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# Alternative RNA splicing in cancer: what about Adult T-cell Leukemia?

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In review

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

### *Author contribution statement*

JMP and JT conceptualized the article. Figures design and writing of the original draft and sections were carried out by JT. Reviews, corrections and editions of the final version were done by JMM and JMP. All authors contributed to this review article and approved the submitted version.

### *Keywords*

Alternative Splicing, Cancer, HTLV-1, tax, HTLV-1 bZIP factor (HBZ)

### *Abstract*

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Eukaryotic cells employ a broad range of mechanisms to regulate gene expression. Among others, mRNA alternative splicing is a key process. It consists of introns removal from an immature mRNA (pre-mRNA) via a transesterification reaction to create a mature mRNA molecule. Large-scale genomic studies have shown that in the human genome, almost 95% of protein-encoding genes go through alternative splicing and produce transcripts with different exons combinations (and sometimes retained introns), thus increasing the proteome diversity. Considering the importance of RNA regulation in cellular proliferation, survival, and differentiation, alterations in the alternative splicing pathway have been linked to several human cancers, including adult T-cell leukemia (ATL). ATL is an aggressive and fatal malignancy caused by the Human T-cell leukemia/lymphoma virus type 1 (HTLV-1). HTLV-1 genome encodes for two oncoproteins: Tax and HBZ, both playing significant roles in the transformation of infected cells and ATL onset. Here, we review current knowledge on alternative splicing and its link to cancers and reflect on how dysregulation of this pathway could participate in HTLV-1-induced cellular transformation and adult T-cell leukemia development

### *Contribution to the field*

The acquisition of drug resistance is a significant problem in the treatment of cancer including ATL. Many cellular mechanisms of drug resistance have been identified, most of which result from changes in gene and protein expression. While changes at the transcriptional level have been duly noted, it is primarily the alternative processing of pre-mRNA into mature mRNA or alternative splicing that regulates the cell's response to chemotherapeutic agents. Alternative splicing involves the differential inclusion or exclusion of exonic sequences into the mature transcript, generating different mRNA templates for protein production. This regulatory mechanism enables the production of many different protein isoforms from the same gene. Here in this review, we review current knowledge on dysregulation of alternative splicing pathways that could participate in HTLV-1-induced cellular transformation and we reflect on how the two HTLV-1 encoded oncoprotein Tax and HBZ could participate in HTLV-1-induced chemoresistance.

# Alternative RNA splicing in cancer: what about Adult T-cell Leukemia?

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7 **Keywords: HTLV-1, Alternative splicing, Oncogenesis, Leukemia, Chemoresistance**

8 **Abstract**

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18 **cell leukemia** virus type 1 (HTLV-1). HTLV-1 genome encodes for two oncoproteins: Tax and  
19 HBZ, both playing significant roles in the transformation of infected cells and ATL onset. Here, we  
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33 induced cellular transformation and we reflect on how the two HTLV-1 encoded oncoprotein Tax  
34 and HBZ could participate in HTLV-1-induced chemoresistance.

## 35 1 INTRODUCTION

36 Cancer burden remains today's second worldwide leading cause of mortality, with up to 10 million  
37 deaths reported in 2020 (1). Among other known risk factors, a substantial part of almost 15% of  
38 cancers is directly linked to infectious agents, especially viruses (2–4). To date, seven viruses have  
39 been described as oncogenic for humans, among which are DNA viruses: Human Papillomavirus  
40 (HPV), Epstein-Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Hepatitis B  
41 virus (HBV) and Merkel cell polyomavirus (MCPyV); one RNA virus: Hepatitis C virus (HCV);  
42 and one retrovirus: Human T-cell leukemia/lymphoma virus type 1 (HTLV-1) (3,5). HTLV-1 is a  
43 member of the *Retroviridae* family and was first discovered in the early '80s (6,7). Later on, it has  
44 been linked to a rare cancer named adult T-cell leukemia/lymphoma (ATL) (8,9) as well as to the  
45 HTLV-1 associated myelopathy or tropical spastic paraparesis (HAM/TSP) inflammatory disease  
46 (10,11). HTLV-1 infects roughly 10 million people in localized endemic clusters, the main ones  
47 being Southwest Japan, the Caribbean area, Central and South America, and West Africa (12). Most  
48 HTLV-1 infected individuals are asymptomatic, but 5% of the latently infected are at risk of  
49 developing ATL (13,14) and another 5% of HAM/TSP (11).

## 50 2 HTLV-1 INFECTION AND ONCOGENES

51 Even though HTLV-1 can infect multiple types of immune cells (lymphocytes, dendritic cells,  
52 macrophages...), the main targets remain the CD4<sup>+</sup> effector/memory T lymphocytes (15). HTLV-  
53 1 9kb-long proviral genome is composed of a positive-sense single-stranded RNA molecule, framed  
54 by two Long Terminal Repeat (LTR) regions in 5' and 3' (16), and randomly integrates into the  
55 host cell genome (17,18). Of note, retroviral LTRs are known to harbor bi-directional promoters  
56 (19). The neosynthesized viral particles can spread via a cell-cell contact through a viral synapse  
57 (20) but it is the clonal expansion of infected cells that is responsible for the proviral burden (21).  
58 HTLV-1 genome contains several regions encoding for either structural proteins such as Gag

59 (structural protein), Pro (protease), Pol (reverse transcriptase), and Env (envelope protein), or  
60 auxiliary and regulatory proteins like Tax and Rex. All the latter genes are expressed through  
61 transcription initiation in the promoter harbored by the 5'-LTR (22). However, a major study  
62 published in 2002 described a tenth protein-encoding gene with a transcription initiating in the 3'-  
63 LTR (23). This antisense transcript and protein is named HBZ for HTLV-1 Basic leucine Zipper  
64 (bZIP) transcription factor. HTLV-1 genome organization was summarized in **Figure 1**. So far,  
65 many studies have proven the crucial role of both Tax and HBZ in HTLV-1-associated persistence,  
66 pathogenesis, cellular transformation, and ATL development (24,25).

