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Review

Long interspersed nuclear element-1 mobilization as a target in cancer diagnostics, prognostics and therapeutics

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ABSTRACT

Long Interspersed Nuclear Element-1 (*LINE-1*) are DNAs that compromise 17% of our genome. *LINE-1* expression is triggered by environmental stressors and accomplished through its demethylation leading to genomic instability. Expression of *LINE-1* is regulated in adult somatic tissues through several endogenous defensive mechanisms, but is found to be associated with tumorigenesis in several cancers. This finding, has inspired the use of different indicators of *LINE-1* activation, as biomarkers in cancer diagnostics and even therapeutic targets in recent years. The objective of this review is to provide a critical examination of LINE-1 elements as companion cancer diagnostic/prognostic biomarkers and anti-cancer drug targets. In our view, there's great potential for LINE-1 serving at both forefronts, but there is a need for more mechanistic studies in the clinic as well as on the bench research to validate *LINE-1* activation elements as cancer biomarkers or therapeutic targets; in different cancer types and/or stages of the disease. In this context, development of minimally invasive, reliable and sensitive diagnostic tools for *LINE-1* activation elements for clinical use, is of priority.

1. Introduction

Transposons are DNA elements that can change their position within a genome (hopping genes), thus creating or reversing mutations [1] [2]. Transposons are present in all forms of life, from bacteria to human. It is estimated that approximately 44% of the human genome is comprised of transposons [3], separated by Class I (retrotransposons) and Class II (DNA transposons). Class I transposons are transcribed from DNA to RNA and then reverse-transcribed from RNA to DNA. This newly formed DNA can then be inserted to the genome DNA via a "copy and paste" mechanism, causing irregular gene expression. Class II transposons, on the other hand, do not need an RNA intermediate and can insert themselves into the genome using several transposase enzymes via a "cut and paste" mechanism. The majority of transposons in human genome are Class I (42.2% versus 2.8% of the total genome) [1] [4].

Long Interspersed Nuclear Element-1 (*LINE-1*) is one of the most abundant Class I retrotransposable elements comprising 17% of our genome with around 500,000 copies [5]. *LINE-1* is approximately 6000 base pairs long. It duplicates itself via an RNA intermediate and two encoded proteins: Open Reading Frame 1 (ORF1), *a* high-affinity RNA binding chaperone protein; and the Open Reading Frame 2 (ORF2) that has reverse transcriptase (RT) and endonuclease enzymatic activity [6].

Both ORF1 and ORF2 are known to play major roles in transposition. ORF1 is a 338 amino acid (~40 KDa) protein, with conserved C terminus among different species. ORF1 will form trimers at the highly variable N-coiled-coiled domain, which can bind to RNA in conjunction with ORF2, a 150 KDa protein, in *cis* format forming a LINE-1

Abbreviations: LINE1, Long Interspersed Nuclear Element 1; ORF1, Open reading Frame 1; ORF2, Open reading Frame 2; RNP, Ribonucleoprotein; TPRT, Target Primed Reverse Transcription; IHC, Immunohistochemistry; AQAMA, Absolute Quantitative Analysis of Methylated Alleles; ADH, Atypical ductal Hyperplasia; FEA, Flat Epithelial Atypia; DCIS, Ductal Carcinoma in situ; AJCC, American Joint Committee on Cancer; ESCC, Esophageal Squamous Cell Carcinoma; COBRA, Combined Bisulfite Restriction Analysis; MDR1, Multidrug Resistance Gene 1; AR, Androgen Receptor; TKI, Tyrosine Kinase Inhibitor; EMT, Epithelial Mesenchymal Transition; TGFβ1, Transforming Growth Factor beta 1; VEGFR, Vascular Endothelial Growth Factor Receptor 2; PDGFRB, Platelet-derived Growth Factor Receptor Beta; ARE, Androgen Response Element; CROP, Cisplatin-Resistance-Associated Overexpressed Protein; RT, Reverse Transcriptase; NIS, sodium/iodide symporter; NNRTI, non-Nucleoside Reverse Transcriptase Inhibitor; HAART, Highly Active Antiretroviral Therapy; EGFR, Epidermal Growth Factor Receptor; NSCLC, Non-small-cell Lung Carcinoma; HDAC, Histone Deacetylases

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ribonucleoprotein (RNP). This RNP is then transferred to the nucleus where ORF2 nicks nuclear DNA to make a single strand of DNA from the LINE-1 RNA template via target primed reverse transcription (TPRT). While it is known that ORF2 acts via TPRT through both endonuclease and reverse transcriptase activity, the mechanism of action of ORF1 in retrotransposition is perceived to be indirect, through regulation of ORF2 expression, recruitment of ORF2 to the RNP, delivering the RNP to the chromosomal DNA, and/or assisting strand exchanges during TPRT [7].

LINE-1 is highly active during early embryogenesis contributing to genetic sorting, but its activity is suppressed in somatic tissues, suggesting tight host regulatory mechanisms for retrotransposon control [8]. Environmental stressors such as oxidative and genotoxic stress, UV light, ionizing radiation, environmental hydrocarbons, infection, and injury, can reactivate *LINE-1*. Activated *LINE-1* and subsequent insertion gives rise to aberrant cellular phenotypes through functional knockout of genes, genetic mutations, or action as a new promoter for increased gene expression; all leading to DNA instability. Thus, it is not surprising that *LINE-1* and its role in carcinogenesis has become a major field of study in the past decade [2].

Irregular activation of *LINE-1*, has been implicated in tumorigenesis and tumor progression in different types of cancer [9] [10]. However, direct evidence validating *LINE-1* activity as a diagnostic/prognostic biomarker and/or therapeutic target in cancer is limited [11] [12]. The objective of this review is to provide a critical overview on the "status quo" of *LINE-1* and its associated elements (Fig. 1) as a potential companion biomarker in cancer diagnostic and prognostics, as well as a target for cancer treatment.

2. Common methods for quantitation of *LINE-1* activation in clinical specimens

Methods quantifying *LINE-1* activation are either based on assessing the hypomethylation status of *LINE-1*, or levels of *LINE-1* RNA, ORF1 or ORF2 proteins in tissue or blood samples. The most common quantification methods for the above targets are summarized in Table 1.

Quantification of the methylation status of *LINE-1* retrotransposable elements has historically been used to either determine the status of LINE-1 mobilization and retrotransposon activity or estimate global hypomethylation within samples [13,14].

To properly assess hypomethylation of *LINE-1* in tissue, a sample must be obtained from a clean tumor biopsy with little contamination of peritumoral tissue. The microenvironment of a tumor is not the same as the surrounding tissue, so a biopsy containing both (tumor and surrounding tissue), or samples from different regions of the tumor may show differing levels of hypomethylation due to the increased physiological stress within a tumor core as compared to the peripheral tissue. The increased variability may reduce the usefulness of findings. To address this, several recent publications have implicated circulating cell-free DNA as a potential prognostic and diagnostic target for several conditions, including breast, lung and colon cancer, and have promoted its potential as a minimally invasive screening technique [15–18].

Although the use of hypomethylation detection methods is very common, this technique does not provide *LINE-1* specific results as it does not ascertain the activation and mobilization of *LINE-1*. Measuring the specific products of *LINE-1* transcription and translation, i.e., ORF1 and ORF2, eliminates this concern and provides a more accurate understanding of the impact of *LINE-1* activation on cancer progression. Furthermore, proteins may have the advantage of being readily accessible in tissues as well as body fluids. The latter is important as it provides means for non-invasive sample acquisition from patients (from blood, plasma or other fluids) for use in protein detection and quantification.

Immunofluorescence staining and immunohistochemistry (IHC) of human tissues has been used widely for the detection and quantification of ORF1 in different types of cancers [19] [20]. Measuring ORF1 concentration in human blood has also been very recently reported using ELISA [21]. Similar to ORF1, antibody detection methods have also been used to quantify ORF2 expression in tissues via IHC [19].

3. *LINE-1* expression and activation as a companion biomarker in Cancer diagnosis

3.1. *LINE-1* hypomethylation status

LINE-1 hypomethylation may be an initiating factor in carcinogenesis, as significant demethylation is seen in pre-malignant lesions before cancer transformation in some cancer types [22]. Barchitta et al. conducted a meta-analysis study to determine if *LINE-1* hypomethylation had diagnostic value for cancer [22]. Nineteen unique articles were selected that included twenty different cancers and several methods for *LINE-1* hypomethylation detection. In addition, the data analyzed in this meta-analysis included both leukocytic blood and tissue samples. Overall, a total of 2554 cancer samples and 3553 normal samples were included. The authors concluded that *LINE-1* methylation levels were 6.4% lower in cancer patients compared to normal patients. This difference was significant in tissue, but not in blood [22].

Colorectal cancer is one of the most studied cancers for *LINE-1* hypomethylation status. *LINE-1* hypomethylation, as measured by absolute quantitative analysis of methylated alleles (AQAMA) realtime PCR, was shown to be an early event in colon cancer progression. *LINE-1* hypomethylation could be used to differentiate adenomas from normal colon mucosa because methylation was significantly lower in adenomas compared to normal tissue [23]. However, there was no significant difference in *LINE-1* hypomethylation between adenoma and carcinoma tissue implying the potential of *LINE-1* hypomethylation as an early biomarker of colorectal cancer, even before transition from adenoma to carcinoma. The ability to detect pre-cancerous adenomas is of great importance because of the curative potential of early surgical interventions in colorectal cancer and reduced risk of developing fatal colon adenocarcinoma [24].

Similar to colon cancer, *LINE-1* hypomethylation is an early event in breast tissue transformation, and is detected in atypical ductal hyperplasia (ADH) or flat epithelial atypia (FEA) [25]. In line with the observation in colon cancer, *LINE-1* methylation does not decrease significantly in the transition from hyperplastic states to breast carcinoma [25]. However, van Hoesel et al. reported conflicting findings that indicated the methylation levels of normal and ADH tissues were comparable, with significant changes only present in ductal carcinoma in situ (DCIS) and AJCC Stage 1 breast cancer [26]. Regardless, *LINE*-methylation status was found to be associated with breast cancer in both studies, indicating its potential as a companion diagnostic marker.

Esophageal cancer is among the top ten most fatal cancers, with a 5-year survival rate of 43% [27]. Early diagnosis of esophageal cancer improves 5-year survival, highlighting the importance of a reliable diagnostic biomarker for this cancer type. Unfortunately, the sensitivity of endoscopy screening for esophageal cancer is poor, with up to 40% of patients with surgically proven invasive disease presenting with a negative endoscopy [28]. Furthermore, there are no diagnostic biomarkers for esophageal cancer currently in use, and proposed biomarkers like COX2 and NF-κB (general markers of inflammation) are not specific to diagnosis of esophageal cancer [28]. Several studies have indicated that *LINE-1* is hypomethylated in esophageal squamous cell carcinoma (ESCC). Suggesting a possible environmental implication of LINE-1 in pathogenesis in esophageal cancer, Shigaki and authors described a significant correlation between smoking history and hypomethylation of non-cancerous esophageal mucosae in 109 patients with esophageal squamous cell carcinoma [29]. Using the combined bisulfite restriction analysis (COBRA) technique, Chalitchagorn et al. found an average10.58% decrease in *LINE-1* methylation in cancerous esophageal tissue compared to normal esophageal tissue [30]. Furthermore, 50.10%–77.39% of *LINE-1* was demethylated in ESCC [30]. Zhu et al.

