochem Biophys Res Commun. 2009;384(3):383–8.
21. Wu Z, Jiang Q, Clarke PR, Zhang C. Phosphorylation of Crm1 by CDK1–cyclin-B promotes Ran-dependent mitotic spindle assembly. J Cell Sci. 2013;126(15):3417–28.
22. Gilistro E, de Turris V, Damizia M, et al. Importin-β and CRM1 control a RANBP2 spatiotemporal switch essential for mitotic kinetochore function. J Cell Sci. 2017;130(15):2564–78.
23. Nord MS, Bernis C, Carmona S, Garland DC, Travesa A, Forbes DJ. Exportins can inhibit major mitotic assembly events in vitro: Membrane fusion, nuclear pore formation, and spindle assembly. Nucleus. 2020;11(1):178–93.
24. Yang B, Chen J, Li X, et al. TNPO1-mediated nuclear import of ARID1B promotes tumor growth in ARID1A-deficient gynecologic cancer. Cancer Lett. 2021;515:14–27.
25. Yu T, Ran L, Zhao H, et al. Circular RNA circ-TNPO3 suppresses metastasis of GC by acting as a protein decoy for IGF2BP3 to regulate the expression of MYC and SNAIL. Mol Ther Nucl Acids. 2021;26:649–64.
26. Gong L, Wen T, Li Z, et al. TNPO2 operates downstream of DYNC1I1 and promotes gastric cancer cell proliferation and inhibits apoptosis. Cancer Med. 2019;8(17):7299–312.
27. Xu X, Zhang X, Xing H, et al. Importin-4 functions as a driving force in human primary gastric cancer. J Cell Biochem. 2019;120(8):12638–46.
28. Chang Z, Miao X, Zhao W. Identification of prognostic dosage-sensitive genes in colorectal cancer based on multi-omics. Front Genet. 2020;10:1310.
29. Kuusisto HV, Wagstaff KM, Alvisi G, Roth DM, Jans DA. Global enhancement of nuclear localization-dependent nuclear transport in transformed cells. Faseb J. 2012;26(3): 1181–93.
30. Chen M, Nowak DG, Narula N, et al. The nuclear transport receptor Importin-11 is a tumor suppressor that maintains PTEN protein. J Cell Biol. 2017;216(3):641–56.
31. Zhao J, Shi L, Zeng S, et al. Importin-11 overexpression promotes the migration, invasion, and progression of bladder cancer associated with the deregulation of CDKN1A and THBS1. Urologic oncology: Seminars and original investigations: 2018. Elsevier, United States. 2018; p. 311.e311–311.e313.
32. Lorenzato A, Martino C, Dani N, et al. The cellular apoptosis susceptibility CAS/CSE1L gene protects ovarian cancer cells from death by suppressing RASSF1C. Faseb J. 2012;26(6): 2446–56.
33. Angus L, van der Watt PJ, Leaner VD. Inhibition of the nuclear transporter, Kpnβ1, results in prolonged mitotic arrest and activation of the intrinsic apoptotic pathway in cervical cancer cells. Carcinogenesis. 2014;35(5):1121–31.
34. Verrico A, Rovella P, Di Francesco L, et al. Importin-β/karyopherin-β1 modulates mitotic microtubule function and taxane sensitivity in cancer cells via its nucleoporin-binding region. Oncogene. 2020;39(2):454–68.
35. Baumhardt JM, Walker JS, Lee Y, et al. Recognition of nuclear export signals by CRM1 carrying the oncogenic E571K mutation. Mol Biol Cell. 2020;31(17):1879–91.
36. Taylor J, Sendino M, Gorelick AN, et al. Altered nuclear export signal recognition as a driver of oncogenesis altered nuclear export signal recognition drives oncogenesis. Cancer Discov. 2019;9(10):1452–67.
37. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell. 2011;144(5):646–74.
38. Yu H, Wu S, Liu S, et al. Venetoclax enhances DNA damage induced by XPO1 inhibitors: A novel mechanism underlying the synergistic antileukaemic effect in acute myeloid leukaemia. J Cell Mol Med. 2022;26(9):2646–57.
39. Murugaesu N, Wilson GA, Birkbak NJ, et al. Tracking the genomic evolution of esophageal adenocarcinoma through neoadjuvant chemotherapytracking the genomic evolution of esophageal adenocarcinoma. Cancer Discov. 2015;5(8): 821–31.
40. Achimas-Cadariu P, Kubelac P, Irimie A, Berindan-Neagoe I, Rühli F. Evolutionary perspectives, heterogeneity and ovarian cancer: A complicated tale from past to present. J Ovarian Res. 2022;15(1):67.

41. Kırlı K, Karaca S, Dehne HJ, et al. A deep proteomics perspective on CRM1-mediated nuclear export and nucleocytoplasmic partitioning. Elife. 2015;4:e11466.
42. Lee Y, Baumhardt JM, Pei J, Chook YM, Grishin NV. pCRM1exportome: Database of predicted CRM1-dependent nuclear export signal (NES) motifs in cancer-related genes. Bioinformatics. 2020;36(3):961–3.
43. Stelma T, Chi A, van der Watt PJ, Verrico A, Lavia P, Leaner VD. Targeting nuclear transporters in cancer: Diagnostic, prognostic and therapeutic potential. IUBMB Life. 2016;68(4):268–80.
44. Cai X, Liu X. Inhibition of Thr-55 phosphorylation restores p53 nuclear localization and sensitizes cancer cells to DNA damage. Proc Natl Acad Sci U S A. 2008;105(44):16958–63.
45. Nair JS, Musi E, Schwartz GK. Selinexor (KPT-330) induces tumor suppression through nuclear sequestration of IκB and downregulation of SurvivinSelinexor induces sarcoma tumor suppression through Survivin. Clin Cancer Res. 2017;23(15): 4301–11.