## 67 **2.1 Tax**

68 Tax was initially described as the viral transactivation protein of HTLV-1 because of its capacity  
69 to promote 5'-LTR transcription (26). Thus, it is an essential pawn in viral replication and *de novo*  
70 infection. Previous *in vivo* studies have also shown T-cell leukemia or lymphoma induction in  
71 transgenic mice when Tax alone is expressed (27) or simultaneously with HBZ (28). By modulating  
72 various cellular pathways, such as the NF-KB (29) or AP-1 (30) pathways, implicated in cell  
73 proliferation, apoptosis, and genomic stability, Tax plays a key role in cellular transformation and  
74 leukemogenesis onset (22). However, because it is a major antigen targeted by cytotoxic T  
75 lymphocytes, its expression is very often silenced in ATL cells. Indeed, nonsense mutations of the  
76 *tax* gene (31) and deletion and/or hyper-methylation of the 5'-LTR (32) explain why so few ATL  
77 patients display a stable Tax expression. Overall, HTLV-1 transactivator Tax remains necessary for  
78 the early steps of infection and cellular transformation onset, but not for their maintenance over  
79 time. Nonetheless, minor and sporadic expression of Tax is sufficient to trigger the antiapoptotic  
80 machinery. This mechanism once stimulated, continues even after Tax expression is switched off  
81 again, and allows the infection persistence (33). In other words, Tax transient silencing allows  
82 HTLV-1 stably infected cells to evade the immune system detection and to clonally expand.

## 83 **2.2 HBZ**

84 Unlike Tax, HTLV-1 antisense protein HBZ is expressed in all ATL cells. Over the years,  
85 diversified techniques were used to confirm both Tax silencing and HBZ strong expression in ATL  
86 cells isolated from leukemic patients (34–36). **Most recently, Matsuo et al. described a new  
87 intragenic enhancer region near the 3'-LTR that actively promotes and maintains antisense  
88 transcription, further confirming HBZ persistence over time (37).** After its discovery in 2002 (23),  
89 another study manage to describe different transcripts, hence different potential protein isoforms of

90 HBZ [**Figure 1**]. Indeed, three transcripts were characterized in different T cell lines: one unspliced  
91 isoform usHBZ and two spliced HBZ\_SP1 and HBZ\_SP2. However, only usHBZ and HBZ\_SP1  
92 can be detected at the protein level in ATL patients' PBMCs, HBZ\_SP1 being the isoform with the  
93 strongest expression (38,39). Like Tax, HBZ plays a crucial role in HTLV-1 pathogenesis and ATL  
94 development as both mRNA and protein expression of HBZ were shown to induce T cell  
95 proliferation; and the knockdown of *hbz* gene leads to cell death in infected/ATL cell lines (35,40).  
96 Because retroviral infection/leukemic environment is so complex and HBZ functions so pleiotropic,  
97 it is quite difficult to have a global view of HBZ's particular role in oncogenesis. *In vivo* experiments  
98 using HBZ-expressing transgenic mice have previously shown induction of the chemokine receptor  
99 CCR4 which resulted in a promotion of T-cell proliferation and migration, both needed for  
100 leukemogenesis (41). Moreover, our group showed that *hbz* gene expression leads to an increase in  
101 the AP-1 transcription factor JunD expression that correlates with the appearance of transformed  
102 cell features (42). We also described how HBZ induces the expression of a N-terminal truncated  
103  $\Delta$ JunD isoform. This isoform is unresponsive to the tumor suppressor *menin*, thus leading to cell  
104 proliferation and transformation (43). In addition, HBZ is responsible for the disruption of the  
105 microRNAs network leading to DNA-strand breaks and general genetic instability (44,45). Barbeau  
106 and Mesnard previously thoroughly reviewed the different ways HBZ uses to down-regulate the  
107 viral sense transcription, meaning Tax expression, to help evade host immune surveillance. This  
108 down-regulation occurs simultaneously with antisense transcription promotion, hence its own, to  
109 maintain and amplify its effects (46). Taken together, those data give us an overview on the central  
110 role, among many other cellular pathways (25), HBZ plays in the oncogenic process leading to  
111 ATL.

### 112 3 RNA SPLICING AND CANCER

113 As mentioned before, RNA splicing is a key mechanism for gene expression regulation, allowing  
114 one gene to generate several distinct mature mRNAs. According to large-scale genomic studies,  
115 approximately 95% of human protein-encoding genes are subjected to alternative splicing (47),  
116 hence greatly increasing the cellular transcriptome and the proteome diversity. Considering the  
117 importance of RNA regulation in multiple cellular pathways such as differentiation, proliferation,  
118 survival, and death, any aberration in the splicing process could lead to various diseases occurrence.  
119 Alternative splicing is a highly regulated mechanism involving a massive protein complex called  
120 the spliceosome. Spliceosomal assembly requires a series of steps and intermediate complexes and  
121 starts at the transcription site [**Figure 2**]. The spliceosome is composed of five small nuclear  
122 ribonucleoprotein particles (snRNPs): U1, U2, U4, U5, and U6; combined with roughly 300

123 associated proteins, and catalyzes pre-mRNA splicing reactions (48,49). In short, it recognizes the  
124 splicing donor and acceptor sites and takes care of removing introns (non-coding sequences) to  
125 ligate exons together. A more detailed explanation of the splicing process can be found in **Figure**  
126 **2**. Different kinds of splicing events can occur (listed in **Figure 3A**), therefore broadening the  
127 number of potential mature mRNAs and with that, protein isoforms, from a single coding gene.  
128 Alternative splicing is regulated by trans-acting regulatory proteins, also called splicing factors  
129 (SFs), binding to cis-acting regulatory sequences. SFs such as SR (serine/arginine-rich) proteins  
130 and hnRNPs (heterogeneous nuclear ribonucleoproteins) are RNA-binding proteins and are  
131 considered as enhancers (50,51) and silencers (52,53) respectively since SR proteins are typically  
132 recruited to **ISEs and ESEs (respectively intronic & exonic splicing enhancers)** while hnRNPs  
133 usually bind to ISSs and ESSs [**Figure 3B**]. Disruptions of the splicing mechanism and regulation  
134 have been documented in many pathologies, ranging from genetic (54–56) and autoimmune (57,58)  
135 diseases, to cancers; the latter being the center of discussion in the following sections.

