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UNDERSTANDING THE PATHWAY

Targeting Cancer at the Nuclear Pore

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Nuclear protein transport regulates the function of cellular proteins in a compartmentalization-dependent fashion.[1] Proteins require an active form of transport through the nuclear pore that involves specialized transporters belonging to the karyopherin family (Fig 1).[2] The import of the majority of the cytosolic proteins is regulated by importins through nuclear localization signal recognition.[3] The export of nuclear proteins is almost exclusively mediated through exportin 1 (XPO1), also known as chromosome maintenance region 1 (CRM1), through the recognition of a conserved nuclear export signal (NES).[2] Nuclear export is a crucial aspect in the biology of any protein because mislocalization causes their functional inactivation. Most of the transcription factors and tumor suppressor proteins (TSPs), such as p53, IkB, p27, Rb, prostate apoptosis response-4 (PAR-4), and others, require nuclear retention and sequence-specific alignment to DNA to induce gene expression changes and transcriptional regulation of targets. It is logical to assume that the aberrant expression of nuclear transporters would result in unusual import or export of proteins, leading to their functional inactivation. Supporting this, there is ample evidence that cancer cells harbor an unusually higher expression of XPO1, making it an attractive therapeutic target to restore the proper localization of TSPs.[4]

XPO1/CRM1 can transport more than 200 target proteins.[5] Many of these cargoes are TSPs and transcription factors that can affect the majority of cancer hallmarks, driving the interest in the pharmaceutical industry as a target worth pursuing.[5] The first agent, leptomycin B (LMB), a natural product (originally developed as an antifungal compound), could specifically inhibit CRM1 export function.[6] LMB covalently binds to the NES recognizing Cys528 amino acid in XPO1/CRM1, thereby effectively blocking its target recognition.[7] Nevertheless, LMB showed numerous secondary effects and proved to be highly toxic and, therefore, was discontinued from a single phase I clinical trial.[8] Later, some modified forms of LMB (eg, leptomycin A) with better toxicity profiles were also tested preclinically.[9] Ratjadone is another natural product that works as a CRM1 inhibitor in a fashion similar to LMB.[10] In parallel, CBS9106 was developed as a potent CRM1 inhibitor[11] with a mechanism distinct from that of LMB.[12]

Around 2010, a series of specific inhibitors of nuclear export (SINEs) developed at Karyopharm Therapeutics (KPT) in Natick, Massachusetts (now located in Newton, MA), were presented at the American Society of Hematology annual meeting. These agents were shown to effectively block nuclear export function by binding to the Cys528 amino acid in the NES-recognizing domain of XPO1. Unlike irreversibly binding LMB, the SINE compounds bind Cys528 in XPO1 in a slowly reversible fashion. Their specificity has been validated using CRISPR/Cas9 genome editing where a single mutation in Cys528 renders the drug ineffective.[13] These agents showed cancer cell selectivity and broad activity against a spectrum of solid tumors and hematologic cell lines as single agents. Numerous groups in heme models have demonstrated that SINE could synergize with chemotherapeutic agents such as cyclophosphamide, doxorubicin, vincristine, and prednisone; BRAF inhibitors; mammalian target of rapamycin inhibitors; and dexamethasone. Studies also showed synergy with gemcitabine and platinum compounds in solid tumor models (reviewed in Turner et al[14]). SINEs could also suppress cancer stemness and reverse epithelial-to-mesenchymal transition, which is an event considered critical to metastasis.[15] These multimodel analyses ushered the SINE compound selinexor into multiple phase I and II clinical trials.

