LINE-1 as a therapeutic target for castration-resistant prostate cancer

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1. ABSTRACT

Prostate cancer is the third leading cause of death by cancer in men. Surgery or hormone deprivation usually contains the progression of the local forms of the disease. In metastatic situations, chemotherapy or second generation hormone therapies are used with an overall survival that never exceeds 36 months when tumors become resistant to castration. In the search for new alternatives, clinical trials with various classes of anticancer drugs have been performed, including chemotherapies, targeted therapies with kinase inhibitors, radium-223, or immunotherapies with somehow limited efficacy. Targeting LINE-1 with reverse transcriptase inhibitors was also proposed as an attractive strategy as retrotransposons may play a role in the initiation and the progression of prostate cancers. After reviewing the biological rational to use RT inhibitors in the treatment of prostate cancers, we will discuss the results of the phase II trial evaluating the efficacy of Efavirenz in the treatment of castration-resistant prostate cancers with a particular emphasis on pharmacokinetics data that were obtained. We will also discuss the positioning of other RT inhibitors in the current therapeutic armamentarium.

2. INTRODUCTION

2.1. Epidemiology of prostate cancers

Prostate cancer is the most commonly diagnosed cancer in men aged over 50 years old. An estimated 1.1 million men worldwide were diagnosed with prostate cancer in 2012, accounting for 15% of all cancers in men. With an estimated 307,000 deaths in 2012, it is the third leading cause of death from cancer in men (http://globocan.iarc.fr). It represents a greater health concern in industrialized countries as compared to developing countries, in which the percentage of patients with prostate cancer is only 4% (1). Advanced age is the primary risk factor as approximately 80% of cases and 90% of deaths occur in men over the age of 65, but other environmental factors or lifestyles were also involved in the etiology of prostate cancers such as obesity, smoking, consumption of alcohol or anti-inflammatory drugs, Vitamine D and E, sexual activity, or exposure to pesticides (2,3). A potential impact of specific single nucleotide polymorphisms in susceptibility genes to those risk factors was also evidenced with more than 70 SNPs that were associated with an increased risk to develop a prostate cancer (4).
2.2. The genetic background of prostate tumors

Prostate cancer is a hormone-dependent cancer that displays a high level of heterogeneity. Since 1972, prostate tumors are classified using the anatomo-pathological Gleason grading system that reflects the differentiation state of tumor cells within tumor biopsies and that is still playing a determinant role to orient the treatment strategy (5). However, the development of new generation sequencing technologies and the systematic analyses of large panels of tumor samples led to the identification of genetic alterations that were used to define tumor subtypes with potential driving molecular lesions that could specifically be targeted (6–9). Apart from TP53 mutations and PTEN loss that are observed in a large percentage of prostate cancers (6,7), recurrent alterations have been described. They include fusions between the promoter region of androgen-regulated genes that are highly expressed in normal prostate such as TMPRSS2 (transmembrane serine protease 2) or SLC45A3 (solute carrier transporter 45A3), and the coding region of transcription factors of the ETS family, in particular ERG, ETV1 and ETV4 (10). These rearrangements are detected in more than 50% of prostate tumors in which these ETS genes are usually overexpressed (11,12). The ETS negative tumors include 10% of tumors with either SPOP mutations, overexpression of SPINK1 or disruption of CDH1 (accounting for ~20% of tumors), tumors with activating mutations of the RAS-RAF pathway, tumors with FGFR2 overexpression and tumors with IDH1 mutations. For the remaining 25% of prostate cancer samples, mutations that could explain tumor onset and/or tumor progression were either unique or unclear (10). Nevertheless, these data are in line with the future view of cancer treatment and the development of precision medicine.

3. THE TREATMENTS OF PROSTATE CANCER

3.1. Drugs that are clinically approved

While surgery or radiotherapy can be curative for the local forms of prostate cancers, androgen deprivation therapy using LHRH antagonists or agonists represents the gold standard for the treatment of high risk prostate cancers. These drugs block the testicular production of testosterone by preventing the release of luteneizing hormone secretion by a competitive inhibition for agonists of LHRH or a negative feedback mechanism for LHRH agonists (13). However, blockage of the androgen signaling axis is only temporary active and patients become resistant to castration (14). This pointed toward the need for drug alternatives to prolong survival of metastatic castration-resistant prostate cancer (mCRPC) patients.