### 136 **3.1 Splicing alterations in cancer onset and development**

137 Alternative splicing is an efficient and key mechanism for proteomic variety. Hence, cancer cells  
138 could divert and take advantage of this process to produce aberrant proteins with additional, deleted,  
139 or modified biological functions, thus contributing to oncogenesis. We discuss here at what level  
140 pre-mRNA splicing can be altered and the implication of this misregulation in cancer onset and/or  
141 development. The first alteration level would be mutations in the splicing core machinery, namely  
142 the spliceosome components. This aspect, even if it seems obvious, has not been extensively studied  
143 in **cancer biology**, as mutations in the spliceosomal assemble often lead to cell death. Using whole-  
144 genome sequencing, Yoshida *et al.* revealed frequent mutations for at least six spliceosome-related  
145 proteins-encoding genes in myeloid neoplasm samples (59). Among others, U2AF35, a key factor  
146 in the early steps of spliceosome assembly [**Figure 2**] is redundant. Its mutations induce defects in  
147 3'SS recognition and lead to major intron retention and with that, the introduction of termination  
148 codons in the mRNA, conducting to mRNA decay. Besides, mutations also significantly affected  
149 SF3B1, a component of U2 snNRP, in myeloid neoplasms but also in uveal melanomas, which it  
150 was more thoroughly studied (60,61). Globally, alterations of any splicing machinery component  
151 are linked to cell death rather than growth (59,62); however, they are still related to hematological  
152 disorders such as acute myeloid leukemia (59,63).

153 The second level of RNA splicing alterations resides in mutations, or at least differential  
154 expression, of the trans-acting regulatory factors mentioned before [**Figure 3B**]. This particular  
155 aspect has been extensively assessed in many cancers already, with a focus on the two main families

156 of splicing factors: SR and hnRNP proteins. As an example, SRSF1 (SF2/ASF) is particularly  
157 overexpressed in diverse human tumors such as breast (64,65) or lung cancers (64,66). Its  
158 expression both *in vitro* and *in vivo* allows cells to proliferate and transform; and its deletion leads  
159 to a reversal effect (64), making it a defined proto-oncogene. Other SR proteins like SRSF3  
160 (SRp20) and SRSF6 have been classified as proto-oncogenes as well, mostly in lung and colon  
161 cancers (67–69). Regarding the hnRNP family of proteins, recent genome-wide and pan-cancer  
162 omics data reveal that global overexpression of hnRNPs is linked with poor cancer prognosis.  
163 hnRNP A1, for instance, is highly expressed in most cancers (70) but seems to be a key proto-  
164 oncogene in particular in lung cancers where it antagonizes SRSF1 functions and deregulates the  
165 global RNA splicing environment (71,72). We won't be further detailing the links between splicing  
166 factors alterations and oncogenesis, as current knowledge has been broadly documented by others  
167 (73–75). Overall, even if SFs can act as oncogenes, it is the interplay between them and their  
168 expression balance that will determine the outcome, meaning the carcinogenic potential of the cells  
169 or not (71,76).

170 Finally, the last level of splicing dysregulation we wanted to mention is regarding known  
171 cellular oncogenes and/or newly oncogenic splice variants. Because RNA splicing can produce  
172 protein isoforms with modified functions, alteration in this mechanism could lead to new isoforms,  
173 with potentially higher transformation properties. Hence, cancer-related protein variants could  
174 confer survival advantages to cells and be preferentially selected. A good example we can highlight  
175 is p53. p53 is known as a master regulator of many cellular pathways ranging from cell-cycle  
176 control, DNA repair, and apoptosis to immunity (77,78). It has been shown that p53 is regulated by  
177 splicing as several protein isoforms have been described, associated with different cell fate  
178 regulation (79–81). p53 is generally called and considered a tumor suppressor for its DNA repair  
179 and cell-cycle controlling activities, however, in a cancer-related matter, p53 displays a differential  
180 expression of its isoforms (82–84), and a particular one is often mentioned:  $\Delta 133p53$ . This variant  
181 is promoting tumor progression and angiogenesis in mice models and escapes cellular senescence;  
182 making it a potential prognosis marker (79,85,86). This example illustrates the infinite possibilities  
183 of RNA splicing misregulation of key cellular genes in cancer development.

184 Taken together, these samples of explanation give us insights into how alternative splicing is  
185 altered at different levels in cancers. Adding to the complexity, this mechanism has also been  
186 described as implicated in various chemoresistance mechanisms (87–89) (not detailed here).  
187 Therefore, RNA splicing modulation is, without a doubt, a crucial element to consider in the  
188 development of new therapeutic strategies to prevent, ameliorate treatment efficiency, and fight  
189 cancer. In the remaining sections, we approach the peculiar matter of viral oncogenes and especially  
190 those of HTLV-1.