46. Wang J, Sun T, Meng Z, et al. XPO1 inhibition synergizes with PARP1 inhibition in small cell lung cancer by targeting nuclear transport of FOXO3a. Cancer Lett. 2021;503:197–212.
47. Aloisi A, Di Gregorio S, Stagno F, et al. BCR-ABL nuclear entrapment kills human CML cells: Ex vivo study on 35 patients with the combination of imatinib mesylate and leptomycin B. Blood. 2006;107(4):1591–8.
48. Turner JG, Marchion DC, Dawson JL, et al. Human multiple myeloma cells are sensitized to topoisomerase II inhibitors by CRM1 inhibition. Cancer Res. 2009;69(17):6899–905.
49. Miloudi H, Leroy K, Jardin F, Sola B. STAT6 is a cargo of exportin 1: Biological relevance in primary mediastinal B-cell lymphoma. Cell Signal. 2018;46:76–82.
50. Gaubatz S, Lees JA, Lindeman GJ, Livingston DM. E2F4 is exported from the nucleus in a CRM1-dependent manner. Mol Cell Biol. 2001;21(4):1384–92.
51. Martinez I, Hayes KE, Barr JA, et al. An Exportin-1-dependent microRNA biogenesis pathway during human cell quiescence. Proc Natl Acad Sci. 2017;114(25):E4961–70.
52. Thomas F, Kutay U. Biogenesis and nuclear export of ribosomal subunits in higher eukaryotes depend on the CRM1 export pathway. J Cell Sci. 2003;116(12):2409–19.
53. Wu J, Bao A, Chatterjee K, Wan Y, Hopper AK. Genome-wide screen uncovers novel pathways for tRNA processing and nuclear–cytoplasmic dynamics. Genes Dev. 2015;29(24): 2633–44.
54. Volpon L, Culjkovic-Kraljacic B, Sohn HS, Blanchet-Cohen A, Osborne MJ, Borden KL. A biochemical framework for eIF4E-dependent mRNA export and nuclear recycling of the export machinery. RNA. 2017;23(6):927–37.
55. Culjkovic B, Topisirovic I, Skrabanek L, Ruiz-Gutierrez M, Borden KL. eIF4E is a central node of an RNA regulon that governs cellular proliferation. J Cell Biol. 2006;175(3):415–26.
56. Siddiqui N, Borden KL. mRNA export and cancer. Wiley Interdiscip Rev RNA. 2012;3(1):13–25.
57. Tabe Y, Kojima K, Yamamoto S, et al. Ribosomal biogenesis and translational flux inhibition by the selective inhibitor of nuclear export (SINE) XPO1 antagonist KPT-185. PloS One. 2015;10(9):e0137210.
58. Hill R, Cautain B, De Pedro N, Link W. Targeting nucleocytoplasmic transport in cancer therapy. Oncotarget. 2014;5(1):11.
59. Van Der Watt PJ, Maske CP, Hendricks DT, et al. The Karyopherin proteins, Crm1 and Karyopherin β1, are overexpressed in cervical cancer and are critical for cancer cell survival and proliferation. Int J Cancer. 2009;124(8):1829–40.
60. Noske A, Weichert W, Niesporek S, et al. Expression of the nuclear export protein chromosomal region maintenance/exportin 1/Xpo1 is a prognostic factor in human ovarian cancer. Cancer. 2008;112(8):1733–43.
61. Inoue H, Kauffman M, Shacham S, et al. CRM1 blockade by selective inhibitors of nuclear export attenuates kidney cancer growth. J Urol. 2013;189(6):2317–26.
62. Gao W, Lu C, Chen L, Keohavong P. Overexpression of CRM1: A characteristic feature in a transformed phenotype of lung carcinogenesis and a molecular target for lung cancer adjuvant therapy. J Thorac Oncol. 2015;10(5):815–25.
63. Zhou F, Qiu W, Yao R, et al. CRM1 is a novel independent prognostic factor for the poor prognosis of gastric carcinomas. Med Oncol. 2013;30(4):1–7.
64. Shen A, Wang Y, Zhao Y, Zou L, Sun L, Cheng C. Expression of CRM1 in human gliomas and its significance in p27 expression and clinical prognosis. Neurosurgery. 2009;65(1):153–60.
65. Van der Watt PJ, Zemanay W, Govender D, Hendricks DT, Parker M, Leaner VD. Elevated expression of the nuclear export protein, Crm1 (exportin 1), associates with human oesophageal squamous cell carcinoma. Oncol Rep. 2014;32(2): 730–8.
66. Zheng Y, Gery S, Sun H, Shacham S, Kauffman M, Koeffler HP. KPT-330 inhibitor of XPO1-mediated nuclear export has anti-proliferative activity in hepatocellular carcinoma. Cancer Chemother Pharmacol. 2014;74(3):487–95.
67. Schmidt J, Braggio E, Kortuem K, et al. Genome-wide studies in multiple myeloma identify XPO1/CRM1 as a critical target validated using the selective nuclear export inhibitor KPT-276. Leukemia. 2013;27(12):2357–65.
68. Kojima K, Kornblau SM, Ruvolo V, et al. Prognostic impact and targeting of CRM1 in acute myeloid leukemia. Blood. 2013;121(20):4166–74.
69. Yoshimura M, Ishizawa J, Ruvolo V, et al. Induction of p53-mediated transcription and apoptosis by exportin-1 (XPO 1) inhibition in mantle cell lymphoma. Cancer Sci. 2014; 105(7):795–801.
70. van der Watt PJ, Leaner VD. The nuclear exporter, Crm1, is regulated by NFY and Sp1 in cancer cells and repressed by p53 in response to DNA damage. Biochim Biophys Acta Gene Regul Mech. 2011;1809(7):316–26.
