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Therapeutic Targeting of Nuclear Export Inhibition in Lung Cancer

Arjun Gupta, MD[1], **Jessica M. Saltarski, BA**[2], **Michael A. White, PhD**[2,3], **Pier P. Scaglioni, MD**[1,2], and **David E. Gerber, MD**[1,2,4]

[1]Department of Internal Medicine, UT Southwestern Medical Center

[2]Harold C. Simmons Comprehensive Cancer Center, UT Southwestern Medical Center

[3]Pfizer, Inc

[4]Department of Clinical Sciences, UT Southwestern Medical Center

Abstract

Intracellular compartmentalization and trafficking of molecules plays a critical role in complex and essential cellular processes. In lung cancer and other malignancies, aberrant nucleocytoplasmic transport of tumor suppressor proteins and cell cycle regulators results in tumorigenesis and inactivation of apoptosis. Pharmacologic targeting of this process, termed selective inhibition of nuclear export (SINE), has demonstrated anti-tumor efficacy in preclinical models and human clinical trials. Exportin-1 (XPO1)—which serves as the sole exporter of several tumor suppressor proteins and cell cycle regulators, including retinoblastoma (Rb), adenomatous polyposis coli (APC), p53, p73, p21, p27, FOXO, STAT3, IKB, topoisomerase II and PAR-4—is the principal focus of SINE drug development. The most extensively studied SINE to date, the XPO1 inhibitor selinexor (KPT-330; Karyopharm Therapeutics, Inc., Newton, MA), has demonstrated single-agent anticancer activity and synergistic effects in combination regimens against multiple cancer types, with principal toxicities of low-grade cytopenias and gastrointestinal effects. SINE may have particular relevance in KRAS-driven tumors, for which this treatment strategy demonstrates significant synthetic lethality. A multi-center phase 1/2 clinical trial of selinexor in previously treated advanced *KRAS* mutant non-small cell lung cancer is underway.

Keywords

Adenocarcinoma; Exportin-1; KRAS; Pathway; Selinexor; Targeted therapy; XP01

Corresponding author: David E. Gerber, MD, Division of Hematology-Oncology, Harold C. Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Mail Code 8852, Dallas, TX 75390-8852, Tel. (214) 648-4180, Fax. (214) 648-1955, david.gerber@UTSouthwestern.edu.

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Intracellular compartmentalization and trafficking of molecules plays a critical role in complex and essential cellular processes. Aberrant nucleocytoplasmic transport of tumor suppressor proteins and cell cycle regulators—mediated by importins and exportins—can result in tumorigenesis and inactivation of apoptosis. Several malignancies, including lung cancer, feature over-expression of these nuclear transport receptors. Pharmacologic targeting of this process has demonstrated anti-tumor efficacy. In this review, we describe the mechanism, function, and therapeutic targeting of nuclear transport, with particular focus on application in lung cancer.

Nuclear export machinery

The nuclear envelope, comprising an inner and outer membrane, prevents the unrestricted diffusion of molecules larger than 40 kilodalton between the nucleus and the cytoplasm. This regulated nuclear-cytolasmic transport of proteins and other molecules plays a key role in cell functioning.[1] Within the nuclear envelope, nuclear pore complexes provide an aqueous channel for the active transport of molecules. The karyopherin-B protein family, which includes both importins and exportins, facilitates transport across these nuclear pores.[2] Cargo proteins destined for nuclear export have specific leucine-rich amino acid sequences known as nuclear export signals (NES), which are recognized by exportin proteins.[3] Nuclear-cytoplasmic transport is an active process, requiring energy provided by RanGTP. A complex between the cargo protein, the exportin molecule, and RanGTP is formed and transported across the nuclear pore complex to the cytoplasm.[4] In the cytoplasm, RanGTPase causes hydrolysis of the RanGTP, releasing and the cargo (which remains in the cytoplasm) and exportin protein (which is recycled back to the nucleus) (Figure 1A).[5] At least 7 eukaryotic exportins have been identified (Table 1). While most of these are responsible for transport,[6] Exportin-1 (XPO1, also known as chromosomal region maintenance 1, or CRM1) is a more ubiquitous receptor protein responsible for transporting approximately 220 proteins.[7,8]