### 191 **3.2 Focus on viral oncogenes**

192 Oncogenic viruses are yet another particular case. As mentioned before, there are seven known  
193 viruses capable of inducing cancer in humans; each of them expressing its own viral oncogenes  
194 (90–92). As the splicing mechanism is greatly impaired in many cancers, knowing if and how those  
195 viral genes are regulated at the splicing level seemed relevant in order to assess their global  
196 oncogenic properties. Indeed, splicing promotes proteome diversity by producing protein isoforms  
197 with potentially different biological functions. Hence, regulation of viral oncogenes by splicing  
198 might be critical for their associated pathogenesis and oncogenesis. We tried to review here whether  
199 the said viral oncogenes' expression is regulated by alternative splicing or not, and if it is the case,  
200 what kind of alternative splicing events (ASEs) occur [Table 1]. Surprisingly, splicing of those  
201 viral oncogenes is globally well documented, except for Hepatitis B and C viruses for which we  
202 did not find clear information in the literature. Then, we went even further by looking at whether  
203 or not the same viral oncogenes were known splicing regulators. Overall, most of the mentioned  
204 oncogenes' expression is indeed regulated via different ASEs, and some are even known to alter  
205 the splicing mechanism themselves. This knowledge corroborates the current thinking about the  
206 link between RNA splicing dysregulation and pathogenesis, especially oncogenesis, as virally-  
207 induced cancers are no exception. In the last section, we focus on HTLV-1 particular case and  
208 review current insights on splicing regulation in HTLV-1 infection, focusing on its two oncogenes  
209 *tax* and *hbz*.

### 210 **4 RNA SPLICING IN HTLV-1 INFECTION**

211 Despite a growing interest in understanding the link between splicing mechanism and cancer onset  
212 and evolution, not much has been done concerning HTLV-1 and related ATL. However, Morteux's  
213 team study from 2014 did provide a few elements of clarity on the matter. Using microarray  
214 comparative analysis, the authors showed that HTLV-1 infected but untransformed cells display a  
215 significant modification in alternative exons usage (AEU). Similarly, ATL (leukemic patients) cells  
216 exhibited the same behavior but at a higher level, as the authors calculated an 11-fold increase in  
217 AEU events (93). When looking at the >300 genes affected by those splicing events, mostly cancer  
218 pathway-related genes can be found. This leads us to wonder how HTLV-1-infected cells can use  
219 these alterations to their advantage, and if it plays a bigger role in the pathogenesis and ATL onset  
220 than just promoting proteome diversity and cell plasticity. **As mentioned before, most HTLV-1**  
221 **regulatory and auxiliary genes undergo splicing for mRNA maturation before translation into one**

222 unique protein [Figure 1]; but only *hbz* produces different protein isoforms. Splicing alterations in  
223 HTLV-1 could then slightly affect the good expression of viral genes, compared to genes involved  
224 in major cellular pathways. Hence, it is legitimate now to consider the role of HTLV-1 regulatory  
225 proteins in the modulation of alternative splicing pathways.

226 The role of Rex in HTLV-1 pathogenesis has been extensively reported already. Indeed, it is  
227 known for mediating the transport of unspliced or incompletely spliced viral structural proteins out  
228 of the nucleus to promote viral particle formation (94,95). Although its potential role in splicing  
229 modulation had not been tackled in the HTLV-1 infection context yet, few studies did look into it,  
230 but in the HTLV-2 framework. Bakker *et al.* described decades ago that HTLV-2 Rex is a potent  
231 inhibitor of splicing *in vitro* and at an early step in spliceosomal assembly. Therefore, this inhibition  
232 of early spliceosome assembly by Rex may be responsible for the differential accumulation of  
233 unspliced transcripts, leading the latter to being transported out of the nucleus (96). With that in  
234 mind, we can consider that Rex could possess such splicing regulation function in the HTLV-1  
235 context as well. In the remaining sections, we review current knowledge about Tax and HBZ's  
236 potential role in splicing regulation and reflect on how dysregulation of this pathway, maybe by  
237 those two oncogenes, could participate in HTLV-1-induced cellular transformation and adult T-cell  
238 leukemia/lymphoma development.

#### 239 **4.1 Splicing modulation by Tax**

240 Regarding Tax and considering everything that was mentioned so far, it seems natural to question  
241 whether or not this viral transactivator and oncogene plays any role in the splicing mechanism  
242 regulation. Ben Ameur *et al.* were the first to tackle this particular aspect (97). Using RNA-  
243 sequencing analyses in reporter cells with a transient expression of Tax, the authors described that  
244 >900 genes were affected at the splicing level. Among all the splicing-altered genes recorded, only  
245 a minority of those were also altered at the expression level, meaning that Tax greatly impacts  
246 alternative splicing, independently from its transcription-mediation function. Half of the splicing  
247 modifications were also detected in ATL patients' samples, confirming the previous results.  
248 Considering that no significant modification occurred in splicing factors-encoding genes upon Tax  
249 expression, the authors found that Tax-induced splicing alteration was dependent on NF-KB  
250 activation. In addition to the well-described functions of Tax on this major pathway (29,98,99), Tax  
251 activates NF-KB transcription factor RelA, which locally recruits the auxiliary spliceosome  
252 component DDX17. This splicing regulator then modulates splicing via its RNA helicase activity  
253 (100). This study constitutes the first step for deciphering an additional regulation role of Tax, this  
254 time in the splicing modulation of HTLV-1-infected cells.

255 Later on, the same team assessed the occurrence of different splicing events (see the 6 first  
256 events listed in **Figure 3A**) upon Tax expression (101). Results showed that Tax mostly promotes  
257 exon inclusion in their model; and that the splicing targets were enriched for cancer-related genes.  
258 Co-IP assays could confirm the interaction between Tax and U2AF65 (U2AF large sub-unit, see  
259 **Figure 2**) mentioned before, adding to the list of spliceosome-related interactants of Tax. Taken  
260 together, those two studies indicate a non-negligible role of Tax on alternative splicing regulation  
261 of mostly cancer-related genes, in a DDX17 and U2AF65-dependant manner (100,101). These  
262 results contribute to enriching the diverse functions of Tax in HTLV-1-mediated pathogenesis and  
263 especially leukemogenesis.