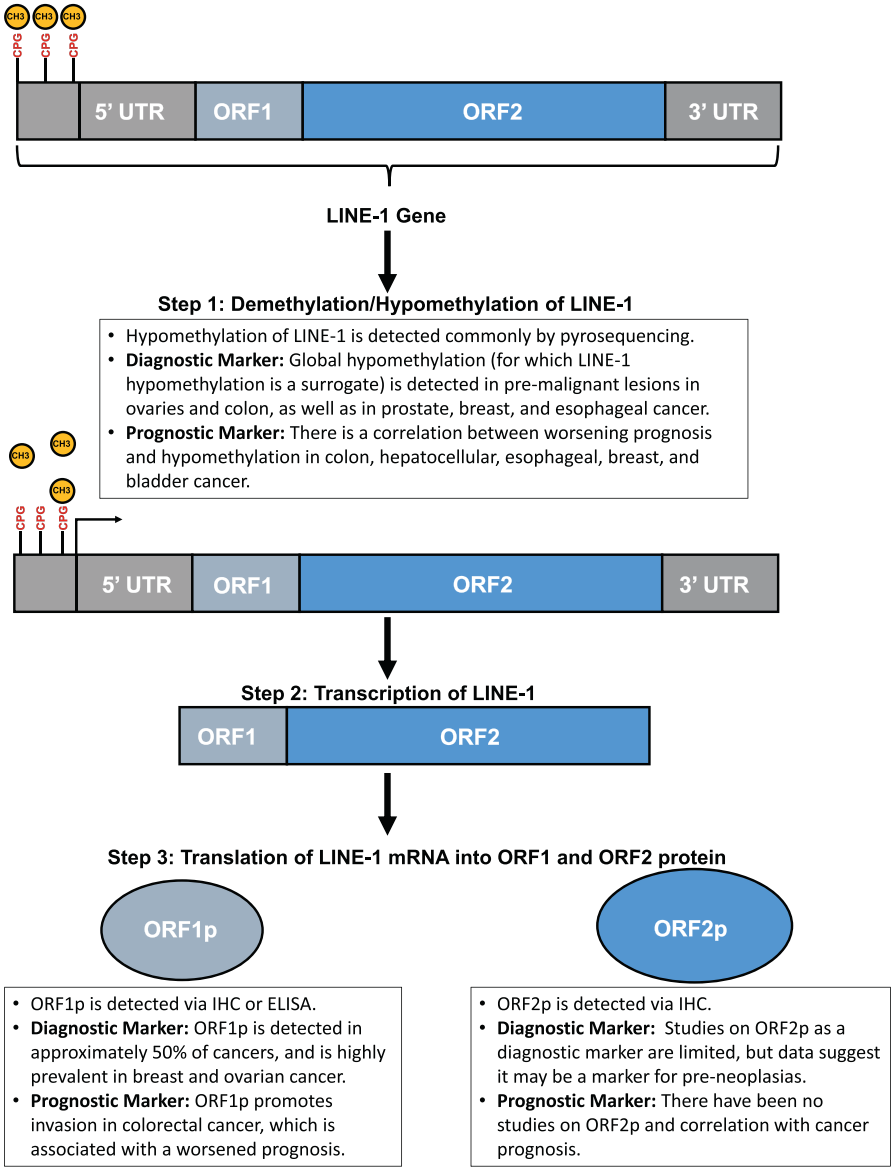

Fig. 1. Overview of LINE-1 as a diagnostic and prognostic marker for cancer. During oncogenesis, *LINE-1* gets demethylated. In this hypomethylated state, expression of *LINE-1* mRNA is increased, ultimately resulting in increased protein expression of ORF1 and ORF2. *LINE-1* hypomethylation and ORF1p/ORF2p levels have been studied as potential diagnostic and prognostic markers of several types of cancer.

further confirmed *LINE-1* hypomethylation in 310 cases of ESCC using real-time methylation specific PCR, and indicated that the total methylation of *LINE-1* was significantly decreased in ESCC tissue compared to non-tumor tissue [27].

Assessment of *LINE-1* methylation status has also been performed in prostate and ovarian cancer tissue samples, albeit to a lesser extent than

Table 1
Most common targets for the quantification of LINE-1 mobilization in clinical cancer specimens.

Target LINE-1 element	Clinical specimens	Common methods	Advantages	Disadvantages
LINE-1 methylation status	Tissue Plasma/Serum	Bisulfite Sequencing Methylation-specific PCR Primers or ELISA Cell-free DNA methods	- Routinely in use	- Not a specific indicator of *LINE-1* activity - Sample non-homogeneity, - Invasive
LINE-1 RNA	Tissue Plasma/Serum	RT-qPCR, ELISA No Published Cell-Free Methods	- Specific marker of Line-1 expression	- Experimental methods
ORF 1 expression	Tissue Plasma/Serum	IHC ELISA	- Specific marker of LINE-1 activity and post-translational control - Noninvasive sampling possible	- Needs mAb development or highly specific polyclonal antibody
ORF 2 expression	Tissue Plasma/Serum	IHC Not reported	- Specific marker of LINE-1 activity and post-translational control	- Needs mAb development - Requirement for high assay sensitivity

the cancers discussed above. Using methylation-sensitive restriction enzyme digestion of DNA isolated from tissue and a 32P-labeled probe specific for *LINE-1*, Schulz et al. found that 17/55 (31%) of prostate carcinomas examined had > 10% decrease in *LINE-1* methylation [31]. Furthermore, DNA methylation in this study was correlated to chromosome 8 instability, highlighting the potential use of *LINE-1* methylation status in identifying the pathogenesis of prostate cancer, as well [31]. Hypomethylated status of *LINE-1* in prostate cancer has been further confirmed by Santourlidis et al. who found 17/32 (53%) of examined prostate cancer tissues had significant hypomethylation of *LINE-1* as evidenced by PCR [32].

Pattamadilok et al. showed that *LINE-1* hypomethylation was an early event in the initiation of ovarian cancer [33]. Using COBRA-LINE-1 for methylation studies, they concluded that normal endometrial tissue had approximately 80% methylation of *LINE-1*. Whereas in tissues from patients with endometriosis, a risk factor for endometriosis-associated ovarian cancer, methylation averaged only 74.3% [33].

While the above studies support *LINE-1* as a biomarker in cancer diagnosis, contradictory results have been reported in the literature [34]. This might be due to low number of patients examined. Nevertheless, one must practice caution interpreting the reported results of hypomethylation data as for many cancers, *LINE-1* methylation status may serve as a surrogate marker for activation of other cancer related genes that are responsible for tumorigenesis. This is a major disadvantage for the use of *LINE-1* hypomethylation status as a biomarker in cancer diagnosis and prognosis.

3.2. ORF1 expression

Hypomethylation of *LINE-1* leads to increased activity of the retrotransposon and, consequently, an increase in translation of ORF1 and ORF2. In a seminal study by Rodic et al. utilizing tissue biopsies from a myriad of cancers as well as normal tissue from the surrounding tumor area, 47% of the 1027 neoplasms examined had detectable ORF1 as measured by IHC [35]. This was in contrast to the non-neoplastic tissue, which either expressed ORF1 in very low levels or not at all.

High levels of ORF1 was shown to be more prevalent in certain cancers, like breast and ovarian cancer. Other cancers such as renal, liver, and cervical cancer had little to no expression of ORF1 [11,35]. Rodic et al. reported that 90% of the breast cancer tissue samples examined, were highly positive for ORF1 [35]. Similarly, Chen et al. reported that ORF1 level is very high in ductal carcinoma in situ (DCIS) [36]. Furthermore, a correlation between ORF1 expression levels and loss of TP53 led the authors to conclude that ORF1 upregulation was an acquired feature in tumorigenesis [36]. Chen et al. further solidified the potential diagnostic value by reporting homogeneity of ORF1 expression throughout tumor tissue especially in very small biopsy samples, and possibly even in residual disease [36].

Rodic et al. also reported that out of 31 cases of ovarian cancer examined, approximately 90% were positive for ORF1 via IHC, and this was further confirmed by Ardeljan et al. [11,35]. Like breast cancer, ORF1 level was highest in high-grade ovarian carcinoma, and also correlated to the loss of TP53 [35].

The expression of ORF1 in prostate cancer is not fully confirmed. Ardeljan et al. reported ORF1 positivity in 41% of all prostate cancer tissue samples examined [11]. Clearly, more comprehensive studies are required before the extent of ORF1 expression can be fully elucidated in prostate cancer, including the state of disease and location of tissue section within or around the tumor boundaries. We have recently reported success in the development of a blood-based test to quantify ORF1 which was able to successfully delineate suspected prostate cancer patients who had PSA values in the “gray zone” of 4–10 ng/mL [21]. Same method of quantification has also been used by our group to quantify ORF1 levels in the blood of a group of smokers presenting for CT scan for lung cancer. Using the CT scan report, 83 subjects were initially grouped into two major categories: those with negative chest CT result (52 individulals) and others who were considered to be positive (31 individuals). The mean ORF-1 concentration in subjects with suspicious nodule(s) based on CT scan who were recommended for further follow up ($n = 31$) and those with negative lung CT scans ($n = 52$) were 14.2 ± 13 and 8.5 ± 8.0 ng/mL, respectively ($P = .024$, Mann-Whitney Rank Sum) [37].

3.3. ORF2 expression

While a large body of work has focused on *LINE-1* methylation and ORF1 expression, the data on ORF2 as a diagnostic marker of cancer is limited. Based on the present data, ORF2, similar to *LINE-1* hypomethylation, may be a better diagnostic marker for pre-neoplasias. In addition, since *LINE-1*'s “copy and paste” mechanism of action relies heavily on ORF2's reverse transcriptase activity, ORF2 expression could serve as a functional biomarker of *LINE-1* activity. However, there are challenges in measuring ORF2 expression. Concentrations of ORF2 have been reported to be 10,000-fold less than ORF1 [21]. Furthermore, while there are a handful of reliable ORF1 antibodies for IHC and we have recently reported one for an ELISA-based assay [21], very few antibodies are available for ORF2 detection and quantification.

De Luca et al. was one of the first groups to develop an ORF2 antibody for IHC. They showed high expression of ORF2 in transitional colon mucosa and no expression in normal colon mucosa [19]. ORF2 was also detected in prostate intraepithelial neoplasias (PIN). The authors, therefore, concluded that ORF2 expression occurs early in the tumorigenesis process, and may be a useful early diagnostic marker of colon and prostate neoplasia.