The two recent articles published in *Journal of Clinical Oncology* (*JCO*) present the results of multi-institutional clinical studies on selinexor.[16,17] The first *JCO* article, by Abdul-Razak et al,[16] presents results from a multicenter phase I study that evaluated selinexor (KPT-330) and determined the recommended phase II dose. The study enrolled 189 patients with advanced solid tumors who received selinexor (3 to 85 mg/m^2) in either 3- or 4-week continuous cycles. The study used gene expression to evaluate pre- and post-treatment levels of XPO1 mRNA in patient-derived leukocytes.[16] The tumor biopsies were also examined by immunohistochemistry for changes in markers consistent with XPO1 inhibition. The main outcomes include the observation of dose-dependent elevations in XPO1 mRNA in leukocytes up to a dose level of 28 mg/m^2 before reaching a plateau. In line with the proposed mechanism of action of the drug, paired tumor biopsies showed nuclear accumulation of key TSPs, reduction of cell proliferation, and induction of apoptosis. Among 157 patients evaluable for response, one complete and six partial responses were observed (n = 7, 4%), with 27 patients (17%) achieving stable disease for ≥ 4 months.[16]

In the second *JCO* article, Gounder et al[17] evaluated pharmacodynamic changes in tumor biopsies of 54 patients treated with oral selinexor administered twice per week (administered on days 1 and 3) at one of three doses (30 mg/m^2, 50 mg/m^2, or a flat dose of 60 mg) either continuously or on a schedule of 3 weeks on and 1 week off. Pharmacokinetic analysis was performed under fasting and feeding states (low- *v* high-fat content) and using various formulations of selinexor (tablet, capsule, or suspension).[17] Most strikingly, there