The first chemotherapy that was approved for the treatment of advanced prostate cancer was mitoxantrone in 1998 (15). The microtubule poison docetaxel was approved only in 2004 and until recently it was the gold standard as first-line chemotherapy. Patients who received docetaxel and prednisone had a strong biological response and showed a statistically significant improvement in median survival of 2.5 months only (16,17). In 2010, cabazitaxel, another taxane that is less susceptible to drug transport by ABC transporters, was approved as second-line chemotherapy as it improved survival after treatment with docetaxel and was also beneficial to mCRPC patients who became resistant to docetaxel (18). The discovery that castration-resistant prostate tumors growth could still rely on the production of androgen by adrenal glands or by cancer cells themselves, led to the paradoxical hypothesis that mCRPC were not entirely refractory to hormone blockage, which further translated into the approval of two new generation hormone therapies: abiraterone acetate which inhibits CYP17A1, a key enzyme involved in androgen synthesis, and enzalutamide, an androgen receptor antagonist that competes for the ligand binding domain of the androgen receptor and inhibits multiple steps of the androgen-signaling pathway (19,20). Both abiraterone acetate and enzalutamide are conferring an additional increase of survival of approximately 5 months in post chemotherapy treatment (21,22), but their use in earlier stages of the disease, prior to chemotherapy (23,24), or in hormone-sensitive patients (25,26), could significantly increase overall survival, which may restructure the cards in terms of future treatment strategies.

Radium-223 dichloride is a radioactive isotope that was specifically developed for its activity against bone metastases (27). Its cytotoxic effect is linked to the generation of DNA double-strand breaks. It was approved in 2013 but is only prescribed for patients with bone metastases who are unfit for docetaxel, based on the results of a phase III trial showing a survival benefit of approximately 3 months when compared to placebo (28), but it is not accessible for all industrialized countries.

Sipuleucel-T is another alternative that was approved in 2010 for asymptomatic or minimally-symptomatic mCRPC (29). It is a cellular immunotherapy that can be considered as a vaccine. It is obtained from patients’ blood mononuclear cells along with antigen-presenting cells that are activated by a chimeric protein referred to as PA2024 (a fusion between prostatic acide phosphate and GM-CSF). Its approval was based on a phase III trial showing a prolonged overall survival of 4.1 months (30). Because of its complex manufacturing and its low therapeutic impact, it is mainly prescribed in the US.

3.2. Drugs that are in development

Despite the approval of new AR antagonists such as enzalutamide, tumors invariably develop...
resistance mechanisms. A comprehensive description of these mechanisms is out of the scope of this work but excellent reviews addressing this topic have been recently published (31–34). Usually, resistance to castration is predominantly related to alterations of the androgen axis signaling through amplification or mutations of the androgen receptor, to the expression of spliced-variants lacking the ligand binding domain of AR or to deregulation of AR co-factors (overexpression of AR co-activators and/or downregulation of AR corepressors). This conducted to the development of more potent antagonists such as apalutamide (ARN-509) that is the most advanced drug showing a robust response in phase II trial with >50% PSA decline in approximately half of the patients at 12 weeks (35). In order to target AR splicing variants lacking the ligand binding domain, new strategies were also envisaged and led to the identification of small molecules targeting the N-terminal domain of the receptor such as EPI-001 (36), or targeting protein chaperones such as HSP27, HSP70, HSP90 and clustatin involved in the maintenance of AR integrity and/or AR-mediated transcriptional activity (see (37) for review). While EPI-0056, an EPI-001 derivative deprived of PPARγ activity has just entered phase I/II clinical trial, HSP inhibitors that were developed to target all forms of AR, showed modest antitumor activity and relatively poor tolerability (37). Among all HSP inhibitors that were clinically tested, only OGX-011 targeting clustatin advanced to phase III trial in combination with docetaxel but failed to improve overall survival (38), and results of phase II trial with the antisense oligonucleotide OGX-427 targeting HSP27 are awaited based on a decrease of prostate tumor markers in phase I (39). Because targeting both full length and truncated forms of AR such as AR-V7 spliced variant remains a biologically relevant strategy, new generation HSP inhibitors are still under evaluation (37).