4. *LINE-1* expression and activation as a biomarker in Cancer prognosis

4.1. LINE-1 hypomethylation

Similar to cancer diagnosis, the body of evidence supporting the prognostic potential of LINE-1 has primarily focused on identifying the methylation status of the promoter, while less attention has been paid to the *LINE-1* protein products. For many types of cancer, hypomethylation of *LINE-1* has been shown to correlate with cancer prognosis. One example is colorectal cancer prognosis that is shown to correlate well with the hypomethylation level of *LINE-1* or global hypomethylation [38–41]. Ognio and authors measured *LINE-1* hypomethylation as a prognostic indicator for colon cancer by examining 643 colon cancers from two cohorts at different stages of disease [41]. They found decreased methylation correlated to worsening prognosis. A receiver operator characteristic curve indicated that a 30% decrease in global methylation levels correlated to a colon-cancer specific hazard ratio of 2.37 and a global mortality hazard ratio of 1.85. More recently, Kaneko et al., identified hypomethylation of *LINE-1* in primary tumors from 40 colorectal cancer patients (used as a surrogate for global hypomethylation) as an independent prognostic marker in colon cancer [40]. In 2017, Swets et al. considered that unlike colon cancers, rectal cancers have low rates of microsatellite instability-driven tumors, thereby hypothesizing they would be less prone to *LINE-1* activation as an initiating event in tumorigenesis [42]. In line with this understanding, a significant correlation between *LINE-1* hypomethylation and overall survival of stage II (or lower) colon cancer patients (not colon and rectal cancer cases) was observed.

The risk of developing aggressive hepatocellular carcinoma has been linked to *LINE-1* hypomethylation [43]. Zhu et al. demonstrated that hypomethylation of three specific sites on the 5′-untranslated region of *LINE-1* were highly associated with poor prognosis, advanced stage, risk of metastasis and vascular invasion [27]. Further, the authors also reported that *LINE-1* hypomethylation is associated with activation of the c-Met oncogene due to *LINE-1* insertion upstream of c-Met, a high-affinity receptor for hepatocyte growth factor and critical element

in growth and metastasis of hepatic tumors. Gao and authors also report poor prognoses in patients showing marked hypomethylation of two 5-UTR sites not investigated in the Zhu manuscript [44].

Esophageal, breast, bladder and lung cancers show significantly worsening prognosis when *LINE-1* is hypomethylated [25,26,29,45–47]. For instance, early progression of gastric cancer from metaplasia to adenoma has been associated with *LINE-1* hypomethylation in tissue, where methylation levels < 50.90% were significantly associated with decreased overall survival and disease-free survival [48]. Breast cancer progression has also been linked to a progressive decrease in *LINE-1* methylation levels [25] particularly in primary tumors [26]. *LINE-1* methylation was reported to be decreased in esophageal squamous cell carcinoma (ESCC) compared to normal tissue in 310 patients [49]. In addition, a decrease in *LINE-1* methylation was associated with worsened prognosis. In patients who had a total methylation index (MI) of < 0.78, the mean survival was 34 months, while patients with an MI of > 0.78 had a mean overall survival of 43 months [49].

4.2. ORF1 expression

The importance of ORF1 to *LINE-1* function, lends to the hypothesis that ORF1 may serve as a biomarker of LINE-1 activity and be of value as a companion diagnostic and prognostic biomarker in the clinic. Direct evidence validating the value of ORF1 as a biomarker in different types of cancer are somewhat limited, however. In this context, Su et al. reported a close association between expression of ORF1 and stage of malignant germ cell tumors at the time of diagnosis. They highlighted that this increased expression was in contrast to the peritumoral normal tissue, which had low or undetectable levels of expression [50]. Harris and authors, on the other hand, demonstrated that nuclear localization of ORF1 to be associated with poor prognosis in breast cancer. Further, individuals expressing high levels of nuclear ORF1 were more likely to have distant metastasis and significantly reduced overall survival and disease-free survival [51]. Chen and authors built upon the work of Harris et al. by investigating the association of not only ORF1, but also ORF2 localization with cancer prognosis [36]. They reported that nuclear localization, but not cytoplasmic expression, was strongly correlated to survival, and that nuclear localization of both proteins increased as prognosis worsened. Interestingly, the authors report that expression of ORF1 and ORF2 in pre-invasive cancers (ductal carcinoma in situ) was primarily cytoplasmic and only a subset of invasive carcinomas exhibited nuclear ORF1 and ORF2, and this expression was strongly associated with a poor prognosis.

4.3. ORF2 expression

Few authors have examined the correlation of ORF2 and cancer prognosis. De Luca et al. describe enhanced expression of *LINE-1*-encoded ORF2 in early stages of colon and prostate transformation [19]. As mentioned previously, Chen et al. describe the importance of nuclear localization of ORF2 to the prognostic value of this protein in cancer [36]. In non-neoplastic breast tissue, they report frequently undetectable levels of ORF2 in either the cytoplasm or nucleus, while nuclear expression of ORF2 (in addition to ORF1) was found to be an ominous prognostic marker. Interestingly, their findings describe the majority of ORF1&2 being cytoplasmic in non-invasive ductal carcinoma in situ (DCIS) and not associated with an increase in morbidity and mortality. The authors surmised this could be due to the fact that *LINE-1* activity is frequently detected in early tumor development. By the time the tissue has escaped the clonal expansion stage, the increased genetic diversity afforded by retrotransposon activity may be unnecessary or even harmful to tumor growth, albeit the low number of DCIS samples included in the study may not have been sufficient to make a more specific claim.

De Luca and authors developed a highly-sensitive monoclonal antibody to quantify and localize ORF2 in early stages of colon and prostate transformation [19]. They report similar findings to what Chen et al. reported in breast cancer; in prostate and colon cancer, normal tissues express ORF2 at very low or even undetectable levels, while expression increases as the cancer progresses. Furthermore, they found evidence to support a biphasic nature of ORF2 expression in carcinogenesis, with (1) an initial burst of LINE-1 activity which produced large amounts of ORF1 and ORF2 as tissues transform from normal to precancerous, and (2) a second, broader wave corresponding to massive retrotransposition events as tissues progress from pre-cancerous to overtly cancerous.

5. *LINE-1* expression and activation as a companion diagnostics of response to Cancer therapy

Acquired or inherent drug resistance within subpopulations of heterogeneous tumor cells presents a significant impediment to successful cancer treatment. There is limited clinical data examining *LINE-1* expression and activity as a biomarker for resistance to cancer therapy, but several cell-based studies have implied *LINE-1* dysregulation to be mechanistically linked to therapy resistance in cancer cells.

One of the few clinical studies examining *LINE-1* and its role in drug resistance focuses on esophageal squamous cell carcinoma (ESCC) [49]. In addition to the association of *LINE-1* methylation status with cancer prognosis, a correlation with the hypomethylation index in cancer tissues and increased expression of MDR1 has been reported in a population of 310 ESCC patients. MDR1 is one of the main mechanisms for drug resistance of cancer cells, as overexpression of this protein increases the amount of the cancer drug that is pumped out of the cancer cell. Although the authors do not show a direct relationship between increased *MDR1* expression and *LINE-1* decreased methylation, they did show that in the few cases of ESCC where *LINE-1* was hypermethylated, these patients had decreased expression of *MDR1* [49], implying an association between *LINE-1* hypomethylation and multi-drug resistance in ESCC.

Mechanistic studies on cell lines implying a role for *LINE-1* expression and mobilization as a biomarker of resistance to conventional chemotherapeutics are more abundant in the literature. The majority of this work focuses on *LINE-1*'s role in overexpression of ATP-binding cassette transporter (ABC) MDR1, androgen resistance in prostate cancer, resistance to tyrosine kinase inhibitors (TKIs), resistance to cisplatin, or other platinum drugs.

For instance, *LINE-1* has been proposed to play a role in androgen-independent activation of androgen receptor (AR) in prostate cancer [52]. *LINE-1* can randomly insert into the genome and promote the overexpression of AR as well as create splice variants of the AR gene that may be ligand-independent [52]. This, in turn, activates several pathways that increase cell proliferation and growth. Lu et al. have shown that the coiled-coil domain of ORF1 directly binds to AR and aids in translocation of AR from the cytoplasm to the nucleus [53]. Ultimately, this results in activation of AR-mediated cellular proliferation pathways, even in the absence of AR ligand, leading to development of castration-resistant prostate cancer [53].

Another example from Reyes-Reyes et al. who have shown *LINE-1* dependent epithelial to mesenchymal transition (EMT) and malignancy in human bronchial epithelial cells (BEAS-2B) through a TGFβ1-*LINE-1* mechanism [54]. When BEAS-2B cells were treated with TGFβ1, EMT was marked by an increase in vimentin and a decrease in epithelial-associated cadherin [54]. Furthermore, both ORF1 and ORF2 mRNAs were upregulated when cells were treated with TGFβ1. To further confirm the role of *LINE-1* in the TGFβ1 signaling pathway, an ORF1-specific siRNA was introduced into the cells. This siRNA caused complete knockdown of ORF1, but did not affect TGFβ1 levels, indicating that *LINE-1* is downstream of TGFβ1. Overexpression of either wild-type *LINE-1* or mutant *LINE-1* lacking RT activity was sufficient to induce EMT in the BEAS-2B cells. Sunitinib maleate, a VEGFR2/PDGFRB

inhibitor, did not decrease proliferation in BEAS-2B cells over-expressing either wild-type or mutant *LINE-1* [54]. In addition, resistance to sunitinib maleate was marked by an increase in (phospho) ERK1/2 and (phospho)AKT, two survival pathways that are commonly upregulated during the course of drug resistance in cancer cells [54]. Thus, *LINE-1* plays a direct role in EMT as a downstream component of TGFβ1 signaling and promotes drug resistance by over-activation of survival pathways. Surprisingly, this effect occurs even without intact RT activity.

Feng et al. investigated the association between *ORF1* expression and resistance to epirubicin, cisplatin, and paclitaxel in hepatocellular carcinoma (HCC) HepG2 cells [55]. They found a 3-fold increase in the IC_{50} of cisplatin in cells transfected with *ORF1* compared to control, a modest increase in IC_{50} of epirubicin, and a statistically insignificant change in the IC_{50} of paclitaxel. Transfection of cells with *ORF1* siRNA significantly decreased the IC_{50} for all three drugs by up to 9-fold [55]. Overexpression of ORF1 also caused an increase in cell proliferation, increased expression of the MDR1 protein, and promoted transcription of the androgen response element (ARE), indicating a direct role for ORF1 in mediating drug resistance. Furthermore Feng et al. found that the increase in proliferation was likely due to downregulation of p21, p15, and p27 proteins which are involved in checkpoint inhibition of the cell cycle [55]. Overexpression of ORF1 decreased apoptosis in HepG2 cells when they were treated with epirubicin, cisplatin, or paclitaxel and this was marked by an increase in BCL-2 protein. Finally, co-immunoprecipitation studies with ORF1 constructs have indicated a physical interaction between ORF1 and cisplatin-resistance-associated overexpressed protein (CROP or LUC7L3) [1]. Taken together, these data indicate a clear role for ORF1 in chemotherapeutic resistance, although the full mechanism is yet to be elucidated.

More recently, the association between *LINE-1* expression and effectiveness of cancer immunotherapeutics have attracted attention [56]. In these cases, *LINE-1* hypomethylation may be a positive indicator of response, perhaps through potential increase in immunogenicity of tumor. In this context, *LINE-1* can serve as a therapeutic target particularly in metastatic cells. This aspect is discussed more towards the end of the following section.