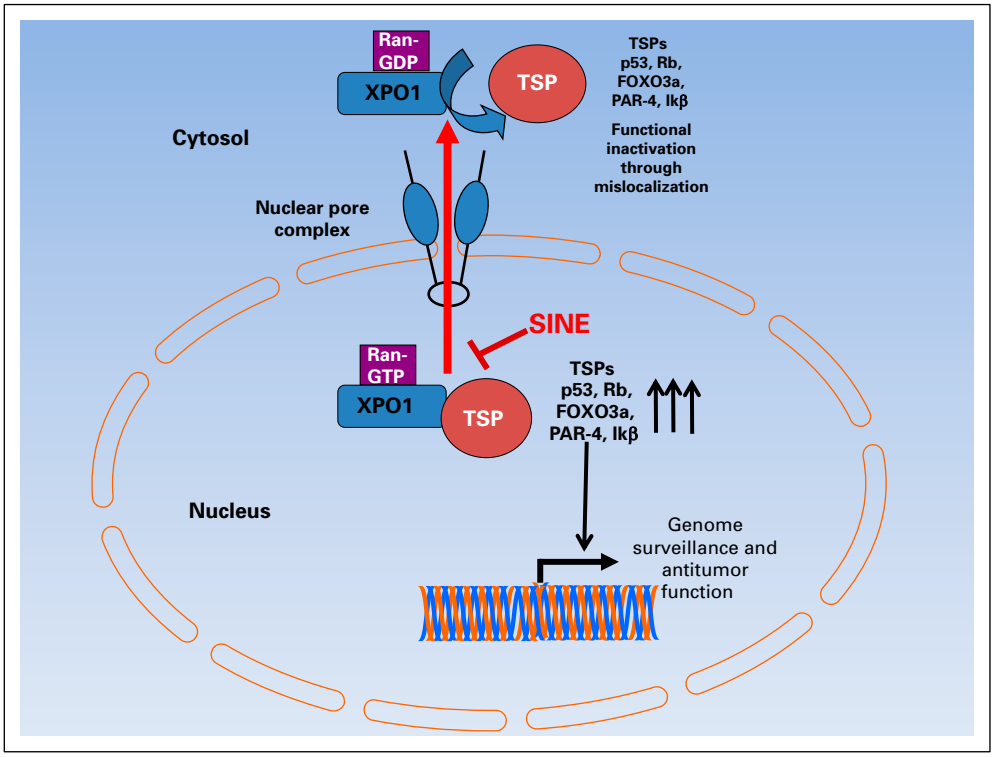

Fig 1. Nuclear protein export is a druggable avenue in cancer. Proteins with size larger than 40 kDa are exported from the nucleus to the cytoplasm with the aid of specialized carrier protein exportin 1 (XPO1), also known as chromosome maintenance region 1 (CRM1). XPO1 recognizes a nuclear export signal (NES) sequence in the target protein. NES is a short amino acid sequence of a few hydrophobic residues in the majority of proteins. XPO1 recognizes this NES, allowing attachment that results in movement of cargo protein from cell nucleus to the cytoplasm through the nuclear pore complex. Nuclear export of proteins occurs first through the binding of Ran-GTP (a G protein) to XPO1. Such binding causes structural alteration in XPO1, thereby increasing its affinity toward protein that is to be exported. Once the cargo protein is bound, the Ran-exportin-cargo complex moves out of the nucleus through the nuclear pore in a process that is controlled by the guanosine triphosphate (GTP)–guanosine diphosphate (GDP) concentration gradient. Once in the cytosol, the GTPase activating proteins (GAPs) then hydrolyze the Ran-GTP to Ran-GDP, and this causes structural alteration and subsequent XPO1 release. In the absence of Ran, the XPO1 molecule loses affinity for the nuclear cargo as well. Tumor suppressor proteins (TSPs) such as p53, forkhead box O3 (FOXO3a), prostate apoptosis response 4 (PAR-4), cyclin-dependent kinase inhibitor 1B (p27), retinoblastoma (Rb), and others are exported continuously through hyperactivation of XPO1 in cancer. Such excessive export leads to their functional inactivation through mislocalization. Specific inhibitor of nuclear export (SINE) compounds block nuclear export by binding to the NES recognizing Cys528 in CRM1, resulting in nuclear accumulation of important genome surveillance transcription factors and TSPs.

was evaluable nuclear retention of TSPs in paired tumor biopsies alongside decreased cell proliferation, increased apoptosis, and stromal deposition.[17] Although none of the 52 patients evaluated showed objective response (Response Evaluation Criteria in Solid Tumors [RECIST] version 1.1), 33% of the patients showed stable disease. Similar to the findings of Abdul-Razak et al,[16] this study also reported drug-related grade 1 or 2 adverse events such as nausea, vomiting, anorexia, and fatigue, which were well managed with supportive care. The commonly reported grade 3 or 4 toxicities in this study were anemia, thrombocytopenia, fatigue, leukopenia, and lymphopenia. Selinexor was better tolerated when administered as a flat dose on an intermittent schedule. Encouragingly, clinically significant major organ or cumulative toxicities were rare. It is likely that the recent introduction of KPT-8602 (an analog of selinexor with better toxicity profile) into a phase I trial (ClinicalTrials.gov identifier: NCT02649790) is to specifically address and overcome these toxicity-related issues of the parent compound.[18,19]

These are encouraging findings and could form the basis for the expansion into phase II clinical studies. Such efforts are under way, and a number of phase IB/II studies are ongoing, including studies of selinexor, gemcitabine, and nanoparticle albumin-bound paclitaxel in metastatic pancreatic cancer (NCT02178436), gliomas (NCT01986348), and gynecologic and metastatic breast malignancies (NCT02025985); selinexor plus sorafenib combination in leukemia (NCT02530476); and selinexor plus cyclophosphamide, doxorubicin, vincristine, and prednisone in non-Hodgkin lymphoma (forthcoming). In parallel, the SINE analog KPT-335 (verdinexor) has been evaluated in a phase I study with clinical benefits and partial response in spontaneous canine non-Hodgkin lymphoma.[20]

Despite these encouraging results, the optimism is still mixed with caution. Some unwelcomed oncogenes are bound to be also retained in the nucleus, causing an unfavorable balance between TSPs and oncogenes. High-throughput proteomic approaches may help evaluate the consequence of such global nuclear retention of proteins in cancer and normal cells to identify the mechanism of selinexor's cancer cell selectivity. It would also be worthy to investigate the epigenetic changes induced by selinexor in vitro, in vivo, and in patients given the emerging knowledge that aside from proteins, the export of noncoding RNAs is also regulated by XPO1.[21] These approaches are currently under way in our laboratory and may provide clues to better management of toxicity-related issues.

In summary, the recent publication of phase I studies on selinexor has certainly marked a major turn in the field of nuclear export inhibitors. These studies will definitely bolster the expansion phase and perhaps clinical approval of selinexor and related analogs for the treatment of cancer at the nuclear pore.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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