A growing number of studies show that castration resistance could also result from androgen-independent activation of other signaling pathways that are essential for cell growth (40–42). It is indubitable that a better understanding of these mechanisms was the driving force to identify alternative targets that already are or could be druggable. One striking example is the deregulation of the PI3K-AKT-mTOR pathway in castration resistant prostate cancer where PTEN loss or mutations of PI3KCA are frequently observed (43). Carver et al. nicely demonstrated that in a PTEN-null context, AR signaling and PI3K-AKT-mTOR pathway could regulate each other by a reciprocal feedback justifying the use of PI3K or AKT inhibitors alone or in combination with AR blockade to suppress tumor growth (44), justifying several trials that are currently ongoing (see (45) for a recent review).

In the same vein, a series of inhibitors targeting tyrosine kinases have been tested based on the fact that these kinases are either overexpressed or constitutively active in prostate cancer cells and/or in primary tumors or bone metastases (46). Thus, erlotinib or gefitinib targeting EGFR, lapatinib targeting HER2, imatinib targeting PDGFR, dasatinib targeting Abl and Src kinases, and antiangiogenic inhibitors targeting VEGFR such as sorafenib, sunitinib, cediranib, or cabozantinib were evaluated. Intriguingly, the results of these phase II or III trials were rather disappointing with no or modest activity except for cabozantinib that also targets c-MET. Despite a relatively strong preclinical rational, these negative results could be explained, at least in part, by the heterogeneity of the patients’ populations who were enrolled in these studies further emphasizing the need for a better molecular characterization of the tumors and a better selection of the patients who will benefit the most from these targeted therapies. This is even more critical as a growing number of kinase inhibitors will be approved in the years to come.

Even if the link between the immune system and the development of cancers has been known since the mid-90s with the pioneering studies of Erlich and the concept of immunosurveillance of Burnet, the emergence of immune checkpoint blockers as alternative options for the treatment of advance metastatic diseases is more recent. Some of the spectacular results that were observed in preclinical settings and the results of phase I trials, led to an overwhelming number of clinical trials for almost, if not all indications including prostate (47). The first category of drugs includes cytokines and “vaccines” that stimulate the cytolytic activity of lymphocytes or that increase the recruitment of natural cell killers to the tumor site. While none of the tested cytokines could improve survival, the use of vaccines was beneficial as prostate tumors express a large variety of antigens for which specific lymphocytes are already present in patients’ blood. As mentioned earlier, sipuleucel-T remains the sole immunotherapy of this class that is approved in prostate cancer, though new vaccines targeting other antigens are currently investigated such as PSA-TRICOM (PROSTVAC) that advanced to phase III trial based on encouraging results on survival (48). Antibodies targeting CTL-A4, PD1 or PD-L1 belong to another class of immunotherapies that blocks the antitumoral immune response (49). Conversely to melanomas and NSCLC, these molecules did not show any gain in survival or objective response in advanced prostate cancers (50,51). As several antibodies are being developed simultaneously, it remains to be seen whether these new drugs or combinations of these drugs could be more efficient. A third category includes molecules that inhibit the immnosuppressive effect of the microenvironment by limiting T-cell or MDSCs infiltration. This could be achieved with either VEGFR inhibitors such as sunitinib or inhibitors of immune-modulator factors leading to antiangiogenic effects.
such as tasquinimod. However, despite encouraging results in phase II, phase III were negative in mCRPC patients (82). These disappointing results may have to do with a peculiar immunogenicity status of prostate tumors, more specifically a low level of immune cell infiltration (53) and/or a low level of mutation load that is directly related to the efficacy of drugs targeting CTLA4 or PD1/PD-L1 (54). One possibility that is currently envisaged is to increase tumor infiltration using tumor vaccines and to associate anti-CTL-A4 or anti-PD1 drugs. Preliminary results with such kinds of combinations are encouraging and phase II trials are awaited.

