Selinexor
Selective Inhibitor of Nuclear Export (SINE) Compound
Overview

• The cell cycle and role of tumor suppressor proteins (TSPs) and exportin 1 (XPO1) in cancer

• The nuclear pore complex (NPC): the gatekeeper

• Rationale for SINE compounds

• First-in-class SINE compound selinexor: mechanism of action in cancer
The cell cycle and role of TSPs and XPO1 in cancer
The role of TSPs in cell cycle regulation

TSPs function primarily in the nucleus to prevent the genesis of neoplastic clones by regulating cell growth and death pathways\(^1,2\)

Nuclear TSPs exert their apoptotic or cell cycle arrest effects at critical points in the cell cycle\(^2-4\)

Cancer cells export TSPs from the nucleus to the cytoplasm, leading to their functional inactivation\(^1\)

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XPO1 inactivates TSPs in cancer cells through export from the nucleus to the cytoplasm

**XPO1**

- 1 of 8 known nuclear export proteins (exportins 1-7 and transportin)$^1$
- Chaperones more than 200 proteins, including most of the TSPs and growth regulators, to the cytoplasm$^1$
- Only export protein that transports TSPs$^1$
- There are no known redundancies with XPO1$^2,3$
- Overexpressed in all cancers evaluated to date$^1-4$
- Overexpression correlates with poor prognosis and treatment resistance$^5,6-9$
- Cancer cells inactivate TSPs to propagate
  - TSPs/cell cycle regulators include; p53, p73, p21, p27, BRCA1 and 2, FOXO1a, FOXO3a, IκB, PP2Aα, and survivin

XPO1-mediated export inactivates TSPs

XPO1-mediated export of cargo proteins from the nucleus to the cytoplasm

- In cancer, TSPs are often modified and form a complex with RanGTP
- The complex binds to XPO1 and is functionally inactivated after being exported through the NPC to the cytoplasm

Key TSPs transported by XPO1 include p53, p73, p21, p27, BRCA1 and 2, FOXO1a, FOXO3a, IκB, PP2Aα, and survivin
XPO1-mediated export of mRNA supports oncoprotein production

**XPO1/eIF4E-mediated export of mRNA from the nucleus to the cytoplasm**

- Cancer cells over-expressing XPO1 also exhibit increased transport of oncogenic mRNAs out of the nucleus
- Oncogenic mRNAs (c-Myc, cyclin D1, Bcl-2, Bcl-6) require adapter proteins (eIF4E) for export
- mRNAs are “capped”, then bound by eIF4E for export via XPO1 to the cytoplasm
- In the cytoplasm, oncogene mRNAs are translated to oncoproteins
The Nuclear Pore Complex (NPC): the gatekeeper
Overview of the nuclear pore complex (NPC)

NPC structure and molecular composition

- NPCs provide a gateway for trafficking proteins between the nucleus and cytoplasm
- XPO1 mediated TSP transport is key to maintenance of normal cellular homeostasis
- NPC is unidirectional: exportins for export of proteins from the nucleus, importins for import

Changes in the abundance or function of components of the NPC in tumor cells can dysregulate:
  - Cell migration
  - Intracellular signaling
  - DNA repair
  - Cell division
  - Gene regulation and expression

Inhibition of XPO1 restores the activity of TSPs and reduces oncoprotein synthesis

- XPO1, by virtue of its non-redundant, chaperone function, controls the nuclear export of TSPs
- More than 14 distinct TSP pathways have been identified that utilize XPO1 exclusively
- Pathways that involve cytokines to promote cell activation, proliferation and resistance are adjunctive to XPO1 activity
- XPO1-mediated nuclear export causes functional TSP inactivation and fosters proto-oncogene translation in malignancies
- Inhibiting XPO1 is a viable strategy to restore the activity of multiple TSPs and reduce oncoprotein synthesis

Rationale for SINE compounds: 

*drugs that stop traffic* 

*at the nuclear border*
SINE compounds force the nuclear retention and activation of TSPs