71. Golomb L, Bublik DR, Wilder S, et al. Importin 7 and exportin 1 link c-Myc and p53 to regulation of ribosomal biogenesis. Mol Cell. 2012;45(2):222–32.
72. Zhao L, Luo B, Wang L, Chen W, Jiang M, Zhang N. Pan-cancer analysis reveals the roles of XPO1 in predicting prognosis and tumorigenesis. Transl Cancer Res. 2021;10(11): 4664–79.
73. Cosson A, Chapiro E, Bougacha N, et al. Gain in the short arm of chromosome 2 (2p+) induces gene overexpression and drug resistance in chronic lymphocytic leukemia: Analysis of the central role of XPO1. Leukemia. 2017;31(7):1625–9.

74. Jardin F, Pujals A, Pelletier L, et al. Recurrent mutations of the exportin 1 gene (XPO1) and their impact on selective inhibitor of nuclear export compounds sensitivity in primary mediastinal B-cell lymphoma. Am J Hematol. 2016;91(9): 923–30.
75. Puente XS, Pinyol M, Quesada V, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature. 2011;475(7354):101–5.
76. Camus V, Stamatoullas A, Mareschal S, et al. Detection and prognostic value of recurrent exportin 1 mutations in tumor and cell-free circulating DNA of patients with classical Hodgkin lymphoma. Haematologica. 2016;101(9):1094.
77. Lin D-C, Hao J-J, Nagata Y, et al. Genomic and molecular characterization of esophageal squamous cell carcinoma. Nat Genet. 2014;46(5):467–73.
78. Cimica V, Chen H-C, Iyer JK, Reich NC. Dynamics of the STAT3 transcription factor: Nuclear import dependent on Ran and importin-β1. PloS One. 2011;6(5):e20188.
79. Kurisaki A, Kose S, Yoneda Y, Heldin C-H, Moustakas A. Transforming growth factor-β induces nuclear import of Smad3 in an importin-β1 and Ran-dependent manner. Mol Biol Cell. 2001;12(4):1079–91.
80. Lo HW, Ali-Seyed M, Wu Y, Bartholomeusz G, Hsu SC, Hung MC. Nuclear-cytoplasmic transport of EGFR involves receptor endocytosis, importin β1 and CRM1. J Cell Biochem. 2006;98(6):1570–83.
81. Gomes DA, Rodrigues MA, Leite MF, et al. c-met must translocate to the nucleus to initiate calcium signals. J Biol Chem. 2008;283(7):4344–51.
82. Giri DK, Ali-Seyed M, Li L-Y, et al. Endosomal transport of ErbB-2: Mechanism for nuclear entry of the cell surface receptor. Mol Cell Biol. 2005;25(24):11005–18.
83. Mingot J-M, Vega S, Maestro B, Sanz JSM, Nieto MA. Characterization of Snail nuclear import pathways as representatives of C_2H_2 zinc finger transcription factors. J Cell Sci. 2009; 122(9):1452–60.
84. Van Der Watt PJ, Ngarande E, Leaner VD. Overexpression of Kpnβ1 and Kpnα2 importin proteins in cancer derives from deregulated E2F activity. PloS One. 2011;6(11):e27723.
85. Nevins JR. The Rb/E2F pathway and cancer. Hum Mol Genet. 2001;10(7):699–703.
86. Kent LN, Leone G. The broken cycle: E2F dysfunction in cancer. Nat Rev Cancer. 2019;19(6):326–38.
87. Yang J, Guo Y, Lu C, et al. Inhibition of Karyopherin beta 1 suppresses prostate cancer growth. Oncogene. 2019;38(24): 4700–14.
88. Yang L, Hu B, Zhang Y, et al. Suppression of the nuclear transporter-KPNβ1 expression inhibits tumor proliferation in hepatocellular carcinoma. Med Oncol. 2015;32(4):1–11.
89. Kuusisto HV, Jans DA. Hyper-dependence of breast cancer cell types on the nuclear transporter importin β1. Biochim Biophys Acta Mol Cell Res. 2015;1853(8):1870–8.
90. Zhu J, Wang Y, Huang H, et al. Upregulation of KPNβ1 in gastric cancer cell promotes tumor cell proliferation and predicts poor prognosis. Tumor Biol. 2016;37(1):661–72.
91. He S, Miao X, Wu Y, et al. Upregulation of nuclear transporter, Kpnβ1, contributes to accelerated cell proliferation- and cell adhesion-mediated drug resistance (CAM-DR) in diffuse large B-cell lymphoma. J Cancer Res Clin Oncol. 2016;142(3):561–72.
92. Yan W, Li R, He J, Du J, Hou J. Importin β1 mediates nuclear factor-κB signal transduction into the nuclei of myeloma cells and affects their proliferation and apoptosis. Cell Signal. 2015; 27(4):851–9.
93. Wang T, Huang Z, Huang N, et al. Inhibition of KPNB1 inhibits proliferation and promotes apoptosis of chronic myeloid leukemia cells through regulation of E2F1. Onco Targets Ther. 2019;12:10455.
94. Zhu Z-C, Liu J-W, Li K, Zheng J, Xiong Z-Q. KPNB1 inhibition disrupts proteostasis and triggers unfolded protein response-mediated apoptosis in glioblastoma cells. Oncogene. 2018;37(22):2936–52.
95. Kodama M, Kodama T, Newberg JY, et al. In vivo loss-of-function screens identify KPNB1 as a new druggable oncogene in epithelial ovarian cancer. Proc Natl Acad Sci. 2017; 114(35):E7301–10.
96. Turner JG, Kashyap T, Dawson JL, et al. XPO1 inhibitor combination therapy with bortezomib or carfilzomib induces nuclear localization of IκBα and overcomes acquired proteasome inhibitor resistance in human multiple myeloma. Oncotarget. 2016;7(48):78896–909.