Thus, many alternative strategies have been or are being evaluated to improve survival of mCRPC patients. Apart from “standard” chemotherapies, most of these drugs were developed based on specific gene alterations of the prostate tumors. In the following sections, we will describe the use of LINE-1 inhibitors as an alternative for the treatment of mCRPC. This is a rather unique type of strategy since it is targeting evolutionary conserved retroelements that are highly represented in the human genome and that may have a more predominant role in governing many aspects of cell growth and differentiation as initially thought (55).

4. LINE-1 AS A RATIONAL TARGET FOR PROSTATE CANCER

4.1. Role of LINE-1 in tumorigenesis

LINE-1 or long interspersed nuclear elements-1 are repetitive DNA sequences that belong to the family of retrotransposons, accounting for ~15% of the human genome (56). Among the estimated 500,000 copies that are present in a human genome, only 80 to 100 copies are full length and able to retrotranspose (57,58). LINE-1 encodes a bicistronic RNA transcript that is translated into a 40 kDa RNA-retrotranspose (57,58). LINE-1 RT activIty was correlated with breast cancer progression (84) and with proliferation and differentiation of various human cancer cell lines (85). Overexpression of LINE-1 was associated with hypomethylation of its promoter in both cancer cell lines (71) and a variety of tumor samples (86) with a greater hypomethylation in late stages of colonic carcinogenesis models or prostate cancers with high Gleason scores as compared to dysplatic or normal adjacent tissues (86,87). Therefore, hypomethylation of LINE-1 could be considered as a biomarker of cancer progression as it is associated with poor outcome and/or poor survival in prostate, colon, esophageal or non-small-cell lung cancers (87–90), with recurrences in liver cancer (91), aggressiveness of the tumor in ovarian cancers (75), or with lymph node metastases and resistance to therapy in younger patients with breast cancers (92).

While it is now admitted that LINE-1 reactivation could participate to tumorigenesis, it remains unclear how this process is occuring from a mechanistic standpoint. It was previously shown that expression of LINE-1, in particular ORF2p, was associated with insertional events that could lead to the production of several types of DNA lesions including mutations, deletions or large rearrangements, contributing to genomic instability in vitro and in vivo (93–98). Whether LINE-1-induced tumorigenesis is directly associated to these insertional events via the alteration of oncogenes or tumor suppressor genes functions is a possibility that was envisaged decades ago based on MYC activation and APC inactivation that were respectively observed in breast carcinoma and colon cancer cells (99,100). With new sequencing technologies, additional somatic transpositions of LINE-1 were further evidenced in lung, ovarian and colon cancers with an average number of insertions ranging from 4...
to 100 per tumor and a propensity of these events to occur within genes that are often deregulated in cancer (82,101,102). Insertional events are mostly detected in tumor suppressor genes and accompanied by a decreased expression of the corresponding genes (102,103), but gene activation (namely the ST18 gene) via a repression of its enhancer was also observed in patients with hepatocellular carcinoma (104). While these data argue in favor of transposition itself as a driver of tumorigenesis, it is suggested that such mechanism would be involved in a limited number of cases, especially in a context where multiple gene rearrangements are already present within a tumor (66,105,106). Another alternative would be that LINE-1 reactivation and de novo insertions are simply a consequence of tumorigenesis (55,64). Nonetheless, one has to consider that LINE-1 insertions mainly occur in intronic or intergenic regions that are known to harbor a myriad of transcription factors binding sites, pseudogenes, or other regulatory sequences such as small interfering or long non-coding RNAs. It is not unreasonable to think that disruption of these regulatory elements following LINE-1 insertion may induce a transcriptional reprogramming involving a variety of genes directly or indirectly controlling cell growth. Consistent with this view is the fact that ORF2p could modulate the expression of genes regulating epithelial to mesenchymal transition or stemness in colon cancer cells (62) and that LINE-1 inhibition induced the expression of endogenous miRNAs targeting tumor-suppressor genes in breast cancer cells (107).