- Cancer cells overexpress XPO1, causing increased nuclear export of TSPs
- With TSPs outside the nucleus, cells with damaged DNA resist apoptosis and continue to divide
- SINE compounds inhibit XPO1-mediated nuclear-cytoplasmic transport by covalently binding to the XPO1 cargo binding site
- With SINE therapy, TSPs are retained and accumulate in the nucleus, amplifying their natural apoptotic function in cells with damaged DNA/cancer cells
SINE Compounds reduce oncoprotein synthesis through nuclear retention of their mRNAs

- mRNA is efficiently exported from the nucleus to the cytoplasm via XPO1/elf4E
- Cancer cells overexpressing XPO1 exhibit increased transport of oncogenic mRNAs (e.g. c-Myc) out of the nucleus
- In the cytoplasm, oncogene mRNAs are translated to oncoproteins
- SINE compounds inhibit XPO1/elf4E-mediated nuclear-cytoplasmic transport by covalently binding to the XPO1 cargo binding site
- In the absence of protein translation, oncoprotein levels rapidly decline

2) Cancer cells resist cell death

Reduces elf4E-dependent mRNA export of MYC, Bcl2/ Bcl6, CycD1, (etc) and inhibits NFκB
Selinexor
Selective Inhibitor of Nuclear Export (SINE) Compound
Selinexor
Selective Inhibitor of Nuclear Export (SINE)

Selinexor (KPT-330)

• An oral, first-in-class SINE compound

• Specifically and reversibly binds to the nuclear export protein exportin 1 (XPO1, CRM1) to inhibit the transport of over 200 cargo proteins with nuclear export sequences (NES), including tumor suppressor proteins (TSPs) and eIF4E, the carrier of oncogenic mRNAs

• Established safety profile

• Broad range of anti-tumor activity in hematological and solid malignancies

• 1,300+ patients treated with selinexor as of 1 December 2015, some with over 2 years on therapy

• More than 40 clinical trials ongoing in a broad range of hematologic and solid malignancies to evaluate selinexor as a single agent or in combination with established therapies

Selinexor MoA

Selinexor (KPT-330)

- Forms a covalent adduct at Cys528 in the XPO1 cargo binding pocket
- Inhibits XPO1-mediated nuclear export of TSPs\(^2\,^4\) and oncogenic mRNAs
- Forces G1 and/or G2 phase arrest and apoptosis in cancer cells
- Exhibits minimal toxicity in healthy cells with normal DNA
- Synergizes with chemotherapeutic agents, including many targeted agents\(^1\,^5\) that transiently induce expansion of TSPs
  - Selinexor potentiates TSP surveillance of DNA damage to restore apoptotic pathways and tumor cell sensitivity\(^5\)
  - Selinexor reduces the expression of DNA damage repair (DDR) proteins: MSH2, MSH6, PMS2, MLH1, Rad51 and CHK1\(^6\)
- Therapies, include cytarabine, platinums, taxanes, doxorubicin, etoposide, lenalidomide, dexamethasone, bortezomib, melphalan, topoisomerase I and II inhibitors, tyrosine kinase inhibitors

Selinexor inhibits XPO1 and forces nuclear localization of TSPs

Data on file, Karyopharm Therapeutics, Inc.
XPO1 inhibition impacts TSPs, oncoproteins and cell cycle regulators

Tumor suppressors, oncoproteins, and cell cycle regulators modulated by selinexor treatment

<table>
<thead>
<tr>
<th>Protein</th>
<th>Gene</th>
<th>Function</th>
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</thead>
<tbody>
<tr>
<td>Abl</td>
<td>ABL1</td>
<td>Proto-oncogene</td>
</tr>
<tr>
<td>APC</td>
<td>APC</td>
<td>Tumor suppressor</td>
</tr>
<tr>
<td>Bcl2</td>
<td>BCL2</td>
<td>Inhibitor of apoptosis</td>
</tr>
<tr>
<td>c-Myc</td>
<td>MYC</td>
<td>Proto-oncogene</td>
</tr>
<tr>
<td>Cyclin D1</td>
<td>CCND1</td>
<td>Cell cycle regulator</td>
</tr>
<tr>
<td>FOXO1a</td>
<td>FOXO1</td>
<td>Inducer of apoptosis</td>
</tr>
<tr>
<td>FOXO3a</td>
<td>FOXO3</td>
<td>Inducer of apoptosis</td>
</tr>
<tr>
<td>IkBα</td>
<td>NFKBIA</td>
<td>Inhibitor of NFκB</td>
</tr>
<tr>
<td>p21</td>
<td>CDKN1</td>
<td>Cell cycle regulator</td>
</tr>
<tr>
<td>p27</td>
<td>CDKN1B</td>
<td>Cell cycle regulator</td>
</tr>
<tr>
<td>p53</td>
<td>TP53</td>
<td>Tumor suppressor</td>
</tr>
<tr>
<td>pRB</td>
<td>RB1</td>
<td>Tumor suppressor</td>
</tr>
<tr>
<td>PP2Aα</td>
<td>PPP2CA</td>
<td>Cell cycle regulator</td>
</tr>
<tr>
<td>Survivin</td>
<td>BIRC5</td>
<td>Inhibitor of apoptosis</td>
</tr>
</tbody>
</table>