97. Nie D, Huang K, Yin S, et al. KPT-330 inhibition of chromosome region maintenance 1 is cytotoxic and sensitizes chronic myeloid leukemia to Imatinib. Cell Death Discov. 2018;4(1): 1–12.
98. Chen Y, Camacho SC, Silvers TR, et al. Inhibition of the nuclear export receptor XPO1 as a therapeutic target for platinum-resistant ovarian cancer. Clin Cancer Res. 2017; 23(6):1552–63.
99. Godwin P, Baird A-M, Heavey S, Barr M, O'Byrne K, Gately K. Targeting nuclear factor-kappa B to overcome resistance to chemotherapy. Front Oncol. 2013;3:120.
100. Dolcet X, Llobet D, Pallares J, Matias-Guiu X. NF-kB in development and progression of human cancer. Virchows Arch. 2005;446:475–82.
101. Chi R-PA, van der Watt P, Wei W, Birrer MJ, Leaner VD. Inhibition of Kpnβ1 mediated nuclear import enhances cisplatin chemosensitivity in cervical cancer. BMC Cancer. 2021; 21(1):106.
102. Kashyap T, Argueta C, Aboukameel A, et al. Selinexor, a selective inhibitor of nuclear export (SINE) compound, acts through NF-κB deactivation and combines with proteasome inhibitors to synergistically induce tumor cell death. Oncotarget. 2016;7(48):78883.
103. Galinski B, Luxemburg M, Landesman Y, et al. XPO1 inhibition with selinexor synergizes with proteasome inhibition in neuroblastoma by targeting nuclear export of IkB. Transl Oncol. 2021;14(8):101114.
104. Wang AY, Liu H. The past, present, and future of CRM1/XPO1 inhibitors. Stem Cell Investig. 2019;6:1–9.
105. Newlands E, Rustin G, Brampton M. Phase I trial of elactocin. Br J Cancer. 1996;74(4):648–9.
106. Kudo N, Matsumori N, Taoka H, et al. Leptomycin B inactivates CRM1/exportin 1 by covalent modification at a cysteine residue in the central conserved region. Proc Natl Acad Sci. 1999;96(16):9112–7.

107. Ferreira B, Cautain B, Grenho I, Link W. Small molecule inhibitors of CRM1. Front Pharmacol. 2020;11:625.
108. Kalid O, Toledo Warshaviak D, Shechter S, Sherman W, Shacham S. Consensus induced fit docking (cIFD): Methodology, validation, and application to the discovery of novel Crm1 inhibitors. J Comput Aided Mol des. 2012;26(11):1217–28.
109. Podar K, Shah J, Chari A, Richardson PG, Jagannath S. Selinexor for the treatment of multiple myeloma. Expert Opin Pharmacother. 2020;21(4):399–408.
110. Selinexor. Recruiting, active, not recruiting, enrolling by invitation studies. [cited 2021]. Available from: https://clinicaltrials.gov/ct2/results?term=Selinexor&Search=Apply&recrs=a&recrs=f&recrs=d&age_v=&gndr=&type=&rslt=.
111. Nachmias B, Schimmer AD. Targeting nuclear import and export in hematological malignancies. Leukemia. 2020;34(11):2875–86.
112. Shafique M, Ismail-Khan R, Extermann M, et al. A phase II trial of Selinexor (KPT-330) for metastatic triple-negative breast cancer. Oncologist. 2019;24(7):887–e416.
113. Rashid NS, Hairr NS, Murray G, et al. Identification of nuclear export inhibitor-based combination therapies in preclinical models of triple-negative breast cancer. Translat Oncol. 2021;14(12):101235.
114. Maji S, Panda S, Samal SK, et al. Bcl-2 antiapoptotic family proteins and chemoresistance in cancer. Adv Cancer Res. 2018;137:37–75.
115. Lin KH, Winter PS, Xie A, et al. Targeting MCL-1/BCL-XL forestalls the acquisition of resistance to ABT-199 in acute myeloid leukemia. Sci Rep. 2016;6(1):1–10.
116. Luedtke DA, Niu X, Pan Y, et al. Inhibition of mcl-1 enhances cell death induced by the Bcl-2-selective inhibitor ABT-199 in acute myeloid leukemia cells. Signal Transduct Target Ther. 2017;2(1):1–9.
117. Fischer MA, Friedlander SY, Arrate MP, et al. Venetoclax response is enhanced by selective inhibitor of nuclear export compounds in hematologic malignancies. Blood Adv. 2020;4(3):586–98.
118. Study of Selinexor and Venetoclax in combination with chemotherapy in pediatric and young adult patients with refractory or relapsed acute myeloid leukemia. [cited xxxx]. Available from: https://clinicaltrials.gov/ct2/show/results/NCT04898894.
119. Kumar TS, Kari V, Choudhary B, Nambiar M, Akila TS, Raghavan SC. Anti-apoptotic protein BCL2 down-regulates DNA end joining in cancer cells. J Biol Chem. 2010;285(42):32657–70.
120. Shang E, Zhang Y, Shu C, et al. Dual inhibition of Bcl-2/Bcl-xL and XPO1 is synthetically lethal in glioblastoma model systems. Sci Rep. 2018;8(1):1–11.
121. Tanaka N, Lin JJ, Li C, et al. Clinical acquired resistance to KRASG12C inhibition through a novel KRAS switch-II pocket mutation and polyclonal alterations converging on RAS–MAPK ReactivationClinical acquired resistance to KRASG12C inhibition. Cancer Discov. 2021;11(8):1913–22.