4.2. Targeting LINE-1 in prostate cancers

As already mentioned, numerous studies have suggested a functional link between LINE-1 activity of retrotransposition and the development of prostate cancer with the notion that higher expression of ORF1p and enhanced ORF2p activity in later stages of the disease could be a potential target to inhibit tumor growth. Therefore, numerous studies investigated the biological consequences of LINE-1 inhibition in a variety of preclinical cancer models, including prostate cancer models, using RNA interference-based approaches to downregulate LINE-1 mRNA levels, or pharmacological inhibition of the reverse transcriptase activity with either nucleoside analogs (NRTI) or non-nucleoside inhibitors (NNRTI). Downregulation of LINE-1 expression using siRNA inhibited the growth of human A-375 melanoma and PC3 prostate cancer cell lines, promoted differentiation in vitro and reduced tumorigenic potential in xenografted mice (108,109), strongly supporting a causative role of LINE-1 expression in tumor onset and progression. Similar results were obtained with the NRTI abacavir in medulloblastoma cells (110) and in PC3 prostate cancer cells where it reduced cell growth, migration and invasion and induced senescence and cell death (111). These effects were correlated with a concomitant increase in ORF1 and ORF2 mRNA levels and transcriptional reprogramming of various cellular pathways (111). Other NRTI such as azidothymidine (AZT) and didanosine (DDI) used in combination induced a reduction of telomere length in vitro and a reduction of tumor volume in HCT116 colon cancer-xenografted mice (112). NNRTI such as efavirenz or nevirapine have also been tested on a large spectrum of cancer cell lines including breast (113), colon (114), thyroid (115), cervical cancer (116), pancreatic cancer (117), glioblastoma and osteosarcoma (70) and prostate cancers (108,118). It is surprising to see such an efficiency of these NNRTI as NRTI were shown to be more effective at inhibiting LINE-1 retrotransposition (119,120). This probably suggests that inhibition of retrotransposition itself may not be the sole mechanism involved in the anticancer activity of NNRTI. In the case of prostate cancers, both efavirenz and nevirapine induced a drastic reprogramming of undifferentiated tumor cells with the expression of differentiation markers such as PSA (prostate specific antigen) and a restoration of androgen receptor signaling both in vitro and in xenografted mice (108,118). Interestingly, such an increase in androgen signaling was accompanied by an increase in prostate cancer cell response to the AR natural ligand dihydrotestosterone and an increased susceptibility to the androgen receptor antagonist bicalutamide and to the tubuline poison docetaxel (118). Altogether, these data concur to the notion that pharmacological inhibition of LINE-1 by NNRTI such as nevirapine or efavirenz could not only suppress the growth of castration-resistant tumors by a transcriptional reprogramming of undifferentiated cancer cells, but could also re-sensitize prostate cancer cells to AR antagonists and/or to chemotherapy such as docetaxel.