6. *LINE-1* as a therapeutic target in cancer

It is known that *LINE-1* activation leads to the synthesis of ORF1 and ORF2 that mediate active retrotransposition events only when reverse transcriptase (RT) function is preserved in the cells. Recent studies, however, have provided evidence that the genome reprogramming by *LINE-1* can involve selective deregulation of genes via RT-independent mechanisms [57].

LINE-1 expression and activation has been associated with an increase in cancer cell proliferation [58], tumorigenesis [59] [60], differentiation [61], EMT transformation [62] and metastasis [63] in different types of cancer (reviewed in [64]). Several studies have shown overexpression of LINE-1 transcribed factors in different cancer cell lines compared to normal cells, as well [65]. The question is whether differences in the activation and expression of *LINE-1* in cancer versus normal cells can be exploited to develop new cancer therapeutics.

Despite indications of *LINE-1* expression and activity in cancer cells or cancerous tissue, the direct evidence confirming a causative (driver) role for *LINE-1* mobilization in cancer emergence and progression, validating *LINE-1* as a therapeutic target, are not that frequent in the literature. Besides, recent data shows a discrepancy over the role of *LINE-1* expression in different subpopulations of heterogeneous tumors [57].

As an evidence of direct involvement of *LINE-1* expression in cancer, inactivation of specific RT-encoding ORF2 elements using antisense oliogonucleotides has been shown to inhibit the proliferation of human hepatoma cells (Hep3B) [65]. In another study, inhibition of reverse transcriptase (RT) coding *LINE-1* elements by RNA interference (RNAi) reduced proliferation, induced morphological differentiation and reprogrammed gene expression in melanoma and prostate carcinoma cell lines [58]. Specifically, double stranded RNA oligonucleotides against *LINE-1* expression that reduced expression of *ORF1* and *ORF2* by 80% in A375 cells compared to control cells receiving irrelevant RNA oligonucleotides, showed a significant reduction in the expression of RT proteins, increased differentiation, reduced growth, and reduced expression of *c-myc* and *cyclin-D*. The effects were in line and comparable with those seen following treatment of cells with pharmacological inhibitors of RT, nevirapine and efavirenz. *LINE-1* expression has also been reported to affect cell adhesion, inflammation and cellular metabolism process in Hep G2 cells [57]. Apostolou et al. have reported a ten-fold increase in the number of cells undergoing apoptosis, a 5-fold increase in the number of dead cells, a decrease in the expression of genes for two transcription factors of embryonic stem cells, *NANOG* as well as *Sox2* genes, and a decrease in the expression of epithelial to mesenchymal transformation (EMT) markers following suppression of *LINE-1 ORF2* by siRNA [66]. However, it is not clear how stemness is being identified and maintained *in vitro* in this study.

In accordance with the activation of *LINE-1* RT enzymes in cancer, several known RT inhibitors have been exploited as potential therapeutic agents in cancer [67]. However, in most studies, a direct association between specific inhibition of RT coded *LINE-1* by the RT inhibitors and their observed anticancer activity has not been established. RT inhibitors, such as nevirapine and efavirenz, induce three major effects in tumor cell lines and *primary* tumor cells from patients: (i) reduce cell proliferation rate; (ii) induce cell differentiation; and (iii) lead to global reprogramming of gene expression [68]. Notably, the effects of RT inhibitors are found to be reversible upon discontinuation of treatment and do not lead to tumor eradication, but rather keep tumor progression in check. For instance, upon treatment with a well-established RT inhibitor, nevirapine, decreased cell growth in 9 different murine and human progenitor and tumorigenic cell lines have been observed [69]. Nevirapine treatment was also shown to facilitate the differentiation of several cells, including C2C7 myogenic precursor cells, multipotent F9 teratocarcinoma, Kasumi-1 cells as well as primary AML blasts. The onset of differentiation was accompanied by reduced cell growth in treated murine C2C7 myogenic progenitor cells and in multipotent F9 teratocarcinoma cells. Moreover, human acute myeloid leukemia (AML) cell lines and primary blasts from two AML patients blocked at different differentiation stages, all underwent differentiation in response to nevirapine exposure.

The same group has reported high levels of RT activity in undifferentiated thyroid tumor cells. Consistent with earlier results, they found RT inhibitors to reversibly reduce tumor growth in human undifferentiated thyroid tumor cells, increase cells in G_0/G_1 phase, with no effect on the level of apoptosis or necrosis. Similar results were reported by Dong et al. following treatment of human thyroid anaplastic carcinoma cells with nevirapine. In this study, there was a synergistic effect between nevirapine and TSH treatment in the induction of the sodium/iodide symporter or NIS gene. The NIS is responsible for the uptake of radio-iodine, potentially making the thyroid cancer cells more responsive to this standard radiation therapy of thyroid cancer [70]. There have also been reports on nevirapine treatment inducing cell differentiation and radioiodine uptake in poorly differentiated thyroid tumors, *in vivo* [71].

In line with the above reports, Sciamanna et al. also observed reversible inhibition of cell proliferation with a particular increase in the proportion of G_0/G_1 cell population, following long term exposure of nevirapine and efavirenz (in addition to siRNA against *LINE-1* expression) in cells from A-375 melanoma, PC3 prostate carcinoma and TVM-A12 primary melanoma-derived cells. Neither inhibitor caused significant induction of cell death in A-375 or PC3 cell lines. Reversible morphological changes consistent with differentiation of cell lines and melanoma primary cells were also observed by electron and confocal microscopy upon treatment of cells with RT inhibitors. The results also

showed reversible induction of androgen receptor (AR) and prostate specific antigen (PSA) genes in PC3 cells, and that of E-cadherin as well as down regulation of *Bcl2, c-myc* genes in melanoma cells following treatment with RT inhibitors.

In line with the *in vitro* findings, nude mice continuously treated with efavirenz at a dose of 20 mg/kg, had inhibited growth of H69, H29, A375 and PC3 tumors. Furthermore, efavirenz-pretreated PC3 cells showed a significantly reduced tumor-forming ability *in vivo*, and xenografts grew more slowly. Although no direct evidence is provided that the function of RT inhibitors in these cases are related to *LINE-1* RT inhibition, the authors argue that the absence of senescence-specific modifications in the cells, and the rapid induction of differentiation, indicate that the RT inhibitors do not target telomerase-associated RT (TERT), and induce a low-proliferating differentiated phenotype rather than senescence. The authors also conclude that the similarity of phenotypes induced by RNAi versus RT pharmacological inhibitors indicates that inhibition of *LINE-1* expression, or of RT activity, is sufficient to delay proliferation and promote differentiation and rule out any role for non-specific drug interactions leading to the above observations [58].

A new family of NNRT inhibitors, namely F_2-DABOs, originally developed as anti-HIV medications have also been tried for the same purpose in human melanoma cell line A375. Lead compounds from this RT inhibitor library have shown anti-proliferative activity, helped facilitate differentiation of cells, and inhibit tumor growth in nude mice [72]. Following these studies, Carlini et al., investigated the anticancer activity of Abacavir, a nucleoside reverse transcription inhibitor (NRTI), against PC3 and LNCaP prostate cancer cell lines [73]. A reduction in cell growth, migration and invasion processes, and induction of senescence and death in prostate cancer cells has been observed following treatment with Abacavir. This was; however, followed by upregulation of *ORF1* and *ORF2* mRNA in these cells in a time and dose dependent manner. The mechanistic reason for this upregulation is not clear. The authors speculated a role for ORF2 and its DNA damaging activities in the induction of senescence and some anti-cancer effects of Abacavir, without conduction of mechanistic studies.

Overall, the basic research findings from the literature points to a benefit for RT inhibitors in reducing tumor growth, or as inducers of cancer cell differentiation that may make cancer cells more susceptible to the effects of chemotherapeutics. Indirect clinical evidence also lends credibility to RT as a target in cancer therapy, albeit lack of evidence associating this to *LINE-1* activation. For instance, high reverse transcriptase titers are found in the plasma of lymphoma and breast cancer patients [74]. Epidemiological studies indicate a 30–50% lower incident of Kaposi's sarcoma [75] and other AIDS-related cancers [76] in patients treated with highly active antiretroviral therapy (HAART). This could be attributed to improved immune reaction in treated patients or suggest a direct inhibitory effect of HAART on the endogenous RT activity in tumor cells. Both nevirapine and efavirenz are approved drugs that have been used in AIDS treatment for several years. Repurposing of these RT inhibitors in cancer therapy is an attractive strategy given the proven safety of these medications.

Despite lack of mechanistic evidence, efavirenz is currently undergoing phase II trials in castration-resistant metastatic prostate cancer. In this trial, patient with highest plasma concentrations of efavirenz (> 3 μg/mL) have shown less cancer progression over those with lower plasma drug levels [77]. Another RT inhibitor, nevirapine, has been used in a patient with dedifferentiated metastatic thyroid carcinoma, where restoration of thyroglobulin expression and radioiodine uptake and also regression of metastatic lesions has been observed [78] [71].

In recent years, other strategies targeting different elements of *LINE-1* has been investigated. Idica et al. reported the discovery of the binding ability miR-128 to *LINE-1* RNA in the *ORF2* coding region sequence, resulting in *LINE-1* repression [79] [80]. Increased miR-128 expression was also shown to reduce nuclear import of ORF1 and inhibition of nuclear import factor Transportin-1 (TNPO1), resulting in significant repression of *LINE-1* mobilization to nucleus and retrotransposition. miR-128 has been demonstrated previously to function as a tumor suppressor, regulate cancer stemness and EMT, and modulate the expression of different genes, including Nanog, HIF-1, VEGF, TGFBR1, and EGFR in cancer [81] [82] [83]. The direct role of *LINE-1* function in these events has not been reported, to the best of our knowledge, to date.

Although the reverse transcriptase machinery remains within ORF2, the loss of specific residues on the chaperone ORF1 can lead to an all-but complete shutdown of retrotransposon activity in cell lines [84,85]. ORF1 protein is also shown to promote proliferation and invasion in colorectal cancer by upregulating the activity of the oncogenic ETS-1 transcription factor, *in vitro* and *in vivo* [86]. Crystal structure of ORF1 has been solved [87]. Mutations in ORF1 serine 119 or di-arginine 261/262 has led to a reduction in the frequency of transposition to $< 0.06\%$ compared to wild type cells. Mutation of an active-site in the reverse transcriptase domain of ORF2 (D702Y) has been reported to knock retrotransposition down to 0.15% of wild type [88] [89]. These findings suggest inhibition of ORF1 and ORF2 might be considered for anti-cancer therapy.

Recent studies by Guler et al., indicate a somewhat contrasting role for the activated *LINE-1*, in EGFR-mutant NSCLC cell line PC9 and its transiently drug tolerant subpopulation that has survived treatment by the EGFR kinase inhibitor, erlotinib. Their data suggested a role for epigenetically repressed *LINE-1* in conferring transient tolerance against cell death by erlotinib in this subpopulation of PC9 cells. The authors suggest drug induced activation of transposable elements, can contribute to the genetic adaptation benefits and cancer cell survival. This has been found in patients that have relapsed on cancer drugs. However, at the same time, transposable elements may provide an advantage reducing the fitness of cancer cells surviving therapy. In this context, the use of histone deacetylase (HDAC) inhibitors and DNA demethylating agents have shown promise in reducing the number of drug tolerant subpopulation of NSCLC [90].