122. Khan HY, Nagasaka M, Li Y, et al. Inhibitor of the nuclear transport protein XPO1 enhances the anticancer efficacy of KRAS G12C inhibitors in preclinical models of KRAS G12C–mutant cancers. Cancer Res Commun. 2022;2(5):342–52.
123. Liu R, Chen Y, Liu G, et al. PI3K/AKT pathway as a key link modulates the multidrug resistance of cancers. Cell Death Dis. 2020;11(9):797.
124. Quintanal-Villalonga A, Taniguchi H, Hao Y, et al. Inhibition of XPO1 sensitizes small cell lung cancer to first-and second-line chemotherapy. Cancer Res. 2022;82(3):472–83.
125. Johnson C, Van Antwerp D, Hope TJ. An N-terminal nuclear export signal is required for the nucleocytoplasmic shuttling of IκBα. Embo J. 1999;18(23):6682–93.
126. Krappmann D, Scheidereit C. Regulation of NF-κB activity by IκBα and IκBβ stability. Immunobiology. 1997;198(1–3):3–13.
127. Traenckner E-M, Pahl HL, Henkel T, Schmidt K, Wilk S, Baeuerle P. Phosphorylation of human I kappa B-alpha on serines 32 and 36 controls I kappa B-alpha proteolysis and NF-kappa B activation in response to diverse stimuli. Embo J. 1995;14(12):2876–83.
128. Crochiere M, Kashyap T, Kalid O, et al. Deciphering mechanisms of drug sensitivity and resistance to selective inhibitor of nuclear export (SINE) compounds. BMC Cancer. 2015;15(1):910.
129. Kashyap T, Muqbil I, Aboukameel A, et al. Combination of selinexor and the proteasome inhibitor, bortezomib shows synergistic cytotoxicity in diffuse large B-cells lymphoma cells in vitro and in vivo. Washington, DC: American Society of Hematology, 2016.
130. Grosicki S, Simonova M, Spicka I, et al. Once-per-week selinexor, bortezomib, and dexamethasone versus twice-per-week bortezomib and dexamethasone in patients with multiple myeloma (BOSTON): A randomised, open-label, phase 3 trial. Lancet. 2020;396(10262):1563–73.
131. Ranganathan P, Kashyap T, Yu X, et al. XPO1 inhibition using Selinexor synergizes with chemotherapy in acute myeloid Leukemia by targeting DNA repair and restoring topoisomerase IIα to the NucleusXPO1 inhibition synergizes with chemotherapy in AML. Clin Cancer Res. 2016;22(24):6142–52.
132. Turner JG, Dawson JL, Grant S, et al. Treatment of acquired drug resistance in multiple myeloma by combination therapy with XPO1 and topoisomerase II inhibitors. J Hematol Oncol. 2016;9(1):73.
133. Vigneri P, Wang JYJ. Induction of apoptosis in chronic myelogenous leukemia cells through nuclear entrapment of BCR–ABL tyrosine kinase. Nat Med. 2001;7(2):228–34.
134. Zheng H-C. The molecular mechanisms of chemoresistance in cancers. Oncotarget. 2017;8(35):59950.
135. Kashyap T, Argueta C, Unger T, et al. Selinexor reduces the expression of DNA damage repair proteins and sensitizes cancer cells to DNA damaging agents. Oncotarget. 2018;9(56):30773.
136. Moore SA, Narayanan D, Simonette RA, et al. Selinexor is a novel inhibitor of DNA damage response in Merkel cell carcinoma. Clin Exp Dermatol. 2022;7:1354–7.
137. Kazim S, Malafa MP, Coppola D, et al. Selective nuclear export inhibitor KPT-330 enhances the antitumor activity of gemcitabine in human pancreatic CancerPotentiation of antitumor activity of gemcitabine by KPT-330. Mol Cancer Ther. 2015;14(7):1570–81.
138. Corno C, Stucchi S, De Cesare M, et al. FoxO-1 contributes to the efficacy of the combination of the XPO1 inhibitor

selinexor and cisplatin in ovarian carcinoma preclinical models. Biochem Pharmacol. 2018;147:93–103.
139. Turner JG, Cui Y, Bauer AA, et al. Melphalan and Exportin 1 inhibitors exert synergistic antitumor effects in preclinical models of human multiple MyelomaSynergism of Exportin 1 inhibitors and Melphalan in human MM. Cancer Res. 2020; 80(23):5344–54.
140. Inoue A, Robinson FS, Minelli R, et al. Sequential administration of XPO1 and ATR inhibitors enhances therapeutic response in TP53-mutated colorectal cancer. Gastroenterology. 2021;161(1):196–210.
141. Keung MY, Wu Y, Badar F, Vadgama JV. Response of breast cancer cells to PARP inhibitors is independent of BRCA status. J Clin Med. 2020;9(4):940.
142. Marijon H, Gery S, Chang H, et al. Selinexor, a selective inhibitor of nuclear export, enhances the anti-tumor activity of olaparib in triple negative breast cancer regardless of BRCA1 mutation status. Oncotarget. 2021;12(18):1749.
143. Selinexor & Talazoparib in advanced refractory solid tumors; Advanced/metastatic triple negative breast cancer (START).
144. Hu J, Zhang Z, Zhao L, Li L, Zuo W, Han L. High expression of RAD51 promotes DNA damage repair and survival in KRAS-mutant lung cancer cells. BMB Rep. 2019;52(2):151–6.
145. Luoto KR, Meng AX, Wasylishen AR, et al. Tumor cell kill by c-MYC depletion: Role of MYC-regulated genes that control DNA double-strand break RepairMYC-regulated DSB repair genes and cell kill. Cancer Res. 2010;70(21):8748–59.
146. Jiang K-l, Tong L-x, Wang T, et al. Downregulation of c-Myc expression confers sensitivity to CHK1 inhibitors in hematologic malignancies. Acta Pharmacol Sin. 2022;43(1):220–8.