Selinexor induces G1 and/or G2 arrest in all cells and apoptosis in only cancer cells

Data on file. Karyopharm Therapeutics Inc.
• Tumor suppressor proteins (TSPs) function primarily in the cell nucleus to prevent the genesis of neoplastic clones by identifying damaged DNA and regulating cell growth and death pathways

• The nuclear export protein exportin 1 (XPO1, CRM1), which is overexpressed in cancer cells, transports tumor TSPs out of the nucleus, leading to their functional inactivation

• Selinexor, an oral, first-in-class SINE (Selective Inhibitor of Nuclear Export) compound, specifically and reversibly binds to XPO1 to block the export of TSPs from the nucleus, restoring their normal genome surveillance functions that lead to the detection and elimination of cancer cells, while largely sparing normal cells

• The safety profile of selinexor has been well established, with over 1,300 patients treated with selinexor in trials as of 1 December 2015, some with over 2 years on therapy

• More than 40 clinical trials are ongoing in a broad range of hematologic and solid malignancies to evaluate selinexor as a single agent or in combination with established therapies
Appendix
# XPO1 overexpression in cancer and anti-cancer effects of XPO1 inhibition

<table>
<thead>
<tr>
<th>Study</th>
<th>Objective</th>
<th>Outcomes</th>
</tr>
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<tbody>
<tr>
<td>Huang 2009¹</td>
<td>Assessed prognostic value of XPO1 expression in pancreatic cancer</td>
<td>XPO1 showed increased expression in pancreatic cancer tissue ((P=0.007)). High XPO1 expression was a prognostic indicator for PFS ((P=0.006)) and OS ((P=0.001)).</td>
</tr>
<tr>
<td>Inoue 2013²</td>
<td>Evaluated efficacy of SINE compounds in renal cell carcinoma</td>
<td>SINE compound induced increased cytotoxicity in renal carcinoma cells, increased apoptosis, and inhibited tumor growth.</td>
</tr>
<tr>
<td>Kojima 2013³</td>
<td>Assessed prognostic significance of XPO1 in acute myeloid leukemia and effects of a SINE compound</td>
<td>High XPO1 expression was associated with short survival; SINE compound–induced apoptosis mainly in a p53-dependent manner.</td>
</tr>
<tr>
<td>Noske 2008⁴</td>
<td>Examined XPO1 expression in ovarian tumors and ovarian cell lines</td>
<td>Cytoplasmic XPO1 expression was related to advanced tumor stage ((P=0.043)), poorly differentiated carcinomas ((P=0.011)), and higher mitotic rate ((P=0.008)); nuclear XPO1 expression was related to poor OS ((P=0.01)).</td>
</tr>
</tbody>
</table>

OS, overall survival; PFS, progression-free survival; SINE, Selective Inhibitor of Nuclear Export; XPO1, exportin-1.