#### 264 **4.2 Insights regarding HBZ**

265 As Tax is mainly expressed in the early stages of infection, or only transiently during the late ones,  
266 it seems necessary to question the role of the other HTLV-1 major viral oncogene on the splicing  
267 regulation matter. Unfortunately, this particular aspect was never approached, or only briefly.  
268 Indeed, Vandermeulen *et al.* also looked into the HBZ effect on splicing and uncovered that HBZ  
269 expression resulted in the occurrence of twice more splicing events compared to Tax (101). Authors  
270 noted opposite effects of Tax and HBZ, especially regarding exon inclusion and skipping  
271 (respectively) in their inducible Jurkat (immortalized T CD4<sup>+</sup> lymphocytes) cells model. However,  
272 data obtained in ATL patients' samples displayed a contrary result and indicated major exon  
273 inclusion promotion. As HBZ is the only viral gene expressed in all ATL cells, we hypothesize that  
274 the HBZ effect on splicing modulation is mainly resulting in this exon inclusion promotion type of  
275 event and that the study model may not be the most accurate for splicing study in an HTLV-1  
276 infection context. **Also, those observations could be confirmed in our experimental model using a**  
277 **bichromatic fluorescent reporter minigene in usHBZ or HBZ\_SP1 stably-expressing cell lines.** Y2H  
278 interactome revealed many HBZ-interactants related to RNA processing, especially splicing factors  
279 of interest such as PCBP1, SRSF2, or U2AF2, some overlapping with Tax. Again, analysis of the  
280 splicing targets revealed that HBZ mainly impacts cancer census genes indicating, in addition to  
281 proliferation and transformation functions previously cited. Hence, HBZ also seems to play a role  
282 in alternative splicing regulation; particularly of oncogenesis-associated genes.

### 283 **5 DISCUSSION AND FUTURE PERSPECTIVES**

284 Alternative pre-mRNA splicing is an essential mechanism that contributes to cellular plasticity and  
285 proteome diversity, giving cells an evolutionary advantage. It has now become clear that

286 dysregulation of this key process greatly participates in transformation and maintenance of cancer  
287 cells. More and more works are contributing to deciphering the molecular mechanisms implicated  
288 in those aberrations, however, even with rapidly-advancing technologies such as high-throughput  
289 whole-genome and RNA-sequencing techniques, fully understanding the contribution of RNA  
290 splicing dysregulation in cancer biology is far from being achieved. In this review, we discussed  
291 the different levels of alternative splicing impairment of and by cellular and/or viral oncogenes and  
292 the consequences in the establishment of a cancer-prone environment. Given all the knowledge  
293 already acquired on the matter, it seems crucial to now consider this RNA splicing alteration aspect  
294 as a target in the development of future therapeutic approaches to either prevent, treat or even re-  
295 sensitize cancer cells to current chemotherapeutic strategies. Indeed, recent studies have highlighted  
296 the role of aberrant splicing in the occurrence of drug resistance mechanisms in many different  
297 cancers. Whether it contributes to chemoresistance by modifying the drug targets' gene expression,  
298 or by diversifying the drug efflux-associated ones, alternative splicing can impair the global gene  
299 expression profile of a cancer cell, thus conferring survival benefits. The context of viral infections,  
300 adding to the already highly complex cancer framework, is even more poorly understood as it is the  
301 theater of an even greater number of splicing alterations. Considering the potential role of viral  
302 oncogenes in modulation of RNA splicing, studying their impact on key cellular pathways seems  
303 relevant to better evaluate the oncogenic and drug resistance properties they could confer to the  
304 infected cells. Regarding the particular case of HTLV-1 infection and related adult T-cell  
305 leukemia/lymphoma, if the molecular mechanisms of cell transformation and cancer onset and  
306 maintenance have been documented before, little is known about its oncogenes *tax* and *hbz* role in  
307 RNA splicing modulation. We reviewed here the current knowledge on the matter and stress the  
308 need of gaining more in-depth insights. As adult T-cell leukemia/lymphoma is a fatal malignancy  
309 for which no efficient treatment nor chemotherapies exist, exploring further the role of *tax* and *hbz*  
310 on alternative splicing regulation could give us the necessary tools to develop new approaches to  
311 treat this cancer.

## 312 **6 AUTHOR CONTRIBUTIONS**

313 JMP and JT conceptualized the article. Figures design and writing of the original draft and sections  
314 were carried out by JT. Reviews, correction and edition of the final version were done by JMM and  
315 JMP. All authors contributed to this review article and approved the submitted version.

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318 **8 TABLE**

In review

	Associated Cancers	Oncogenes	ASEs implicated	Splicing modulation ?
<b>Human DNA Viruses</b>				
Human Papillomavirus (HPV-16, 18, 31, 45)	Cervical, anal, vulvar, vaginal, penile, head and neck, skin cancer	E6, E7	Alternative 3' SS Alternative Poly(A) Sites (102–104)	Yes (105–107)
Epstein-Barr Virus (EBV/HHV-4)	B and T cell, Burkitt's and Hodgkin's lymphomas; nasopharyngeal and gastric cancers	LMP-1, BARF-1	Alternative Promoters (91,108,109)	Implied (110–112)
Kaposi's Sarcoma Herpesvirus (KSHV/HHV-8)	Kaposi's sarcoma, pleural effusion lymphoma	LANA, vCyclin, vFLIP, vIRF2-3, LAMP, vGPCR	Alternative Promoters, 3'SS and Poly(A) sites (91,113–115)	Implied (91,116)
Hepatitis B Virus (HBV)	Hepatocellular carcinoma	HBx	/ (Alternative translation initiation)	Yes (117,118)
Merkel Cell Polyomavirus (MCV/MCPyV)	Merkel Cell Carcinoma	T antigens (large/small)	Alternative 5' SS Intron retention (119–121)	N/D
<b>Human RNA Viruses</b>				
Hepatitis C Virus (HCV)	Hepatocellular carcinoma	Core protein, NS3, NS5A, and NS5B	N/D	Implied (117)
<b>Human Retroviruses</b>				
Human T-cell Leukemia/Lymphoma Virus Type 1 (HTLV-1)	Adult T-cell Leukemia/Lymphoma	Tax, HBZ	Constitutive splicing (122,123) Alternative 5'SS Intron retention (38)	Yes (100,101) Suggested (101)