147. Long H, Hou Y, Li J, Song C, Ge Z. Azacitidine is synergistically lethal with XPO1 inhibitor Selinexor in acute myeloid Leukemia by targeting XPO1/eIF4E/c-MYC signaling. Int J Mol Sci. 2023;24(7):6816.
148. Emdal KB, Palacio-Escat N, Wigerup C, et al. Phosphoproteomics of primary AML patient samples reveals rationale for AKT combination therapy and p53 context to overcome selinexor resistance. Cell Rep. 2022;40(6):111177.
149. Lin KH, Rutter JC, Xie A, et al. P2RY2-AKT activation is a therapeutically actionable consequence of XPO1 inhibition in acute myeloid leukemia. Nat Cancer. 2022;3(7):837–51.
150. Alzahrani A, Natarajan U, Rathinavelu A. Enhancement of MDM2 inhibitory effects through blocking nuclear export mechanisms in ovarian cancer cells. Cancer Genet. 2022;266: 57–68.
151. Nguyen R, Wang H, Sun M, Lee DG, Peng J, Thiele CJ. Combining selinexor with alisertib to target the p53 pathway in neuroblastoma. Neoplasia. 2022;26:100776.
152. Shi Y, Xu S, Li S. Selinexor improves the anti-cancer effect of tucidinostat on TP53 wild-type breast cancer. Mol Cell Endocrinol. 2022;545:111558.
153. Nakayama R, Zhang Y-X, Czaplinski JT, et al. Preclinical activity of selinexor, an inhibitor of XPO1, in sarcoma. Oncotarget. 2016;7(13):16581.
154. Deng M, Zhang M, Xu-Monette ZY, et al. XPO1 expression worsens the prognosis of unfavorable DLBCL that can be effectively targeted by selinexor in the absence of mutant p53. J Hematol Oncol. 2020;13(1):1–5.
155. Suzuki R, Kitamura Y, Ogiya D, Ogawa Y, Kawada H, Ando K. Anti-tumor activity of the pan-RAF inhibitor TAK-580 in combination with KPT-330 (Selinexor) in multiple myeloma. Int J Hematol. 2022;115(2):233–43.
156. Subhash VV, Yeo MS, Wang L, et al. Anti-tumor efficacy of Selinexor (KPT-330) in gastric cancer is dependent on nuclear accumulation of p53 tumor suppressor. Sci Rep. 2018;8(1): 12248.
157. Zhao C, Ma B, Yang Z-Y, et al. Inhibition of XPO1 impairs cholangiocarcinoma cell proliferation by triggering p53 intranuclear accumulation. Cancer Med. 2023;12(5):5751–63.
158. Crochiere M, Senapedis W, Kashyap T, et al. The selective inhibitor of nuclear export compound, selinexor, inhibits NF-κb and induces anti-non-small cell lung cancer activity regardless of p53 status. Int J Cancer Res Mol Mech. 2016; 2.2:2–11.
159. De Cesare M, Cominetti D, Doldi V, et al. Anti-tumor activity of selective inhibitors of XPO1/CRM1-mediated nuclear export in diffuse malignant peritoneal mesothelioma: The role of survivin. Oncotarget. 2015;6(15):13119–32.
160. Balasubramanian SK, Azmi AS, Maciejewski J. Selective inhibition of nuclear export: A promising approach in the shifting treatment paradigms for hematological neoplasms. Leukemia. 2022;36(3):601–12.
161. Mikhael J. Treatment options for triple-class refractory multiple myeloma. Clin Lymphoma Myeloma Leuk. 2020;20(1): 1–7.
162. Etchin J, Berezovskaya A, Conway A, et al. KPT-8602, a second-generation inhibitor of XPO1-mediated nuclear export, is well tolerated and highly active against AML blasts and leukemia-initiating cells. Leukemia. 2017;31(1):143–50.
163. Hing ZA, Fung HYJ, Ranganathan P, et al. Next-generation XPO1 inhibitor shows improved efficacy and in vivo tolerability in hematological malignancies. Leukemia. 2016;30(12): 2364–72.
164. Restrepo P, Bhalla S, Ghodke-Puranik Y, et al. A three-gene signature predicts response to Selinexor in multiple myeloma. JCO Precis Oncol. 2022;6:e2200147.
165. Lagana A, Park S, Edwards D, et al. E2F1 is a biomarker of Selinexor resistance in relapsed/refractory multiple myeloma patients. Blood. 2018;132(suppl 1):3216.
166. Jans DA, Martin AJ, Wagstaff KM. Inhibitors of nuclear transport. Curr Opin Cell Biol. 2019;58:50–60.
167. Kelenis DP, Rodarte KE, Kollipara RK, et al. Inhibition of karyopherin β1-mediated nuclear import disrupts oncogenic lineage-defining transcription factor activity in small cell lung cancer. Cancer Res. 2022;82(17):3058–73.
168. Hintersteiner M, Ambrus G, Bednenko J, et al. Identification of a small molecule inhibitor of importin β mediated nuclear import by confocal on-bead screening of tagged one-bead one-compound libraries. ACS Chem Biol. 2010;5(10):967–79.
169. Soderholm JF, Bird SL, Kalab P, et al. Importazole, a small molecule inhibitor of the transport receptor importin-β. ACS Chem Biol. 2011;6(7):700–8.
170. Li Y-Q, Zheng Z, Liu Q-X, et al. Repositioning of antiparasitic drugs for tumor treatment. Front Oncol. 2021;11:670804.
171. Ha S, Choi J, Min NY, Lee K-H, Ham SW. Inhibition of importin β1 with a 2-aminothiazole derivative resulted in

G2/M cell-cycle arrest and apoptosis. Anticancer Res. 2017; 37(5):2373–9.