4.3. The clinical evaluation of efavirenz in mCRPC patients

The first clinical evidence of the potential benefit to inhibit LINE-1 function to obtain tumor regression was observed with the NNRTI nevirapine in a patient with undifferentiated metastatic thyroid carcinoma who was refractory to iodide fixation. Following three months treatment with nevirapine, a significant re-differentiation of the tumor was noticed and was accompanied with a regression of several metastatic foci (121,122). Similar observations were also reported as isolated case reports for efavirenz, another NNRTI that binds to a hydrophobic pocket adjacent to the enzyme-active site and inhibits the activity of HIV-1 reverse transcriptase in a non-competitive manner (123). The first case described a spectacular prolonged survival of 14 months for a patient who was simultaneously treated with chemotherapy for metastatic non-small-cell lung cancer and with a highly active antiretroviral therapy.
(HAART) including efavirenz for a de novo HIV infection (124). The use of HAART was also associated with a complete regression of a myelodysplastic syndrome in an advanced HIV patient (125) and the regression of an infiltrating cervical spinal mass in a child with advanced HIV without any use of antitumoral treatment (126). Despite the fact that no functional link with LINE-1 inhibition were mentioned in these reports, the long-lasting cytostatic effect and the re-differentiating property of efavirenz that were observed in previous preclinical studies, together with the re-expression of prostate specific markers, strongly argued in favor of the evaluation of its potential clinical benefit in patients with metastatic castration-resistant prostate cancer. Moreover, efavirenz has a long plasma half-life (52 to 76 hours following single doses and 40 to 55 hours after multiple doses), which allows for once-daily dosing. In HIV-infected patients where the recommended adult dosage is 600 mg daily, time-to-peak plasma concentrations are reached in 3 to 5 hours and steady-state plasma concentrations are achieved in 6 to 10 days (127,128). A daily treatment with efavirenz was also extremely efficient to prevent tumour growth in xenografts of PC3 prostate cancer cells whether the treatment was introduced early (1 day) or late (7 days) after inoculation of the mice. Interestingly, the antitumoral effect seemed to be persistent even 14 days after discontinuation of the treatment with only a partial reversal of the cytostatic effect (108). The most commonly occurring adverse effects with efavirenz are related to central nervous system (CNS): dizziness, somnolence, insomnia, abnormal dreaming, confusion, impaired concentration, depression, nervousness, hallucinations, or euphoria (129). These symptoms begin within 1 to 2 hours after efavirenz dosing and generally lessen in frequency and severity over time. Most patients report resolution within few weeks. However, taken at bedtime, efavirenz improves the tolerability of the CNS-related adverse effects.

In the first multicenter phase II clinical trial evaluating efavirenz in mCRPC, 61 patients received 600 mg of the drug per os continuously. The results of the study showed a low PSA response rate (7.6% 95% CI 2.1. – 18.2.) and the median OS was 25.7. months (95% CI 21.1. -34.5.) (130). Despite the fact that primary objective of the trial was not met, it was striking to note a high variability in plasma concentrations of the drug that was actually comparable with previously reported concentrations in HIV patients ranging from 125 to 15,230 ng/ml (131,132). While such variability could be attributable to a poor absorption of the available pharmaceutical, it could also be linked to the presence of genetic polymorphisms in genes involved in the metabolism of xenobiotics such as cytochrome P450 2B6 (CYP2B6) that is known to metabolize efavirenz (133–135). However, the hypothesis of an increase of OS as a result of efavirenz treatment was supported by the exploratory analysis, which evaluated the clinical outcomes as a function of efavirenz plasma concentrations. Patients with plasma concentration >3000 ng/ml (that are similar to those showing a cytostatic effect in vitro), had a higher PFS (median of 17 months) and a higher OS (median of 49 months) as compared to patients with plasma concentrations <1500 ng/ml that would be below the cytostatic threshold with a median PFS of 3 months and a median OS of 18 months (unpublished data). In addition, for 13 patients, the increase in dose from 600 mg/day to 1200 mg/day was accompanied with an increase in PFS (median of 10 months) and OS (median of 30 months). Importantly, preliminary pharmacokinetic data show that at the 1200 mg/day dose of efavirenz, plasma concentration were above 3000 ng/ml for approximately 80% of the patients. The fact that a longer survival of patients with “high” plasma concentrations of efavirenz since the beginning of the treatment was observed as compared to patients for whom the dose was escalated only three months after the beginning of the treatment was consistent with an anticipated cytostatic effect of the drug.