**Table 1 | Recapitulative table of human oncogenic viruses, associated cancers and alternative splicing regulation.** The seven human cancer-inducing viruses are listed and classified here with their respective oncogenes and related cancers. If the said oncogenes are regulated by alternative splicing, the alternative splicing events (ASEs) in question are mentioned and references are found between brackets. Their role in splicing modulation is also precised. N/D = Not Documented.

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## 632 10 FIGURES LEGENDS

633 **FIGURE 1 | Recapitulative diagram of HTLV-1 proviral genome, Open Reading Frames**  
634 **(ORFs), and splicing-regulated genes.** HTLV-1 genome is 9 kb long and flanked on each side  
635 by 5' and 3' Long Terminal Repeats (LTRs). HTLV-1 LTRs are approximately 750 bp in length and  
636 are segmented in U3, R and U5 regions (in order). U3 region usually contains enhancer and  
637 promoter sequences which drive viral transcription; and R domain encodes the 5' capping  
638 sequences (5' cap) and the polyadenylation (pA) signal. The structural genes *gag*, *pro*, *pol* and  
639 *env* are encoded in the 5' part of the provirus, and regulatory and auxiliary proteins are encoded  
640 in the pX region, at the 3' side. The transcription initiates in the promoter-bearing 5'-LTR in 5' → 3'  
641 for all viral genes, except for the antisense transcripts of *hbz*. *hbz* is encoded by the proviral  
642 complementary strand and its transcription, initiated in the 3'-LTR, occurs in an antisense fashion  
643 (3' → 5'). The different Open Reading Frames (ORFs) are indicated in roman numbers and splicing  
644 events are shown by dotted lines.

645

646 **FIGURE 2 | Step-by-step spliceosome assembly and pre-mRNA splicing reaction.** Splicing is  
647 catalyzed by a large protein complex called the spliceosome. Spliceosome assembly requires a  
648 series of steps and intermediate complexes, and starts at the transcription site. It involves 5 small  
649 nuclear ribonucleoprotein particles (snRNPs): U1, U2, U4, U5 and U6 ; combined with roughly 300  
650 associated proteins. Splicing is based on the recognition of 5'SS and 3'SS (splicing sites), also  
651 known as donor or acceptor sites, located at each end of an intron. Several cis-acting regulatory  
652 sequences are necessary such as the branching point sequence (BPS) and the poly-pyrimidine  
653 tract (PPT). Splicing begins with U1 snRNP recognition of the 5'SS and binding onto the **pre-**  
654 **mRNA**. U2 auxiliary factor (U2AF) 65 and 35kDa sub-units then respectively bind the PPT and the  
655 3'SS; and Splicing Factor 1 (SF1) the BPS. These first steps form the **E(arly) complex**, which  
656 converts into pre-spliceosome **complex A** after U2 snRNP recruitment at the BPS and SF1  
657 replacement. U2AF then leaves and U4, U5 and U6 pre-assemble into the tri-snRNP which is  
658 recruited to compose the pre-catalytic **complex B**. Rearrangement and catalytic activation into  
659 **complex B<sup>A</sup>** occur via U1 and U4 release. A first trans-esterification reaction is catalyzed and  
660 leads to the **Complex C** containing free Exon 1 and Intron-Exon 2 fragment. The complex  
661 undergoes additional rearrangement and activation, and the **Complex C<sup>A</sup>** catalyzes the second  
662 trans-esterification reaction to free Exon 2. Both exons are ligated in the **Post-splicing Complex**;  
663 U2, U5 and U6 as well as excised intron are released; and mature mRNA is formed. All snRNPs  
664 are recycled for additional rounds of splicing.

665  
666 **FIGURE 3 | pre-mRNA alternative splicing events and regulation mechanisms. A)** Common  
667 constitutive and alternative splicing events are listed here. Colored boxes represent different  
668 exons, grey lines stand for introns, and dotted lines are splicing events. Exons are usually included  
669 or excluded (skipped) individually, but mutually exclusive exons involve the preferential retention  
670 of one exon at the expense of one or more others. Alternative 5' and 3'SS selection induce exons  
671 modifications, as parts of the exons can be excluded during the process. Introns are mostly  
672 removed from the mRNA but inclusion can occur and often leads to nonsense-mediated decay or  
673 shift in the Open Reading Frame (ORF). Alternative promoters and poly(A) sites selection can also  
674 happen at the splicing level. All those events contribute to the increase of the proteome diversity.  
675 **B)** Alternative splicing is regulated by trans-acting regulatory proteins, named splicing factors  
676 (SFs), binding to cis-acting regulatory sequences. SFs such as SR proteins and hnRNPs are RNA-  
677 binding proteins and are considered as enhancers and silencers respectively, since SR proteins  
678 are typically recruited to ISEs and ESEs while hnRNPs usually bind to ISSs and ESSs. However,  
679 increasing number of studies have revealed more context-dependent regulation roles for each.  
680 Other SFs are also at stake, and it is the balance and interplay between those splicing activators  
681 and repressors, and with the spliceosome components, that determines the splicing donor and  
682 acceptor sites selection for a splicing event to occur.