172. Kim YH, Ha S, Kim J, Ham SW. Identification of KPNB1 as a cellular target of aminothiazole derivatives with anticancer activity. ChemMedChem. 2016;11(13):1406–9.
173. Ha S, Oh J, Jang JM, Kim DK, Ham SW. Synthesis and biological evaluation of 2-aminothiazole derivative having anticancer activity as a KPNB1 inhibitor. Bull Korean Chem Soc. 2016;37(11):1743–4.
174. Van Der Watt PJ, Chi A, Stelma T, et al. Targeting the nuclear import receptor Kpnβ1 as an anticancer therapeutic. Mol Cancer Ther. 2016;15(4):560–73.
175. Ajayi-Smith A, van der Watt P, Mkwanazi N, Carden S, Trent JO, Leaner VD. Novel small molecule inhibitor of Kpnβ1 induces cell cycle arrest and apoptosis in cancer cells. Exp Cell Res. 2021;404(2):112637.
176. Balint E, Vousden K. Activation and activities of the p53 tumour suppressor protein. Br J Cancer. 2001;85(12):1813–23.
177. Jirawatnotai S, Hu Y, Michowski W, et al. A function for cyclin D1 in DNA repair uncovered by protein interactome analyses in human cancers. Nature. 2011;474(7350):230–4.
178. Pyndiah S, Tanida S, Ahmed KM, Cassimere EK, Choe C, Sakamuro D. c-MYC suppresses BIN1 to release poly(ADP-ribose) polymerase 1: A mechanism by which cancer cells acquire cisplatin resistance. Sci Signal. 2011;4(166):ra19.
179. Salvesen GS, Duckett CS. IAP proteins: Blocking the road to death's door. Nat Rev Mol Cell Biol. 2002;3(6):401–10.
180. Wang H, Wang D, Li C, Zhang X, Zhou X, Huang J. High Kpnβ1 expression promotes non-small cell lung cancer proliferation and chemoresistance via the PI3-kinase/AKT pathway. Tissue Cell. 2018;51:39–48.
181. Zhang Y, Bao C, Mu Q, et al. Reversal of cisplatin resistance by inhibiting PI3K/Akt signal pathway in human lung cancer cells. Neoplasma. 2016;63(3):362–70.
182. Sacha T. Imatinib in chronic myeloid leukemia: An overview. Mediter J Hematol Infect Dis. 2014;6(1):1–9.
183. Rodriguez-Bravo V, Pippa R, Song W-M, et al. Nuclear pores promote lethal prostate cancer by increasing POM121-driven E2F1, MYC, and AR nuclear import. Cell. 2018;174(5):1200–15.e1220.
184. Zhao R, Tang G, Wang T, et al. POM121 is a novel marker for predicting the prognosis of laryngeal cancer; Histology and Histopathology, Spain. 2020;35(11):1285–93.
185. Wang T, Sun H, Bao Y, et al. POM121 overexpression is related to a poor prognosis in colorectal cancer. Expert Rev Mol Diagn. 2020;20(3):345–53.
186. Ma H, Li L, Jia L, et al. POM121 is identified as a novel prognostic marker of oral squamous cell carcinoma. J Cancer. 2019;10(19):4473.
187. Kang C, Jia L, Hao L, Zhang N, Liu Y, Zhang L. POM121 promotes the proliferation and metastasis of gastric cancer via PI3K/AKT/MYC pathway. Am J Cancer Res. 2023;13(2):485.
188. Sano R, Reed JC. ER stress-induced cell death mechanisms. Biochim Biophys Acta Mol Cell Res. 2013;1833(12):3460–70.
189. Liu X-D, Ko S, Xu Y, et al. Transient aggregation of ubiquitinated proteins is a cytosolic unfolded protein response to inflammation and endoplasmic reticulum stress. J Biol Chem. 2012;287(23):19687–98.
190. Rashid H-O, Yadav RK, Kim H-R, Chae H-J. ER stress: Autophagy induction, inhibition and selection. Autophagy. 2015; 11(11):1956–77.
191. Ding W-X, Ni H-M, Yin X-M. Absence of Bax switched MG132-induced apoptosis to non-apoptotic cell death that could be suppressed by transcriptional or translational inhibition. Apoptosis. 2007;12:2233–44.
192. Qing G, Li B, Vu A, et al. ATF4 regulates MYC-mediated neuroblastoma cell death upon glutamine deprivation. Cancer Cell. 2012;22(5):631–44.
193. Cang S, Iragavarapu C, Savooji J, Song Y, Liu D. ABT-199 (Venetoclax) and BCL-2 inhibitors in clinical development. J Hematol Oncol. 2015;8(1):1–8.
194. Leverson JD, Phillips DC, Mitten MJ, et al. Exploiting selective BCL-2 family inhibitors to dissect cell survival dependencies and define improved strategies for cancer therapy. Sci Transl Med. 2015;7(279):279ra240.
195. Yang F, Li L, Mu Z, et al. Tumor-promoting properties of karyopherin β1 in melanoma by stabilizing Ras-GTPase-activating protein SH3 domain-binding protein 1. Cancer Gene Ther. 2022;29(12):1939–50.
196. Yang P, Mathieu C, Kolaitis RM, et al. G3BP1 is a tunable switch that triggers phase separation to assemble stress granules. Cell. 2020;181(2):325–45.e328.
197. Zhao J, Fu X, Chen H, et al. G3BP1 interacts with YWHAZ to regulate chemoresistance and predict adjuvant chemotherapy benefit in gastric cancer. Br J Cancer. 2021;124(2):425–36.
198. Twyffels L, Gueydan C, Kruys V. Transportin-1 and Transportin-2: Protein nuclear import and beyond. FEBS Lett. 2014;588(10):1857–68.