Induction of immunogenic cell death by DNA demethylating agents that makes cancer cells visible to cytotoxic T cells has been validate in several cancers [91] [92]. This has inspired combination of these agents with immune-therapeutics in cancer treatment.

7. Conclusions

Evidence from basic science, preclinical research as well as clinical studies, point to an association between *LINE-1* expression/activation and tumorigenesis, cancer progression and response to therapy. Despite ample evidence, the complete picture and mechanistic links between *LINE-1* expression/activation and progression of different cancers or different cancer phenotypes is still unraveled. Translational development of diagnostics tools targeting markers of *LINE-1* activation (e.g., ORF1 and ORF2), can assist validation of *LINE-1* activation as a companion biomarker in cancer diagnostics and treatment. *LINE-1* elements have also shown potential as therapeutic targets in cancer. In this context, RT inhibitors have been used in clinical trials in cancer and shown promise, but a mechanistic link between anti-cancer activity of RT inhibitors and deactivation of ORF2 is yet to be established. In an opposite direction, *LINE-1* hypomethylation through the use of HDAC inhibitors, has also attracted a lot of attention. Although, the major role of *LINE-1* in the activity of these agents, is still not completely established.

8. Future direction in research

The review of the literature shows great potential for LINE-1 pathway components, as biomarkers in cancer diagnosis/prognosis and a target in cancer therapy. However, at the same time, it highlights the need for the generation of more direct and mechanistically linked evidence for the association between *LINE-1* mobilization elements and

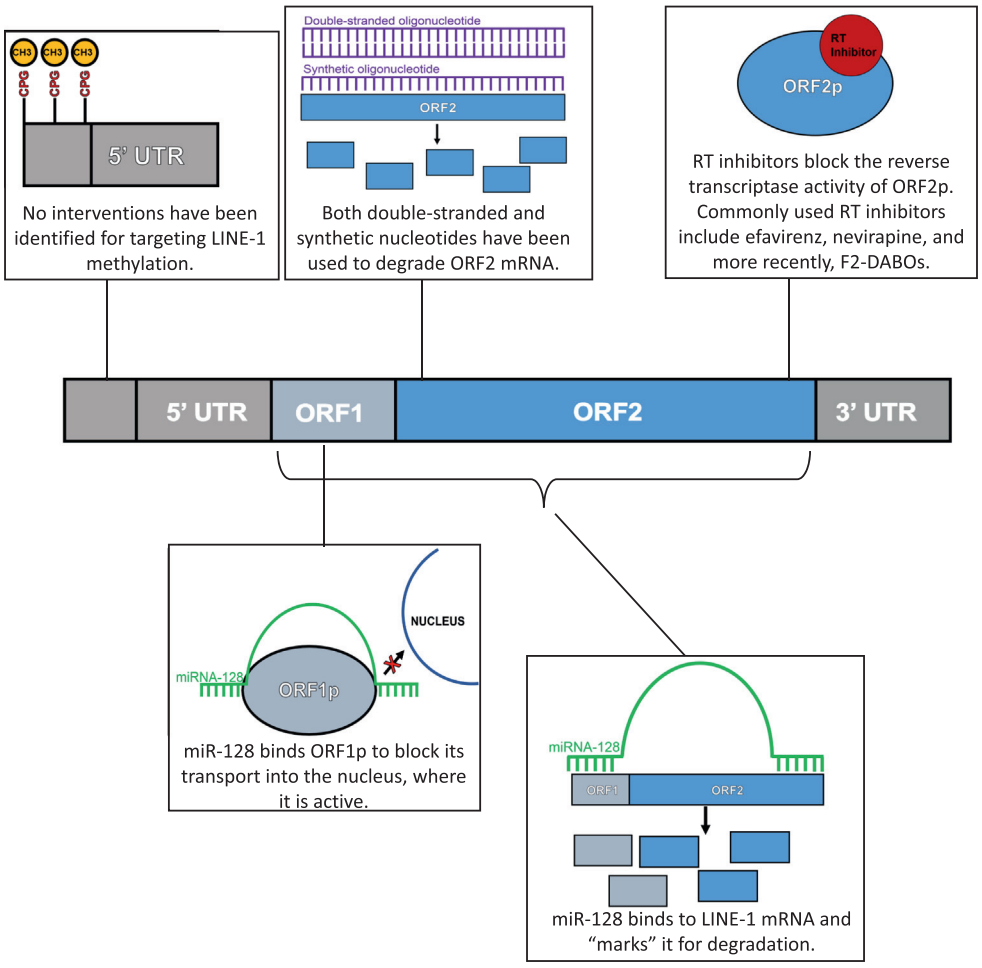

Fig. 2. LINE-1 as a therapeutic target in cancer. Many potential therapeutic interventions have been tested experimentally for targeting the LINE-1 pathway in cancer. Most of the work has focused on inhibition of ORF2p's reverse transcriptase (RT) activity by either oligonucleotides or re-purposed RT inhibitors. In addition, miR-128 is an endogenous regulator of the LINE-1 pathway with therapeutic potential. miR-128 can block translocation of ORF1p to the nucleus in addition to targeting LINE-1 RNA for degradation.

cancer emergence, progression and/or response to therapy in both preclinical basic science and/or clinical investigations. In particular, further research in the following areas seem to have priority. Based on current information, measurement of LINE-1 hypomethylation seem to have utility as a companion diagnostic marker for pre-neoplasias and/or as an indicator of cancer response to therapy in some types of cancer. However, ORF1 expression may be a more reliable, and accessible biomarker of *LINE-1* activation, thus better fitting the criteria of a companion diagnostic biomarker, when compared to *LINE-1* hypomethylation. Furthermore, the application of different LINE-1 elements as a companion diagnostic/prognostic biomarker, seem to be dependent on cancer type, stage of the disease, and other underlying factors. With respect to application of LINE-1 pathway elements as diagnostic and prognostic biomarkers, it would be beneficial, if clinical and basic research investigations could examine the levels of *LINE-1* hypomethylation along with ORF1/ORF2 expression. This will provide a more comprehensive picture of *LINE-1* overall activity. The shortage of information might have been partly attributed to the absence of validated, sensitive and minimally invasive means of quantification for ORF1/ORF2 ratios. With recent progress in the development of sensitive and reliable assays for both proteins, this shortcoming is expected to be overcome in near future [21].

Expression of *LINE-1* may lead to the generation of immune response against developing tumors. As a result, endogenous mechanism that regulate this expression may undergo positive selection during the evolution of the tumor to more advanced stages. This rationale emphasizes the need for mapping of the LINE-1 activation elements during the course of cancer progression in preclinical and clinical studies [92]. Besides, correlations between *LINE-1* expression and its activation elements with tumor immunogenicity (and possibility for the development of neo-antigens), may guide proper use of immunotherapeutic in clinical settings. In this context, combination of chemotherapeutic drugs that can induce expression of LINE-1, with immunotherapeutics can be considered. For instance, in recent studies, carboplatin and anti-programmed death-1 demonstrated enhanced efficacy in lung cancer clinical trials [93]. It has been speculated that the combinatorial effects are due to the ability of carboplatin to induce repeat elements expression.

Because of the relative low expression of ORF2 compared to ORF1, and its almost exclusive expression in neoplastic or pre-neoplastic tissues, ORF2 may serve as a better therapeutic target than diagnostic or prognostic biomarker. In this context, RT inhibitors have been used in clinic and achieved some success. However, a direct relation between RT inhibition and LINE-1 activity has yet to be established. The outcomes from clinical trials may lead to both short term and long term avenues of research. In short term, it may lead to more aggressive pursuit of current RT inhibitors for a repurposed application in therapy of cancer. In this context, tools measuring LINE-1 activation may provide a companion diagnostic approach for selection of patients who are more likely to respond to RT therapy. In the long term, attention can be

paid to development of more specific and potent drugs targeting different elements of LINE-1 pathway activation (Fig. 2), as new cancer preventive or therapeutic medicine.

The potential application of LINE-1 elements as biomarkers for response/no response to cancer therapeutics is implicated through several basic scientific preclinical explorations, mostly at a cellular level. Clinical information on the potential utility of LINE-1 expression and activation elements as biomarkers of precision medicine in cancer are, however limited. If the value of LINE-1 is validated in this front, it can help clinicians to make better decisions on the course and dose of treatment for individual patients, lowering the incident of drugs' side effects while administering more effective anti-cancer regimens. Future investigations, directed towards finding a correlation between LINE-1 activation and chemo- radiation and immunotherapy [94] response in different types of cancer is, thus warranted. In line with clinical investigations in this front, more attention should also be paid to basic scientific research that can unravel the molecular mediators linking *LINE-1* expression to cancer resistance or susceptibility to certain treatments in different cancer types. In parallel, attention should also be paid to the preclinical cell-based assays as well as animal/human studies on the mechanistic links and pathways that governs the temporal regulation of *LINE-1* and its protein constituents during tumorigenesis [10]. Such information will not only validate the utility of LINE-1 pathway as a companion diagnostic biomarker, but also will assist in mapping the most relevant LINE-1 associated biomarker for different types and stages of cancer.

References

[1] R.K. Slotkin, R. Martienssen, Transposable elements and the epigenetic regulation of the genome, Nat. Rev. Genet. 8 (2007) 272–285, https://doi.org/10.1038/nrg2072.

[2] K.H. Burns, Transposable elements in cancer, Nat. Rev. Cancer 17 (2017) 415–424, https://doi.org/10.1038/nrc.2017.35.

[3] R.E. Mills, E.A. Bennett, R.C. Iskow, S.E. Devine, Which transposable elements are active in the human genome? Trends Genet. TIG. 23 (2007) 183–191, https://doi.org/10.1016/j.tig.2007.02.006.

[4] H.H. Kazazian, Mobile elements: drivers of genome evolution, Science 303 (2004) 1626–1632, https://doi.org/10.1126/science.1089670.

[5] J.R. Kemp, M.S. Longworth, Crossing the LINE toward genomic instability: LINE-1 Retrotransposition in Cancer, Front. Chem. 3 (2015) 68, https://doi.org/10.3389/fchem.2015.00068.

[6] C.R. Beck, J.L. Garcia-Perez, R.M. Badge, J.V. Moran, LINE-1 elements in structural variation and disease, Annu. Rev. Genomics Hum. Genet. 12 (2011) 187–215, https://doi.org/10.1146/annurev-genom-082509-141802.

[7] S.L. Martin, The ORF1 protein encoded by LINE-1: structure and function during L1 retrotransposition, J Biomed Biotechnol 2006 (2006) 45621, https://doi.org/10.1155/JBB/2006/45621.

[8] W. Liang, J. Xu, W. Yuan, X. Song, J. Zhang, W. Wei, X.-F. Yu, Y. Yang, APOBEC3DE inhibits LINE-1 Retrotransposition by interacting with ORF1p and influencing LINE reverse transcriptase activity, PLoS One 11 (2016) e0157220, , https://doi.org/10.1371/journal.pone.0157220.

[9] N. Rodić, K.H. Burns, Long interspersed element-1 (LINE-1): passenger or driver in human neoplasms? PLoS Genet. 9 (2013) e1003402, , https://doi.org/10.1371/journal.pgen.1003402.