683 **11 GLOSSARY**

- 684 5'/3'-LTR: 5'/3'-Long Terminal Repeat  
685 5'/3'SS: 5'/3' Splicing site  
686 AEU: Alternative exon usage  
687 AS: Splicing acceptor site  
688 ASE: Alternative splicing event  
689 ATL: Adult T-cell leukemia  
690 BPS: Branching point sequence  
691 DS: Splicing donor site  
692 EBV: Epstein-Barr virus  
693 ESE: Exonic splicing enhancer  
694 ESS: Exonic splicing silencer  
695 HAM/TSP: HTLV-1-associated myelopathy/Tropical spastic paraparesis  
696 HBV: Hepatitis B virus  
697 HBZ: HTLV-1 Basic leucine Zipper (bZIP) transcription factor  
698 HCV: Hepatitis C virus  
699 hnRNPs: Heterogeneous nuclear ribonucleoproteins  
700 HPV: Human papillomavirus  
701 HTLV-1: Human T-cell leukemia/lymphoma virus type 1  
702 ISE: Intronic splicing enhancer  
703 ISS: Intronic splicing silencer  
704 KSHV: Kaposi's sarcoma herpesvirus  
705 MCPyV: Merkel cells polyomavirus  
706 ORF: Open reading frame  
707 PPT: Polypyrimidine tract  
708 SF: Splicing factor  
709 snRNPs: Small nuclear ribonucleoprotein particles  
710 SR proteins: Serine/arginine-rich proteins  
711 SRSF: SR splicing factor  
712 Y2H: Yeast-two hybrid