199. Mathur R. ARID1A loss in cancer: Towards a mechanistic understanding. Pharmacol Ther. 2018;190:15–23.
200. Zhao L, Wu D, Qu Q, Li Z, Yin H. Karyopherin subunit alpha 1 enhances the malignant behaviors of colon cancer cells via promoting nuclear factor-κB p65 nuclear translocation. Dig Dis Sci. 2023;68(7):3018–31.
201. Sang Y, Li Y, Zhang Y, et al. CDK5-dependent phosphorylation and nuclear translocation of TRIM59 promotes macroH2A1 ubiquitination and tumorigenicity. Nat Commun. 2019;10(1):4013.
202. Jiang L, Li D, Wang C, et al. Decreased expression of Karyopherin-α 1 is related to the malignant degree of cervical cancer and is critical for the proliferation of Hela cells. Pathol Oncol Res. 2022;28:1–10.
203. Zhou Y, Liu F, Xu Q, et al. Inhibiting importin 4-mediated nuclear import of CEBPD enhances chemosensitivity by repression of PRKDC-driven DNA damage repair in cervical cancer. Oncogene. 2020;39(34):5633–48.
204. Jögi A, Ehinger A, Hartman L, Alkner S. Expression of HIF-1α is related to a poor prognosis and tamoxifen resistance in contralateral breast cancer. PloS One. 2019;14(12):e0226150.
205. Tsoi H, Man EP, Leung MH, et al. KPNA1 regulates nuclear import of NCOR2 splice variant BQ323636.1 to confer tamoxifen resistance in breast cancer. Clin Transl Med. 2021;11(10):e554.
206. Gong C, Man EP, Tsoi H, et al. 1, a novel splice variant to NCOR2, as a predictor for tamoxifen-resistant breast CancerBQ323636. 1 predicts tamoxifen response in breast cancer. Clin Cancer Res. 2018;24(15):3681–91.

207. Mishra A, Srivastava A, Pateriya A, Tomar MS, Mishra AK, Shrivastava A. Metabolic reprograming confers tamoxifen resistance in breast cancer. Chem Biol Interact. 2021;347:109602.
208. Zhang Y, Song Y, Ren S, et al. GPER-mediated stabilization of HIF-1α contributes to upregulated aerobic glycolysis in tamoxifen-resistant cells. Oncogene. 2023;42(3):184–97.
209. Hodis E, Watson IR, Kryukov GV, et al. A landscape of driver mutations in melanoma. Cell. 2012;150(2):251–63.
210. Ugurel S, Röhmel J, Ascierto PA, et al. Survival of patients with advanced metastatic melanoma: The impact of novel therapies—Update 2017. Eur J Cancer. 2017;83:247–57.
211. Luke JJ, Flaherty KT, Ribas A, Long GV. Targeted agents and immunotherapies: Optimizing outcomes in melanoma. Nat Rev Clin Oncol. 2017;14(8):463–82.
212. Solit DB, Rosen N. Resistance to BRAF inhibition in melanomas. N Engl J Med. 2011;364(8):772–4.
213. Mirzoeva OK, Das D, Heiser LM, et al. Basal subtype and MAPK/ERK kinase (MEK)-phosphoinositide 3-kinase feedback signaling determine susceptibility of breast cancer cells to MEK inhibition. Cancer Res. 2009;69(2):565–72.
214. Wee S, Jagani Z, Xiang KX, et al. PI3K pathway activation mediates resistance to MEK inhibitors in KRAS mutant cancers. Cancer Res. 2009;69(10):4286–93.
215. Lake D, Corrêa SA, Müller J. Negative feedback regulation of the ERK1/2 MAPK pathway. Cell Mol Life Sci. 2016;73(23):4397–413.
216. Adam C, Fusi L, Weiss N, et al. Efficient suppression of NRAS-driven melanoma by Co-inhibition of ERK1/2 and ERK5 MAPK pathways. J Investig Dermatol. 2020;140(12):2455–2465.e2410.
217. Plotnikov A, Flores K, Maik-Rachline G, et al. The nuclear translocation of ERK1/2 as an anticancer target. Nat Commun. 2015;6(1):6685.
218. Arafeh R, Flores K, Keren-Paz A, et al. Combined inhibition of MEK and nuclear ERK translocation has synergistic antitumor activity in melanoma cells. Sci Rep. 2017;7(1):16345.
219. Oral Selinexor. [cited 2023]. Available from: https://www.karyopharm.com/science/pipeline/.
220. ClinicalTrials.gov. Study of the safety, tolerability and efficacy of KPT-8602 in participants with relapsed/refractory cancer indications. 2023.
221. Saito N, Sakakibara K, Sato T, et al. CBS9106-induced CRM1 degradation is mediated by cullin ring ligase activity and the neddylation pathway. Mol Cancer Ther. 2014;13(12):3013–23.
222. ClinicalTrials.gov. A Phase 1 trial of a novel XPO1 inhibitor in patients with advanced solid tumors. 2023.
223. Wang J, Xu Y, Wan H, Hu J. Antibiotic ivermectin selectively induces apoptosis in chronic myeloid leukemia through inducing mitochondrial dysfunction and oxidative stress. Biochem Biophys Res Commun. 2018;497(1):241–7.
224. Kosyna FK, Nagel M, Kluxen L, Kraushaar K, Depping R. The importin α/β-specific inhibitor Ivermectin affects HIF-dependent hypoxia response pathways. Biol Chem. 2015;396(12):1357–67.

How to cite this article: Newell S, van der Watt PJ, Leaner VD. Therapeutic targeting of nuclear export and import receptors in cancer and their potential in combination chemotherapy. IUBMB Life. 2024;76(1):4–25. https://doi.org/10.1002/iub.2773