Role of nuclear export functions in normal cell physiology and cancer

XPO1 is the sole exporter of several tumor suppressor proteins and cell cycle regulators, including retinoblastoma (Rb), adenomatous polyposis coli (APC), p53, p73, p21, p27, FOXO, STAT3, IKB, topoisomerase II and PAR-4.[9] Under physiological conditions, the regulated export of these molecules prevents their over-activity in the nucleus in the absence of oncogenic stimuli or DNA damage. In multiple cancer types, XPO1 overexpression leads to dysregulated export of these tumor suppressor proteins into the cytoplasm where they are unable to exercise their effects, thereby resulting in aberrant growth signaling, inactivation of apoptosis, and tumor initiation and growth (Figure 1B). XPO1 overexpression is also associated with drug resistance due to export of drug targets such as topoisomerase II and galectin-3.[10,11]

Nuclear export targeting

Given the critical role of nuclear export in cell cycle regulation and tumorigenesis, efforts to inhibit XPO1 pharmacologically have been undertaken. First generation XPO1 inhibitors

include natural products such as leptomycin B (Table 1). Leptomycin B irreversibly alkylates an XPO1 cysteine residue (cysteine 528), preventing XPO1 binding to cargo protein nuclear export signals. This in turn leads to inhibition of export complex formation, as well as nuclear retention of tumor suppressor proteins (Figure 1C).[12] Despite promising preclinical studies, strong dose-limiting toxicities (anorexia, nausea) and minimal clinical benefit in early studies limited development of leptomycin B.[13] Newer pharmacological agents, termed selective inhibitors of nuclear export (SINE), reversibly bind the XPO1 cysteine 528 residue. To date, the most extensively studied SINE is selinexor (KPT-330; Karyopharm Therapeutics, Inc., Newton, MA). In multiple *in vitro* and *in vivo* models, selinexor has demonstrated single-agent anticancer activity and synergistic effects in combination regimens (Table 2). Globally, selinexor has been administered to more than 2,100 patients. Common adverse events include low-grade nausea (62%), fatigue (60%), anorexia (51%), thrombocytopenia (42%), and vomiting (37%), which have generally been readily managed with standard supportive care measures.

As monotherapy, selinexor has induced responses in hematologic malignancies and yielded disease control in solid tumors.[14–16] In one study, 31% of evaluable patients had an objective response with use of selinexor across a spectrum of non-Hodgkin's lymphoma subtypes, with a median duration of response exceeding 10 months.[16] In another study, among 157 evaluable patients with advanced or metastatic solid tumors, single-agent selinexor resulted in an objective response rate of 4%, and a stable disease rate of 43%.[17]

Preclinical studies and clinical trials in lung cancer

XPO1 is overexpressed in lung cancer cells, particularly those arising in the setting of Nicotine-derived nitrosamine ketone (NNK, a tobacco carcinogen) exposure.[18] Preclinical studies have demonstrated antitumor activity of SINEs in non-small cell lung cancer (NSCLC) cell lines and xenografts.[19,20] SINEs have shown efficacy against epidermal growth factor receptor (EGFR) inhibitor-resistant NSCLC cell lines in a time- and dose-dependent manner.[20] Synergism with chemotherapy and radiation therapy has been demonstrated in the presence of diverse molecular alterations, including *EGFR, p53, RAS*, and *PIK3CA* mutations.[19,21]

Efficacy against *KRAS* mutant lung adenocarcinoma, a disease setting lacking specific targeted therapies to date, appears particularly promising. In a multi-genomic screen of 4,700 biological processes in more than 100 human NSCLC cell lines, nuclear transport machinery emerged as the sole process exhibiting synthetic-lethal interactions in KRAS-driven cancers.[22] In this study, the primary mechanism of cell kill was intolerance to nuclear accumulation of IκB with consequent inhibition of NFκB transcription activity. Rare cases (<20%) of intrinsic resistance were associated with *FSTL5* mutations and attributed to YAP1 activation. With few exceptions, nuclear export inhibition had limited efficacy against *KRAS* wild type cell lines.

In summary, the broad genomic landscape in lung cancer makes it an attractive clinical setting for SINEs. To date, selinexor trials in advanced squamous cell lung cancer (NCT02536495) and relapsed small cell lung cancer (NCT02351505) have been initiated.[17]

A phase 1/2 trial in previously treated advanced *KRAS* mutant NSCLC is underway (NCT03095612).