[10] A.P. Feinberg, B. Vogelstein, Hypomethylation distinguishes genes of some human cancers from their normal counterparts, Nature 301 (1983) 89–92.

[11] D. Ardeljan, M.S. Taylor, D.T. Ting, K.H. Burns, The human long interspersed Element-1 Retrotransposon: An emerging biomarker of Neoplasia, Clin. Chem. 63 (2017) 816–822, https://doi.org/10.1373/clinchem.2016.257444.

[12] I. Sciamanna, P. Sinibaldi-Vallebona, A. Serafino, C. Spadafora, LINE-1-encoded reverse transcriptase as a target in cancer therapy, Front. Biosci. Landmark Ed. 23 (2018) 1360–1369.

[13] E.C. Scott, E.J. Gardner, A. Masood, N.T. Chuang, P.M. Vertino, S.E. Devine, A hot L1 retrotransposon evades somatic repression and initiates human colorectal cancer, Genome Res (2016), https://doi.org/10.1101/gr.201814.115.

[14] A.S. Yang, M.R.H. Estécio, K. Doshi, Y. Kondo, E.H. Tajara, J.-P.J. Issa, A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements, Nucleic Acids Res. 32 (2004) e38, , https://doi.org/10.1093/nar/gnh032.

[15] I.W.Y. Cheuk, V.Y. Shin, A. Kwong, Detection of methylated circulating DNA as noninvasive biomarkers for breast Cancer diagnosis, J. Breast Cancer 20 (2017) 12–19, https://doi.org/10.4048/jbc.2017.20.1.12.

[16] M. Dunaeva, M. Derksen, G.J.M. Pruijn, LINE-1 Hypermethylation in serum cell-free DNA of relapsing remitting multiple sclerosis patients, Mol. Neurobiol. 55 (2018) 4681–4688, https://doi.org/10.1007/s12035-017-0679-z.

[17] Y. Nagai, E. Sunami, Y. Yamamoto, K. Hata, S. Okada, K. Murono, K. Yasuda, K. Otani, T. Nishikawa, T. Tanaka, T. Kiyomatsu, K. Kawai, H. Nozawa, S. Ishihara, D.S. Hoon, T. Watanabe, LINE-1 hypomethylation status of circulating cell-free DNA in plasma as a biomarker for colorectal cancer, Oncotarget 8 (2017) 11906–11916, https://doi.org/10.18632/oncotarget.14439.

[18] I.V. Gainetdinov, K.Y. Kapitskaya, E.Y. Rykova, A.A. Ponomaryova, N.V. Cherdyntseva, V.V. Vlassov, P.P. Laktionov, T.L. Azhikina, Hypomethylation of human-specific family of LINE-1 retrotransposons in circulating DNA of lung cancer patients, Lung Cancer Amst. Neth. 99 (2016) 127–130, https://doi.org/10.1016/j.lungcan.2016.07.005.

[19] C. De Luca, F. Guadagni, P. Sinibaldi-Vallebona, S. Sentinelli, M. Gallucci, A. Hoffmann, G.G. Schumann, C. Spadafora, I. Sciamanna, Enhanced expression of LINE-1-encoded ORF2 protein in early stages of colon and prostate transformation, Oncotarget 7 (2016) 4048–4061, https://doi.org/10.18632/oncotarget.6767.

[20] R. Sharma, N. Rodić, K.H. Burns, M.S. Taylor, Immunodetection of human LINE-1 expression in cultured cells and human tissues, Methods Mol. Biol. Clifton NJ. 1400 (2016) 261–280, https://doi.org/10.1007/978-1-4939-3372-3_17.

[21] K. Hosseinnejad, T. Yin, J.T. Gaskins, J.L. Bailen, S.A. Jortani, Discovery of the long interspersed nuclear Element-1 activation product [Open Reading Frame-1 (ORF1) protein] in human blood, Clin. Chim. Acta Int. J. Clin. Chem. 487 (2018) 228–232, https://doi.org/10.1016/j.cca.2018.09.040.

[22] M. Barchitta, A. Quattrocchi, A. Maugeri, M. Vinciguerra, A. Agodi, LINE-1 Hypomethylation in blood and tissue samples as an epigenetic marker for Cancer risk: a systematic review and meta-analysis, PLoS One 9 (2014) e109478, , https://doi.org/10.1371/journal.pone.0109478.

[23] E. Sunami, M. de Maat, A. Vu, R.R. Turner, D.S.B. Hoon, LINE-1 Hypomethylation during primary Colon Cancer progression, PLoS One 6 (2011) e18884, , https://doi.org/10.1371/journal.pone.0018884.

[24] D.A. Corley, C.D. Jensen, A.R. Marks, W.K. Zhao, J.K. Lee, C.A. Doubeni, A.G. Zauber, J. de Boer, B.H. Fireman, J.E. Schottinger, V.P. Quinn, N.R. Ghai, T.R. Levin, C.P. Quesenberry, Adenoma detection rate and risk of colorectal cancer and death, N. Engl. J. Med. 370 (2014) 1298–1306, https://doi.org/10.1056/NEJMoa1309086.

[25] S.Y. Park, A.N. Seo, H.Y. Jung, J.M. Gwak, N. Jung, N.-Y. Cho, G.H. Kang, Alu and LINE-1 hypomethylation is associated with HER2 enriched subtype of breast cancer, PLoS One 9 (2014) e100429, , https://doi.org/10.1371/journal.pone.0100429.

[26] A.Q. van Hoesel, C.J.H. van de Velde, P.J.K. Kuppen, G.J. Liefers, H. Putter, Y. Sato, D.A. Elashoff, R.R. Turner, J.M. Shamonki, E.M. de Kruijf, J.G.H. van Nes, A.E. Giuliano, D.S.B. Hoon, Hypomethylation of LINE-1 in primary tumor has poor prognosis in young breast cancer patients: a retrospective cohort study, Breast Cancer Res. Treat. 134 (2012) 1103–1114, https://doi.org/10.1007/s10549-012-2038-0.

[27] C. Zhu, T. Utsunomiya, T. Ikemoto, S. Yamada, Y. Morine, S. Imura, Y. Arakawa, C. Takasu, D. Ishikawa, I. Imoto, M. Shimada, Hypomethylation of long interspersed nuclear element-1 (LINE-1) is associated with poor prognosis via activation of c-MET in hepatocellular carcinoma, Ann. Surg. Oncol. 21 (Suppl. 4) (2014) S729–S735, https://doi.org/10.1245/s10434-014-3874-4.

[28] A.K. Shah, N.A. Saunders, A.P. Barbour, M.M. Hill, Early diagnostic biomarkers for Esophageal adenocarcinoma—the current state of play, Cancer Epidemiol. Prev. Biomark. 22 (2013) 1185–1209, https://doi.org/10.1158/1055-9965.EPI-12-1415.

[29] H. Shigaki, Y. Baba, M. Watanabe, S. Iwagami, K. Miyake, T. Ishimoto, M. Iwatsuki, H. Baba, LINE-1 hypomethylation in noncancerous esophageal mucosae is associated with smoking history, Ann. Surg. Oncol. 19 (2012) 4238–4243, https://doi.org/10.1245/s10434-012-2488-y.

[30] K. Chalitchagorn, S. Shuangshoti, N. Hourpai, N. Kongruttanachok, P. Tangkijvanich, D. Thong-ngam, N. Voravud, V. Sriuranpong, A. Mutirangura, Distinctive pattern of LINE-1 methylation level in normal tissues and the association with carcinogenesis, Oncogene 23 (2004) 8841–8846, https://doi.org/10.1038/sj.onc.1208137.

[31] W.A. Schulz, J.P. Elo, A.R. Florl, S. Pennanen, S. Santourlidis, R. Engers, M. Buchardt, H.-H. Seifert, T. Visakorpi, Genomewide DNA hypomethylation is associated with alterations on chromosome 8 in prostate carcinoma, Genes. Chromosom. Cancer. 35 (2002) 58–65, https://doi.org/10.1002/gcc.10092.

[32] S. Santourlidis, A. Florl, R. Ackermann, H.-C. Wirtz, W.A. Schulz, High frequency of alterations in DNA methylation in adenocarcinoma of the prostate, Prostate 39 (1999) 166–174, https://doi.org/10.1002/(SICI)1097-0045(19990515)39:3<166::AID-PROS4>3.0.CO;2-J.

[33] J. Pattamadilok, N. Huapai, P. Rattanatanyong, A. Vasurattana, S. Triratanachat, D. Tresukosol, A. Mutirangura, LINE-1 hypomethylation level as a potential prognostic factor for epithelial ovarian cancer, Int. J. Gynecol. Cancer 18 (2008) 711–717, https://doi.org/10.1111/j.1525-1438.2007.01117.x.

[34] H.K. Al-Ali, M. Cross, E. Bach, R. Krahl, N. Jaekel, D. Niederwieser, C. Junghanss, G. Maschmeyer, T. Tran, Pretreatment long interspersed element (LINE)-1 methylation levels, not early hypomethylation under treatment, predict hematological response to azacitidine in elderly patients with acute myeloid leukemia, Onco Targets Ther. 741 (2013), https://doi.org/10.2147/OTT.S45459.

[35] N. Rodić, R. Sharma, R. Sharma, J. Zampella, L. Dai, M.S. Taylor, R.H. Hruban, C.A. Iacobuzio-Donahue, A. Maitra, M.S. Torbenson, M. Goggins, I.-M. Shih, A.S. Duffield, E.A. Montgomery, E. Gabrielson, G.J. Netto, T.L. Lotan, A.M. De Marzo, W. Westra, Z.A. Binder, B.A. Orr, G.L. Gallia, C.G. Eberhart, J.D. Boeke, C.R. Harris, K.H. Burns, Long interspersed Element-1 protein expression is a Hallmark of many human cancers, Am. J. Pathol. 184 (2014) 1280–1286, https://doi.org/10.1016/j.ajpath.2014.01.007.

[36] L. Chen, J.E. Dahlstrom, A. Chandra, P. Board, D. Rangasamy, Prognostic value of LINE-1 retrotransposon expression and its subcellular localization in breast cancer, Breast Cancer Res. Treat. 136 (2012) 129–142, https://doi.org/10.1007/s10549-012-2246-7.