XPO1 overexpression in cancer and anti-cancer effects of XPO1 inhibition  

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<tr>
<td>Pathria 2012¹</td>
<td>Investigated effect on human melanoma cell line of inhibiting overexpressed nucleocytoplasmic transport</td>
<td>XPO1 inhibition caused extensive apoptosis in melanoma cells while sparing nontransformed melanocytes and primary lung fibroblasts; apoptosis was preceded by G1 cell cycle arrest and was associated with a nuclear entrapment and down-regulation of survivin.</td>
</tr>
<tr>
<td>Schmidt 2013²</td>
<td>Examined preclinical XPO1 inhibition with a SINE compound in MM cell lines</td>
<td>SINE compound inhibited nuclear export function of XPO1 in human MM cell lines, actively induced apoptosis, and decreased MM cell viability.</td>
</tr>
<tr>
<td>Shen 2009³</td>
<td>Assessed whether XPO1, Ser10-phosphorylated p27, and p27 correlated with each other, with glioma pathological stage, and with patient outcome</td>
<td>XPO1 and p27 expression were associated with glioma grade; high XPO1 expression might be related to poor outcome; level of XPO1 and Ser10 phosphorylated p27 were significantly (P&lt;.001) and inversely correlated with p27 expression. The major finding was that XPO1 correlated with increasing grade of glioma; XPO1 was also an independent prognostic indicator for overall survival (P&lt;0.001).</td>
</tr>
</tbody>
</table>

MM, multiple myeloma; SINE, Selective Inhibitor of Nuclear Export; XPO1, exportin-1.

## XPO1 overexpression in cancer and anti-cancer effects of XPO1 inhibition (cont)

<table>
<thead>
<tr>
<th>Study</th>
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</tr>
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<tbody>
<tr>
<td>Tai 2014&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Characterized XPO1 function in MM, and further investigate the efficacy and molecular mechanisms of SINE compounds in MM</td>
<td>Higher XPO1 was significantly associated with a poor outcome ($P=.024$) for EFS and OS ($P=.044$); XPO1 downregulation with SINE compound decreased MM cell growth and survival and induced potent, rapid apoptosis of MM cells in vitro and in vivo and directly decreased bone resorption.</td>
</tr>
<tr>
<td>van der Watt 2009&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Investigated expression of the Karyopherins, XPO1, Karyopherin b1 (Kpnb1) and Karyopherin a2 (Kpna2), in cervical tissue and cell lines</td>
<td>XPO1, Kpnb1, and Kpna2 were overexpressed in cervical cancer; inhibiting the expression of XPO1 and Kpnb1 but not Kpna2 induced cancer cell death.</td>
</tr>
<tr>
<td>Walker 2013&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Assessed preclinical and clinical efficacy of XPO1-inhibitor selinexor in Ph+ leukemias</td>
<td>XPO1 was upregulated in a BCR-ABL1 kinase-dependent and kinase-independent manner and negatively controlled PP2A tumor suppressor activity; XPO1 inhibitor selinexor antagonized survival of TKI-resistant Ph+ acute leukemias in vitro in CML-BC animals and in a CML-AP patient.</td>
</tr>
</tbody>
</table>

BCR-ABL1, breakpoint cluster region Abelson leukemia homolog 1; CML-BC, chronic myelogenous leukemia-blast crisis; CML-AP, chronic myelogenous leukemia-accelerated phase; EFS, event-free survival; MM, multiple myeloma; OS, overall survival; PFS, progression-free survival; TKI, tyrosine inhibitor; XPO1, exportin-1.

**XPO1 overexpression in cancer and anti-cancer effects of XPO1 inhibition (cont)**

<table>
<thead>
<tr>
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<tr>
<td>Yao, 2009¹</td>
<td>Examined the expression of XPO1 in human osteosarcoma and normal cartilage tissues</td>
<td>High XPO1 expression was observed in osteosarcoma compared with normal tissue and was significantly associated with increased serum level of alkaline phosphatase ($P=.001$); significant association between XPO1 expression and tumor size ($P=.014$) and histological grade ($P=.003$); high XPO1 expression was a significant prognostic indicator for PFS ($P=.016$) and OS ($P=.008$).</td>
</tr>
<tr>
<td>Zhang, 2013²</td>
<td>Investigated the functional significance of XPO1 in MCL</td>
<td>XPO1 was highly expressed in MCL cells and was involved in regulating growth and survival mechanisms through the critical NF-kB survival pathway, independent of p53 status. Inhibition of XPO1 by SINE compounds in MCL cells resulted in significant growth inhibition and apoptosis induction.</td>
</tr>
</tbody>
</table>

MCL, mantle cell lymphoma; OS, overall survival; PFS, progression-free survival; XPO1, exportin-1.