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References

1. Schmidt HB, Gorlich D. Transport Selectivity of Nuclear Pores, Phase Separation, and Membraneless Organelles. Trends Biochem Sci. 2016; 41(1):46–61. [PubMed: 26705895]
2. Ullman KS, Powers MA, Forbes DJ. Nuclear export receptors: from importin to exportin. Cell. 1997; 90(6):967–970. [PubMed: 9323123]
3. Dong X, Biswas A, Suel KE, et al. Structural basis for leucine-rich nuclear export signal recognition by CRM1. Nature. 2009; 458(7242):1136–1141. [PubMed: 19339969]
4. Monecke T, Guttler T, Neumann P, Dickmanns A, Gorlich D, Ficner R. Crystal structure of the nuclear export receptor CRM1 in complex with Snurportin1 and RanGTP. Science. 2009; 324(5930):1087–1091. [PubMed: 19389996]
5. Lui K, Huang Y. RanGTPase: A Key Regulator of Nucleocytoplasmic Trafficking. Mol Cell Pharmacol. 2009; 1(3):148–156. [PubMed: 20300488]
6. Guttler T, Gorlich D. Ran-dependent nuclear export mediators: a structural perspective. EMBO J. 2011; 30(17):3457–3474. [PubMed: 21878989]
7. Turner JG, Sullivan DM. CRM1-mediated nuclear export of proteins and drug resistance in cancer. Curr Med Chem. 2008; 15(26):2648–2655. [PubMed: 18991627]
8. Stade K, Ford CS, Guthrie C, Weis K. Exportin 1 (Crm1p) is an essential nuclear export factor. Cell. 1997; 90(6):1041–1050. [PubMed: 9323132]
9. Fukuda M, Asano S, Nakamura T, et al. CRM1 is responsible for intracellular transport mediated by the nuclear export signal. Nature. 1997; 390(6657):308–311. [PubMed: 9384386]
10. Takenaka Y, Fukumori T, Yoshii T, et al. Nuclear export of phosphorylated galectin-3 regulates its antiapoptotic activity in response to chemotherapeutic drugs. Mol Cell Biol. 2004; 24(10):4395–4406. [PubMed: 15121858]
11. Turner JG, Dawson J, Sullivan DM. Nuclear export of proteins and drug resistance in cancer. Biochem Pharmacol. 2012; 83(8):1021–1032. [PubMed: 22209898]
12. Meissner T, Krause E, Vinkemeier U. Ratjadone and leptomycin B block CRM1-dependent nuclear export by identical mechanisms. FEBS Lett. 2004; 576(1–2):27–30. [PubMed: 15474004]
13. Newlands ES, Rustin GJ, Brampton MH. Phase I trial of elactocin. Br J Cancer. 1996; 74(4):648–649. [PubMed: 8761384]
14. Garzon R, Savona M, Baz R, et al. A phase I clinical trial of single-agent selinexor in acute myeloid leukemia. Blood. 2017
15. Gounder MM, Zer A, Tap WD, et al. Phase IB Study of Selinexor, a First-in-Class Inhibitor of Nuclear Export, in Patients With Advanced Refractory Bone or Soft Tissue Sarcoma. J Clin Oncol. 2016; 34(26):3166–3174. [PubMed: 27458288]
16. Kuruvilla J, Savona M, Baz R, et al. Selective inhibition of nuclear export with selinexor in patients with non-Hodgkin's lymphoma. Blood. 2017
17. Abdul Razak AR, Mau-Soerensen M, Gabrail NY, et al. First-in-Class, First-in-Human Phase I Study of Selinexor, a Selective Inhibitor of Nuclear Export, in Patients With Advanced Solid Tumors. J Clin Oncol. 2016; 34(34):4142–4150. [PubMed: 26926685]
18. 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):815–825. [PubMed: 25629636]