[37] K. Hosseinnejad, M. Bousamra, M. Burch, A. Elliot, D. Eichenherger, S.A. Jortani, Serum Concentrations of Open Reading Frame 1 (ORF1) protein Produced by Activation of Long Interspersed Nuclear Element 1 (L1) In Subject Being Screened for Lung Cancer with CT scan, (2017), p. A-024 https://www.aacc.org/science-and-practice/annual-meeting-abstracts-archive/2017-annual-meeting-abstracts (accessed November 14, 2018).
[38] M. Antelo, F. Balaguer, J. Shia, Y. Shen, K. Hur, L. Moreira, M. Cuatrecasas, L. Bujanda, M.D. Giraldez, M. Takahashi, A. Cabanne, M.E. Barugel, M. Arnold, E.L. Roca, M. Andreu, S. Castellvi-Bel, X. Llor, R. Jover, A. Castells, C.R. Boland, A. Goel, A high degree of LINE-1 hypomethylation is a unique feature of early-onset colorectal cancer, PLoS One 7 (2012) e45357, , https://doi.org/10.1371/journal.pone.0045357.
[39] K. Inamura, M. Yamauchi, R. Nishihara, P. Lochhead, Z.R. Qian, A. Kuchiba, S.A. Kim, K. Mima, Y. Sukawa, S. Jung, X. Zhang, K. Wu, E. Cho, A.T. Chan, J.A. Meyerhardt, C.C. Harris, C.S. Fuchs, S. Ogino, Tumor LINE-1 methylation level and microsatellite instability in relation to colorectal cancer prognosis, J. Natl. Cancer Inst 106 (2014), https://doi.org/10.1093/jnci/dju195.
[40] M. Kaneko, M. Kotake, H. Bando, T. Yamada, H. Takemura, T. Minamoto, Prognostic and predictive significance of long interspersed nucleotide element-1 methylation in advanced-stage colorectal cancer, BMC Cancer 16 (2016) 945, https://doi.org/10.1186/s12885-016-2984-8.
[41] S. Ogino, K. Nosho, G.J. Kirkner, T. Kawasaki, A.T. Chan, E.S. Schernhammer, E.L. Giovannucci, C.S. Fuchs, A cohort study of tumoral LINE-1 hypomethylation and prognosis in colon cancer, JNCI J. Natl. Cancer Inst. 100 (2008) 1734–1738, https://doi.org/10.1093/jnci/djn359.
[42] M. Swets, A. Zaalberg, A. Boot, T. van Wezel, M.A. Frouws, E. Bastiaannet, H. Gelderblom, C.J.H. van de Velde, P.J.K. Kuppen, Tumor LINE-1 methylation level in association with survival of patients with stage II colon cancer, Int. J. Mol. Sci 18 (2016), https://doi.org/10.3390/ijms18010036.
[43] K. Harada, Y. Baba, T. Ishimoto, A. Chikamoto, K. Kosumi, H. Hayashi, H. Nitta, D. Hashimoto, T. Beppu, H. Baba, LINE-1 methylation level and patient prognosis in a database of 208 hepatocellular carcinomas, Ann. Surg. Oncol. 22 (2015) 1280–1287, https://doi.org/10.1245/s10434-014-4134-3.
[44] X. Gao, J. Qu, X. Chang, Y. Lu, W. Bai, H. Wang, Z. Xu, L. An, C. Wang, Z. Zeng, Y. Yang, Hypomethylation of long interspersed nuclear element-1 promoter is associated with poor outcomes for curative resected hepatocellular carcinoma, Liver Int. Off. J. Int. Assoc. Study Liver. 34 (2014) 136–146, https://doi.org/10.1111/liv.12264.
[45] K. Ikeda, K. Shiraishi, A. Eguchi, H. Shibata, K. Yoshimoto, T. Mori, Y. Baba, H. Baba, M. Suzuki, Long interspersed nucleotide element 1 hypomethylation is associated with poor prognosis of lung adenocarcinoma, Ann. Thorac. Surg. 96 (2013) 1790–1794, https://doi.org/10.1016/j.athoracsur.2013.06.035.
[46] S. Iwagami, Y. Baba, M. Watanabe, H. Shigaki, K. Miyake, T. Ishimoto, M. Iwatsuki, K. Sakamaki, Y. Ohashi, H. Baba, LINE-1 hypomethylation is associated with a poor prognosis among patients with curatively resected esophageal squamous cell carcinoma, Ann. Surg. 257 (2013) 449–455, https://doi.org/10.1097/SLA.0b013e31826d8602.
[47] D. van Bemmel, P. Lenz, L.M. Liao, D. Baris, L.R. Sternberg, A. Warner, A. Johnson, M. Jones, M. Kida, M. Schwenn, A.R. Schned, D.T. Silverman, N. Rothman, L.E. Moore, Correlation of LINE-1 methylation levels in patient-matched buffy coat, serum, buccal cell, and bladder tumor tissue DNA samples, Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol. 21 (2012) 1143–1148, https://doi.org/10.1158/1055-9965.EPI-11-1030.
[48] J.M. Bae, S.-H. Shin, H.-J. Kwon, S.-Y. Park, M.C. Kook, Y.-W. Kim, N.-Y. Cho, N. Kim, T.-Y. Kim, D. Kim, G.H. Kang, ALU and LINE-1 hypomethylations in multistep gastric carcinogenesis and their prognostic implications, Int. J. Cancer 131 (2012) 1323–1331, https://doi.org/10.1002/ijc.27369.
[49] J. Zhu, Y. Ling, Y. Xu, M.-Z. Lu, Y.-P. Liu, C.-S. Zhang, Elevated expression of MDR1 associated with Line-1 hypomethylation in esophageal squamous cell carcinoma, Int. J. Clin. Exp. Pathol. 8 (2015) 14392–14400.
[50] Y. Su, S. Davies, M. Davis, H. Lu, R. Giller, M. Krailo, Q. Cai, L. Robison, X.-O. Shu, Expression of LINE-1 p40 protein in pediatric malignant germ cell tumors and its association with clinicopathological parameters: a report from the children's oncology group, Cancer Lett. 247 (2007) 204–212, https://doi.org/10.1016/j.canlet.2006.04.010.
[51] C.R. Harris, R. Normart, Q. Yang, E. Stevenson, B.G. Haffty, S. Ganesan, C. Cordon-Cardo, A.J. Levine, L.H. Tang, Association of nuclear localization of a long interspersed nuclear Element-1 protein in breast Tumors with poor prognostic outcomes, Genes Cancer. 1 (2010) 115–124, https://doi.org/10.1177/1947601909360812.
[52] E.M. Briggs, S. Ha, P. Mita, G. Brittingham, I. Sciamanna, C. Spadafora, S.K. Logan, Long interspersed nuclear element-1 expression and retrotransposition in prostate cancer cells, Mob. DNA 9 (2018), https://doi.org/10.1186/s13100-017-0106-z.
[53] Y. Lu, F. Feng, Y. Yang, X. Gao, J. Cui, C. Zhang, F. Zhang, Z. Xu, J. Qv, C. Wang, Z. Zeng, Y. Zhu, Y. Yang, LINE-1 ORF-1p functions as a novel androgen receptor co-activator and promotes the growth of human prostatic carcinoma cells, Cell. Signal. 25 (2013) 479–489, https://doi.org/10.1016/j.cellsig.2012.11.004.
[54] E.M. Reyes-Reyes, I. Aispuro, M.A. Tavera-Garcia, M. Field, S. Moore, I. Ramos, K.S. Ramos, LINE-1 couples EMT programming with acquisition of oncogenic phenotypes in human bronchial epithelial cells, Oncotarget 8 (2017) 103828–103842, https://doi.org/10.18632/oncotarget.21953.
[55] F. Feng, Y.-Y. Lu, F. Zhang, X.-D. Gao, C.-F. Zhang, A. Meredith, Z.-X. Xu, Y.-T. Yang, X.-J. Chang, H. Wang, J.-H. Qu, Z. Zeng, J.-L. Yang, C.-P. Wang, Y.-F. Zhu, J.-J. Cui, Y.-P. Yang, Long interspersed nuclear element ORF-1 protein promotes proliferation and resistance to chemotherapy in hepatocellular carcinoma, World J. Gastroenterol. 19 (2013) 1068–1078, https://doi.org/10.3748/wjg.v19.i7.1068.
[56] K.B. Chiappinelli, P.L. Strissel, A. Desrichard, H. Li, C. Henke, B. Akman, A. Hein, N.S. Rote, L.M. Cope, A. Snyder, V. Makarov, S. Budhu, S. Buhu, D.J. Slamon, J.D. Wolchok, D.M. Pardoll, M.W. Beckmann, C.A. Zahnow, T. Merghoub, T. Mergoub, T.A. Chan, S.B. Baylin, R. Strick, Inhibiting DNA methylation causes an interferon response in cancer via dsRNA including endogenous retroviruses, Cell 162 (2015) 974–986, https://doi.org/10.1016/j.cell.2015.07.011.
[57] P. Bojang, R.A. Roberts, M.J. Anderton, K.S. Ramos, Reprogramming of the HepG2 genome by long interspersed nuclear element-1, Mol. Oncol. 7 (2013) 812–825, https://doi.org/10.1016/j.molonc.2013.04.003.
[58] I. Sciamanna, M. Landriscina, C. Pittoggi, M. Quirino, C. Mearelli, R. Beraldi, E. Mattei, A. Serafino, A. Cassano, P. Sinibaldi-Vallebona, E. Garaci, C. Barone, C. Spadafora, Inhibition of endogenous reverse transcriptase antagonizes human tumor growth, Oncogene 24 (2005) 3923–3931, https://doi.org/10.1038/sj.onc.1208562.
[59] P. Sinibaldi-Vallebona, P. Lavia, E. Garaci, C. Spadafora, A role for endogenous reverse transcriptase in tumorigenesis and as a target in differentiating cancer therapy, Genes. Chromosom. Cancer. 45 (2006) 1–10, https://doi.org/10.1002/gcc.20266.
[60] E. Oricchio, I. Sciamanna, R. Beraldi, G.V. Tolstonog, G.G. Schumann, C. Spadafora, Distinct roles for LINE-1 and HERV-K retroelements in cell proliferation, differentiation and tumor progression, Oncogene 26 (2007) 4226–4233, https://doi.org/10.1038/sj.onc.1210214.
[61] R. Patnala, S.-H. Lee, J.E. Dahlstrom, S. Ohms, L. Chen, S.T. Dheen, D. Rangasamy, Inhibition of LINE-1 retrotransposon-encoded reverse transcriptase modulates the expression of cell differentiation genes in breast cancer cells, Breast Cancer Res. Treat. 143 (2014) 239–253, https://doi.org/10.1007/s10549-013-2812-7.
[62] Y. Zhu, F. Feng, J. Yu, B. Song, M. Hu, X. Gao, Y. Wang, Q. Zhang, L1-ORF1p, a Smad4 interaction protein, promotes proliferation of HepG2 cells and tumorigenesis in mice, DNA Cell Biol. 32 (2013) 531–540, https://doi.org/10.1089/dna.2013.2097.
[63] K. Hur, P. Cejas, J. Feliu, J. Moreno-Rubio, E. Burgos, C.R. Boland, A. Goel, Hypomethylation of long interspersed nuclear element-1 (LINE-1) leads to activation of proto-oncogenes in human colorectal cancer metastasis, Gut 63 (2014) 635–646, https://doi.org/10.1136/gutjnl-2012-304219.
[64] D. Rangasamy, N. Lenka, S. Ohms, J.E. Dahlstrom, A.C. Blackburn, P.G. Board, Activation of LINE-1 retrotransposon increases the risk of epithelial-mesenchymal transition and metastasis in epithelial cancer, Curr. Mol. Med. 15 (2015) 588–597.
[65] K.W. Kuo, H.M. Sheu, Y.S. Huang, W.C. Leung, Expression of transposon LINE-1 is relatively human-specific and function of the transcripts may be proliferation-essential, Biochem. Biophys. Res. Commun. 253 (1998) 566–570, https://doi.org/10.1006/bbrc.1998.9811.
[66] P. Apostolou, M. Toloudi, M. Chatziioannou, E. Kourtidou, G. Mimikakou, I. Vlachou, A. Chlichlia, I. Papasotiriou, Involvement of retrotransposon L1 in stemness and cellular plasticity, Cell Commun. Adhes. 22 (2015) 1–7, https://doi.org/10.3109/15419061.2014.970270.
[67] M. Landriscina, C. Spadafora, M. Cignarelli, C. Barone, Anti-tumor activity of non-nucleosidic reverse transcriptase inhibitors, Curr. Pharm. Des. 13 (2007) 737–747.
[68] P. Sinibaldi-Vallebona, C. Matteucci, C. Spadafora, Retrotransposon-encoded reverse transcriptase in the genesis, progression and cellular plasticity of human cancer, Cancers. 3 (2011) 1141–1157, https://doi.org/10.3390/cancers3011141.
[69] R. Mangiacasale, C. Pittoggi, I. Sciamanna, A. Careddu, E. Mattei, R. Lorenzini, L. Travaglini, M. Landriscina, C. Barone, C. Nervi, P. Lavia, C. Spadafora, Exposure of normal and transformed cells to nevirapine, a reverse transcriptase inhibitor, reduces cell growth and promotes differentiation, Oncogene 22 (2003) 2750.
[70] J.J. Dong, Y. Zhou, Y.T. Liu, Z.W. Zhang, X.J. Zhou, H.J. Wang, L. Liao, In vitro evaluation of the therapeutic potential of nevirapine in treatment of human thyroid anaplastic carcinoma, Mol. Cell. Endocrinol. 370 (2013) 113–118, https://doi.org/10.1016/j.mce.2013.02.001.
[71] S. Modoni, M. Landriscina, A. Fabiano, A. Fersini, N. Urbano, A. Ambrosi, M. Cignarelli, Reinduction of cell differentiation and 131I uptake in a poorly differentiated thyroid tumor in response to the reverse transcriptase (RT) inhibitor nevirapine, Cancer Biother. Radiopharm. 22 (2007) 289–295, https://doi.org/10.1089/cbr.2006.316.
[72] G. Sbardella, A. Mai, S. Bartolini, S. Castellano, R. Cirilli, D. Rotili, C. Milite, M. Santoriello, S. Orlando, I. Sciamanna, A. Serafino, P. Lavia, C. Spadafora, Modulation of cell differentiation, proliferation, and tumor growth by dihydrobenzyloxopyrimidine non-nucleoside reverse transcriptase inhibitors, J. Med. Chem. 54 (2011) 5927–5936, https://doi.org/10.1021/jm200734j.
[73] F. Carlini, B. Ridolfi, A. Molinari, C. Parisi, G. Bozzuto, L. Toccacieli, G. Formisano, D. De Orsi, S. Paradisi, O.M.V. Grober, M. Ravo, A. Weisz, R. Arcieri, S. Vella, S. Gaudi, The reverse transcription inhibitor abacavir shows anticancer activity in prostate cancer cell lines, PLoS One 5 (2010) e14221, , https://doi.org/10.1371/journal.pone.0014221.
[74] R. Contreras-Galindo, M.H. Kaplan, P. Leissner, T. Verjat, I. Ferlenghi, F. Bagnoli, F. Giusti, M.H. Dosik, D.F. Hayes, S.D. Gitlin, D.M. Markovitz, Human endogenous retrovirus K (HML-2) elements in the plasma of people with lymphoma and breast cancer, J. Virol. 82 (2008) 9329–9336, https://doi.org/10.1128/JVI.00646-08.
[75] S. Portsmouth, J. Stebbing, J. Gill, S. Mandalia, M. Bower, M. Nelson, M. Bower, B. Gazzard, A comparison of regimens based on non-nucleoside reverse transcriptase inhibitors or protease inhibitors in preventing Kaposi's sarcoma, AIDS Lond. Engl. 17 (2003) F17–F22, https://doi.org/10.1097/01.aids.0000076309.76477.f1.
[76] U. Tirelli, D. Bernardi, Impact of HAART on the clinical management of AIDS-related cancers, Eur. J. Cancer Oxf. Engl. 1990 (37) (2001) 1320–1324.
[77] N. Houédé, M. Pulido, L. Mourey, F. Joly, J.-M. Ferrero, C. Bellera, F. Priou, C. Lalet, A. Laroche-Clary, M.C. Raffin, F. Ichas, A. Puech, P.V. Piazza, A phase II trial evaluating the efficacy and safety of efavirenz in metastatic castration-resistant