Together, these data indicate that a higher dosage than 600 mg/day of efavirenz may offer interesting perspectives for the treatment of mCRPC patients. Before the approval of efavirenz for HIV treatment, a phase I study established the safety of 1800 mg/day single dosage and 1200 mg/day repeated dosage in healthy patients. In our phase II study, 13 patients received a dose of 1200 mg/day without any serious adverse event (Grade 3 or 4), indicating that higher dosages are well tolerated in prostate cancer patients despite their relatively advanced age (130). Indeed, although the median age of the treated patients was higher compared to other efavirenz toxicity studies, the safety profile of this NNRTI was confirmed and no new or unexpected adverse events were noticed, confirming that it is overall well tolerated with most adverse events being of grade 1 or 2. Only 10 treatment discontinuations (16.9.%) were caused by toxicities. In view of these results, a new phase I dose escalation study (from 1200 mg to 3000 mg) was conducted using a Baysian model and included 25 patients. A dose of 2200 mg of efavirenz was reached in patients without occurrence of severe adverse events and results of the trial are awaited.

5. CONCLUSION AND PERSPECTIVES

The treatment of castration-resistant prostate cancers started with the use of mitoxantrone in 1998, and no alternatives were available until 2004. The better characterization of castration-resistant tumors at the molecular level was a key step in the identification of potential drugable targets and lead to the approval of new drugs that could substantially increase overall survival of these patients. It is striking to note that numerous trials performed with drugs targeting spe-
pecific alterations of mCRPC showed only modest benefit despite encouraging preclinical data and several interesting responses. This is clearly emphasizing the need for molecular biomarker that could be used to select patients in further clinical trials. The LINE-1 inhibitor efavirenz is clearly another example entering this category with the unmet objectives of the first phase II clinical trial. Dose escalation studies could certainly address pharmacokinetic issues that could explain the lack of response to efavirenz in patients with low plasma concentrations, and results of this trial are awaited. But other alternatives may be envisaged including the investigation of other NRTI or NNRTI compounds that are currently approved in AIDS treatment. Indeed, in vitro cytotoxic activity has been demonstrated for abacavir and nevirapine in prostate cancer cells (111,118), and for zidovudine in prostate and breast cancer cells lines (136). Furthermore, entecavir and lamivudine are currently being tested in adjuvant settings for the treatment of hepatocellular carcinoma (NCT02650271, NCT00555334) or p53 mutant colon cancer patients (NCT03144804) and results are awaited. One would also consider using drug associations either between NRTI and NNRTI in order to potentiate LINE-1 transposition, or between NRTI or NNRTI and currently approved drugs such as docetaxel or enzalutamide based on a previous study demonstrating that interaction of LINE-1 with the adrogen receptor could increase AR transcriptional activity in a ligand-dependent manner (137). The coactivator function of LINE-1 would then represent an interesting option to re-sensitize prostate tumors to AR antagonists. In the meantime, ongoing efforts to identify retroelements loci in cancer genomes will certainly be valuable to identify new cancer biomarkers and new potential targets to counteract resistance to castration. As such LINE-1 inhibitors may still have a place in the current armamentarium, especially in this new era of precision medicine.

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**Abbreviations:** AR: androgen receptor; CDH1: cadherin 1; CNS: central nervous system; EGFR: epidermal growth factor receptor; ERG: ETS-related gene; ETV: ETS variant gene; FGFR: fibroblast growth factor receptor; GM-CSF: granulocyte-macrophage colony-stimulating factor; HAART: highly active antiretroviral therapy; HIV: human immunodeficiency virus; HSP: heat shock protein; IDH1: isocitrate dehydrogenase NADP+ 1; LHRH: luteinizing hormone releasing hormone; LINE-1: long interspersed nuclear elements-1; mCRPC: metastatic castration resistant prostate cancer; mTOR: mammalian target of rapamycin; NNRTI: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; PDGFR: platelet derived growth factor receptor; PI3K: phosphatidylinositol 3 kinase; PPARG: peroxisome proliferator activated receptor gamma; PSA: prostate specific antigen; PTEN: phosphatase and tensin homolog; RT: reverse transcriptase; SLC45A3: solute carrier transporter 45A3; SPINK1: serine peptidase inhibitor kazal type 1; SPOP: speckle type BTB/POZ protein; TMPRSS2: transmembrane serine protease 2; TP53: tumor protein 53; VEGFR: vascular endothelial growth factor receptor

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