19. Sun H, Hattori N, Chien W, et al. KPT-330 has antitumour activity against non-small cell lung cancer. Br J Cancer. 2014; 111(2):281–291. [PubMed: 24946002]
20. Wang S, Han X, Wang J, Yao J, Shi Y. Antitumor effects of a novel chromosome region maintenance 1 (CRM1) inhibitor on non-small cell lung cancer cells in vitro and in mouse tumor xenografts. PLoS One. 2014; 9(3):e89848. [PubMed: 24595136]
21. Cromie MM, Gao W. Epigallocatechin-3-gallate enhances the therapeutic effects of leptomycin B on human lung cancer a549 cells. Oxid Med Cell Longev. 2015; 2015:217304. [PubMed: 25922640]
22. Kim J, McMillan E, Kim HS, et al. XPO1-dependent nuclear export is a druggable vulnerability in KRAS-mutant lung cancer. Nature. 2016; 538(7623):114–117. [PubMed: 27680702]
23. Holzer K, Drucker E, Oliver S, et al. Cellular apoptosis susceptibility (CAS) is overexpressed in thyroid carcinoma and maintains tumor cell growth: A potential link to the BRAFV600E mutation. Int J Oncol. 2016; 48(4):1679–1687. [PubMed: 26892809]
24. Leisegang MS, Martin R, Ramirez AS, Bohnsack MT. Exportin t and Exportin 5: tRNA and miRNA biogenesis — and beyond. Biol Chem. 2012; 393(7):599–604. [PubMed: 22944664]
25. Kurisaki A, Kurisaki K, Kowanetz M, et al. The mechanism of nuclear export of Smad3 involves exportin 4 and Ran. Mol Cell Biol. 2006; 26(4):1318–1332. [PubMed: 16449645]
26. Melo SA, Moutinho C, Ropero S, et al. A genetic defect in exportin-5 traps precursor microRNAs in the nucleus of cancer cells. Cancer Cell. 2010; 18(4):303–315. [PubMed: 20951941]
27. Stuven T, Hartmann E, Gorlich D. Exportin 6: a novel nuclear export receptor that is specific for profilin.actin complexes. EMBO J. 2003; 22(21):5928–5940. [PubMed: 14592989]
28. Mingot JM, Bohnsack MT, Jakle U, Gorlich D. Exportin 7 defines a novel general nuclear export pathway. EMBO J. 2004; 23(16):3227–3236. [PubMed: 15282546]