prostate cancer, Oncologist 19 (2014) 1227–1228, https://doi.org/10.1634/theoncologist.2014-0345.
[78] M. Landriscina, S. Modoni, A. Fabiano, A. Fersini, C. Barone, A. Ambrosi, M. Cignarelli, Cell differentiation and iodine-131 uptake in poorly differentiated thyroid tumour in response to nevirapine, Lancet Oncol. 7 (2006) 877–879, https://doi.org/10.1016/S1470-2045(06)70908-1.
[79] M. Hamdorf, A. Idica, D.G. Zisoulis, L. Gamelin, C. Martin, K.J. Sanders, I.M. Pedersen, miR-128 represses L1 retrotransposition by binding directly to L1 RNA, Nat. Struct. Mol. Biol 22 (2015) 824–831, https://doi.org/10.1038/nsmb.3090.
[80] A. Idica, E.A. Sevrioukov, D.G. Zisoulis, M. Hamdorf, I. Daugaard, P. Kadandale, I.M. Pedersen, MicroRNA miR-128 represses LINE-1 (L1) retrotransposition by down-regulating the nuclear import factor TNPO1, J. Biol. Chem. 292 (2017) 20494–20508, https://doi.org/10.1074/jbc.M117.807677.
[81] Z. Shi, J. Wang, Z. Yan, Y. You, C. Li, X. Qian, Y. Yin, P. Zhao, Y. Wang, X. Wang, M. Li, L.-Z. Liu, N. Liu, B.-H. Jiang, MiR-128 inhibits tumor growth and angiogenesis by targeting p70S6K1, PLoS One 7 (2012) e32709, , https://doi.org/10.1371/journal.pone.0032709.
[82] S. Masri, Z. Liu, S. Phung, E. Wang, Y.-C. Yuan, S. Chen, The role of microRNA-128a in regulating TGFbeta signaling in letrozole-resistant breast cancer cells, Breast Cancer Res. Treat. 124 (2010) 89–99, https://doi.org/10.1007/s10549-009-0716-3.
[83] Y. Zhang, T. Chao, R. Li, W. Liu, Y. Chen, X. Yan, Y. Gong, B. Yin, W. Liu, B. Qiang, J. Zhao, J. Yuan, X. Peng, MicroRNA-128 inhibits glioma cells proliferation by targeting transcription factor E2F3a, J. Mol. Med. Berl. Ger. 87 (2009) 43–51, https://doi.org/10.1007/s00109-008-0403-6.
[84] J.V. Moran, S.E. Holmes, T.P. Naas, R.J. DeBerardinis, J.D. Boeke, H.H. Kazazian, High frequency retrotransposition in cultured mammalian cells, Cell 87 (1996) 917–927, https://doi.org/10.1016/S0092-8674(00)81998-4.
[85] E.M. Ostertag, E.T. Prak, R.J. DeBerardinis, J.V. Moran, H.H. Kazazian, Determination of L1 retrotransposition kinetics in cultured cells, Nucleic Acids Res. 28 (2000) 1418–1423.
[86] M.Y. Li, M. Zhu, F. Feng, F.Y. Cai, K.C. Fan, H. Jiang, Z.Q. Wang, E.Q. Linghu, Long interspersed nucleotide acid element-1 ORF-1 protein promotes proliferation and invasion of human colorectal cancer LoVo cells through enhancing ETS-1 activity, Genet. Mol. Res. GMR. 13 (2014) 6981–6994, https://doi.org/10.4238/2014.April.14.13.
[87] E. Khazina, V. Truffault, R. Büttner, S. Schmidt, M. Coles, O. Weichenrieder, Trimeric structure and flexibility of the L1ORF1 protein in human L1 retrotransposition, Nat. Struct. Mol. Biol. 18 (2011) 1006–1014, https://doi.org/10.1038/nsmb.2097.
[88] G.J. Cost, Human L1 element target-primed reverse transcription in vitro, EMBO J. 21 (2002) 5899–5910, https://doi.org/10.1093/emboj/cdf592.
[89] S.L. Mathias, A.F. Scott, H.H. Kazazian, J.D. Boeke, A. Gabriel, Reverse transcriptase encoded by a human transposable element, Science 254 (1991) 1808–1810.
[90] G.D. Guler, C.A. Tindell, R. Pitti, C. Wilson, K. Nichols, T. KaiWai Cheung, H.-J. Kim, M. Wongchenko, Y. Yan, B. Haley, T. Cuellar, J. Webster, N. Alag, G. Hegde, E. Jackson, T.L. Nance, P.G. Giresi, K.-B. Chen, J. Liu, S. Jhunjhunwala, J. Settleman, J.-P. Stephan, D. Arnott, M. Classon, Repression of stress-induced LINE-1 expression protects cancer cell subpopulations from lethal drug exposure, Cancer Cell 32 (2017) 221–237 e13 https://doi.org/10.1016/j.ccell.2017.07.002.
[91] Y. Saito, T. Nakaoka, K. Sakai, T. Muramatsu, K. Toshimitsu, M. Kimura, T. Kanai, T. Sato, H. Saito, Inhibition of DNA methylation suppresses intestinal tumor organoids by inducing an anti-viral response, Sci. Rep 6 (2016), https://doi.org/10.1038/srep25311.
[92] N. Desai, D. Sajed, K.S. Arora, A. Solovyov, M. Rajurkar, J.R. Bledsoe, S. Sil, R. Amri, E. Tai, O.C. MacKenzie, M. Mino-Kenudson, M.J. Aryee, C.R. Ferrone, D.L. Berger, M.N. Rivera, B.D. Greenbaum, V. Deshpande, D.T. Ting, Diverse repetitive element RNA expression defines epigenetic and immunologic features of colon cancer, JCI Insight 2 (2017), https://doi.org/10.1172/jci.insight.91078.
[93] C.J. Langer, S.M. Gadgeel, H. Borghaei, V.A. Papadimitrakopoulou, A. Patnaik, S.F. Powell, R.D. Gentzler, R.G. Martins, J.P. Stevenson, S.I. Jalal, A. Panwalkar, J.C.-H. Yang, M. Gubens, L.V. Sequist, M.M. Awad, J. Fiore, Y. Ge, H. Raftopoulos, L. Gandhi, Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study, Lancet Oncol. 17 (2016) 1497–1508, https://doi.org/10.1016/S1470-2045(16)30498-3.
[94] N.C. Sheppard, R.B. Jones, B.J. Burwitz, F.A. Nimityongskul, L.P. Newman, M.B. Buechler, J.S. Reed, S.M. Piaskowski, K.L. Weisgrau, P.A. Castrovinci, N.A. Wilson, M.A. Ostrowski, B. Park, D.F. Nixon, E.G. Rakasz, J.B. Sacha, Vaccination against endogenous retrotransposable element consensus sequences does not protect rhesus macaques from SIVsmE660 infection and replication, PLoS One 9 (2014) e92012, , https://doi.org/10.1371/journal.pone.0092012.