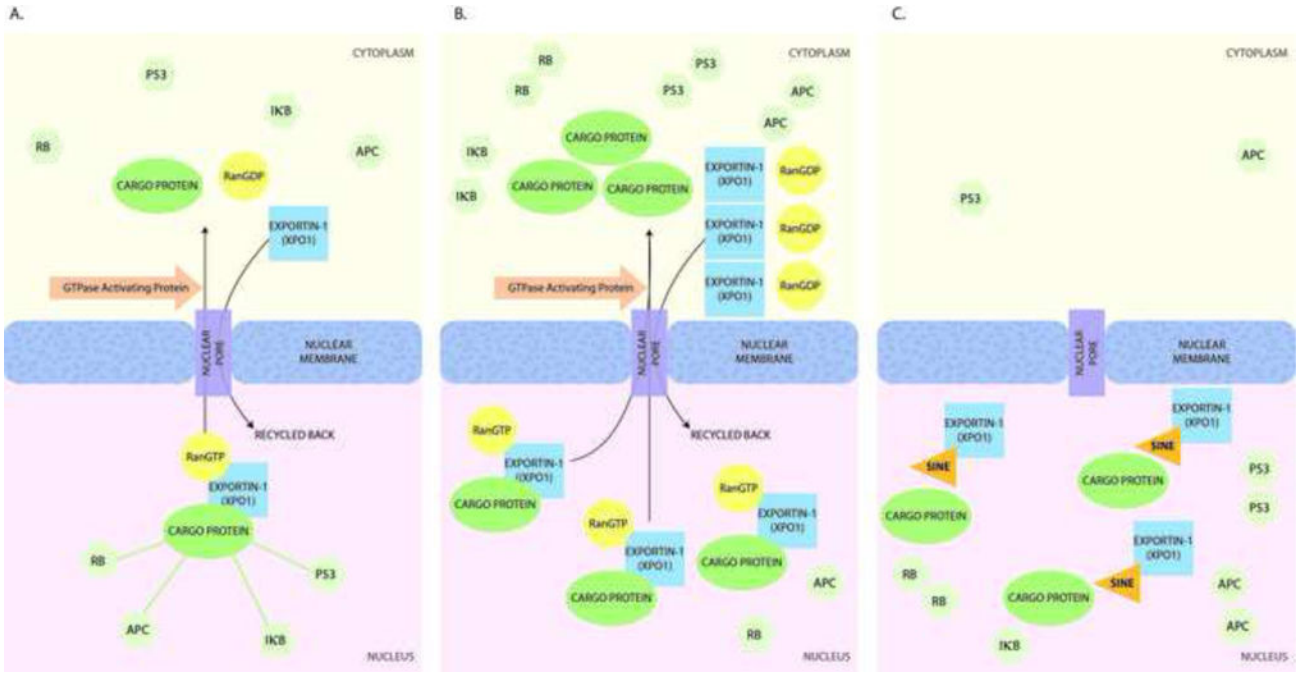

Figure 1. Export through the nuclear pore complex

(A) Physiologic state. Export complexes containing Exportin-1, a cargo protein, and RanGTP are transported across the nuclear pore due to the RanGTP: RanGDP gradient. RanGTPase hydrolyzes RanGTP in the cytoplasm, leading to dissociation of the complex in the cytoplasm. *(B) Cancer.* Up-regulation of Exportin-1 results in dysregulated cytoplasmic transport of cell-cycle regulators, leading to their accumulation in the cytoplasm and inability to exert their effects. In turn, this state leads to aberrant growth signaling, inactivation of apoptosis, and tumor initiation and growth. *(C) Pharmacologic inhibition.* Selective Inhibitors of Nuclear Export (SINE) bind to Exportin-1 (XPO1) and prevent its interaction with cargo proteins, thereby inhibiting nuclear export. Cell-cycle regulators are retained in the nucleus, leading to growth inhibition.

Table 1

Characteristics of exportin molecules

Exportin protein	Alternative name	Chromosome	Cargo	Role in cancer	Reference
Exportin-1 (XPO1)	Chromosome region maintenance 1 (CRM1) protein	2p15	>200 macromolecules, including protein and RNA (including several tumor suppressor genes and cell cycle regulators, such as p53, p21, Rb, APC, FOXO)	Upregulated in multiple cancer types; associated with tumorigenesis and drug resistance	8
Exportin-2	Cellular apoptosis susceptibility (CAS) protein, Chromosome segregation 1-like protein	20q13.13	Importin-alpha	Overexpressed in thyroid cancer	23
Exportin 3, (Exportin-T)	Karyopherin-beta, tRNA exportin	12q14.2	tRNA	–	24
Exportin-4	KIAA1721, FLJ13046,	13q12.11	Broad substrate specificity including SMAD3, eIF5A	–	25
Exportin-5	Ran-binding protein 21, KIAA1291	6p21.1	Proteins bearing a double-stranded RNA binding domain and double-stranded RNAs, micro-RNA precursors, tRNA, eIF1A	Inactivating mutations associated with microsatellite instability	26
Exportin-6	Ran-binding protein 20, KIAA0370, FLJ22519	16p11.2	Actin	–	27
Exportin-7	Ran-binding protein 16, KIAA0745	8p21.3	eIF4A1, ARHGAP1, VPS26A, VPS29, VPS35 and SFN, p50RhoGAP	–	28

APC, Adenomatous polyposis coli; ARHGAP1, Rho GTPase activating protein 1; eIF, eukaryotic translation initiation factor; FOXO, Forkhead box O; Rb, retinoblastoma; RNA, ribonucleic acid; SFN, Stratifin; SMAD3, Mothers against decapentaplegic homolog 3; tRNA, transfer RNA; VPS, Vacuolar protein sorting-associated protein

Table 2

Inhibitors of nuclear export tested in humans

Drug	Parent company	Molecular Target	Trial phase	Indication for human use	Clinical trials.gov Identifier(s)
First generation nuclear export inhibitor					
Leptomycin (also called elactocin)	Warner-Lambert	Exportin-1	1	Advanced refractory solid cancers	
Second generation nuclear export inhibitors					
SL-801	Stemline Therapeutics, Inc.	Exportin-1	1	Advanced Solid Tumors	NCT02667873
Selinexor (KPT-330)	Karyopharm Therapeutics, Inc	Exportin-1	2/3	Acute myelogenous leukemia, myelodysplastic syndrome, T-cell lymphoma, B-cell lymphoma, chronic lymphocytic leukemia, multiple myeloma, glioblastoma, gynecological cancers, lung cancer, head and neck cancer, sarcoma, melanoma, breast cancer, prostate cancer, colon cancer, pancreatic cancer, gastric cancer, esophageal cancer, salivary gland tumors	(>50 trials total). Lung cancer trials: NCT02536495, NCT02351505, NCT03095612, NCT02250885, NCT02213133
Verdinexor (KPT-335)	Karyopharm Therapeutics, Inc	Exportin-1	1	Antiviral agent (approved for canine lymphoma)	NCT02431364
KPT-8602	Karyopharm Therapeutics, Inc	Exportin-1	1/2	Relapsed refractory Multiple myeloma	NCT02649790

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