Figure 1.TIF

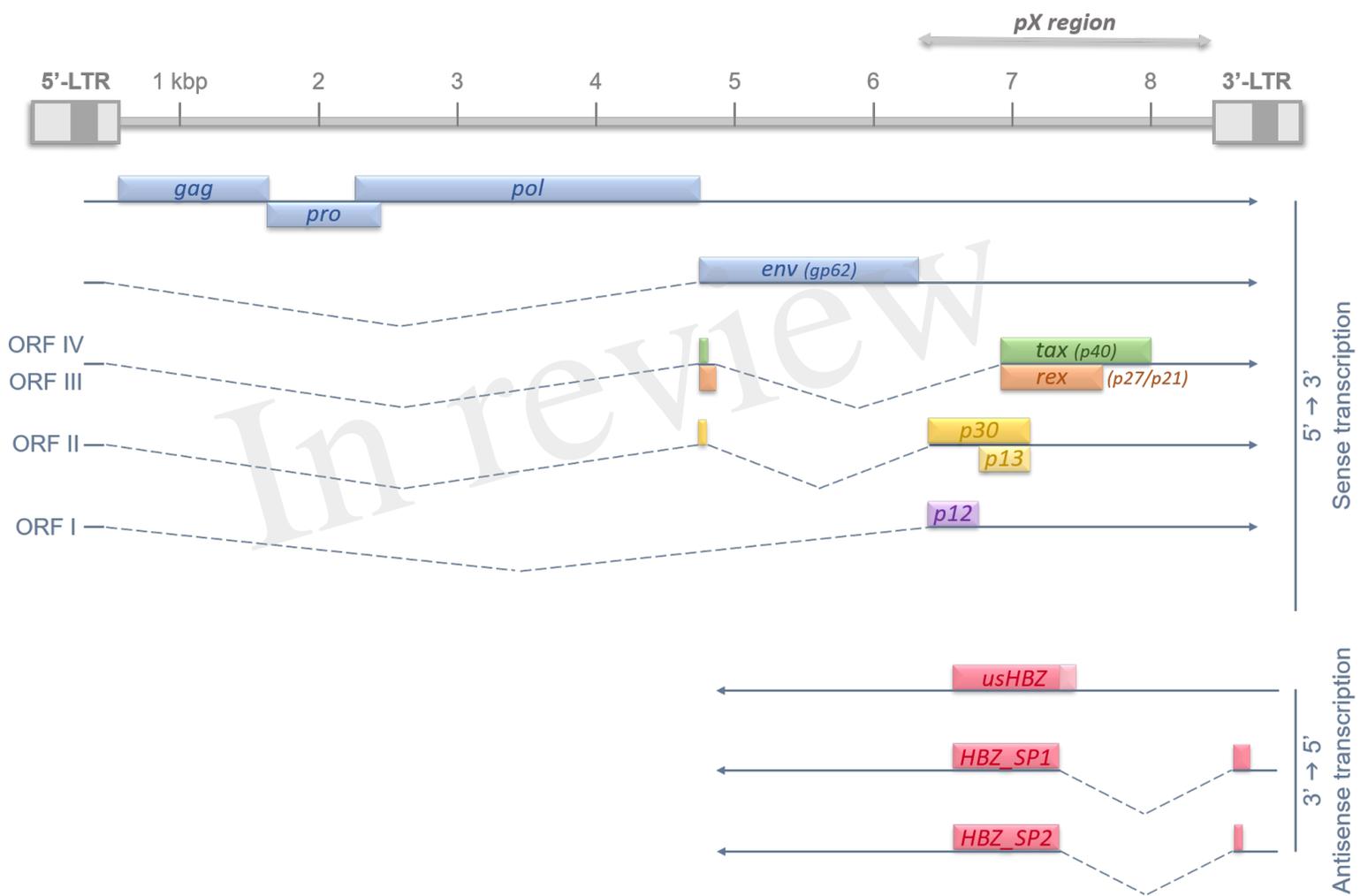


Figure 2.TIF

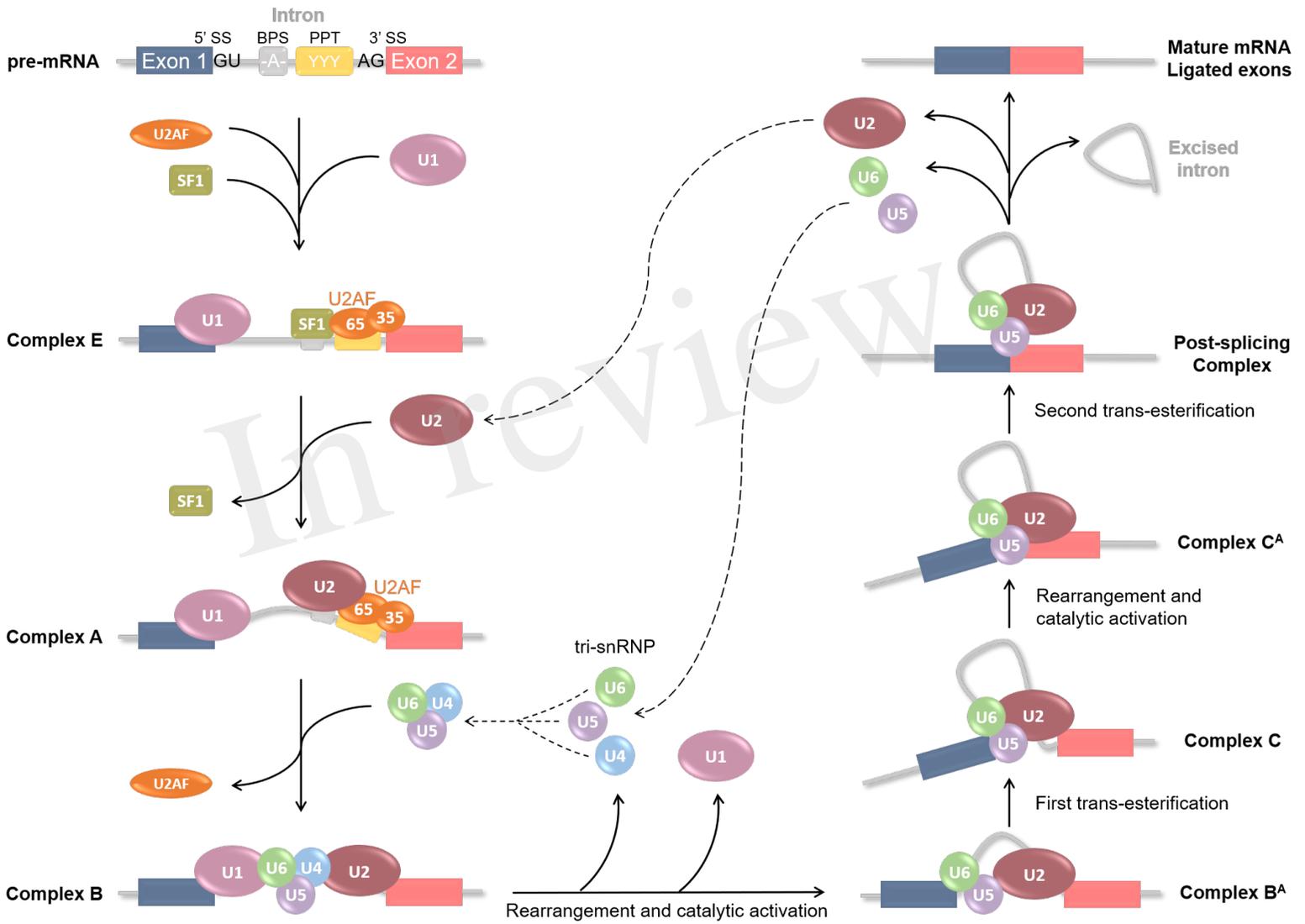
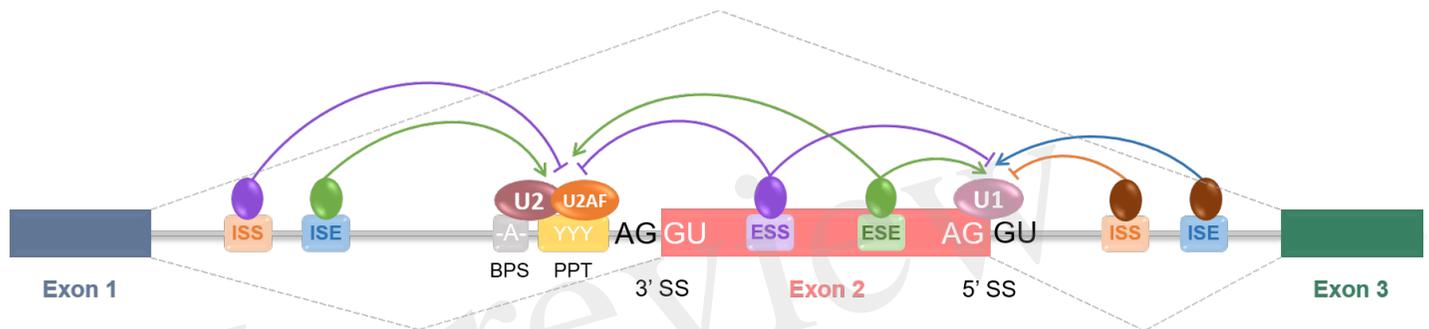


Figure 3.TIF



Figure 4.TIF



<b>Cis-acting regulatory elements</b>	<b>ISS</b> Intronic Splicing Silencer	<b>3' SS</b> 3' Splicing Acceptor Site	<b>Trans-acting regulatory proteins</b>
	<b>ISE</b> Intronic Splicing Enhancer	<b>5' SS</b> 5' Splicing Donor Site	
	<b>ESS</b> Exonic Splicing Silencer	<b>YYY</b> Poly Pyrimidine Tract	
	<b>ESE</b> Exonic Splicing Enhancer	<b>-A-</b> Branching Point Sequence	

<p>→ Activation/Enhancement</p> <p>↔ Inhibition/Silencing</p>	<p>U1, U2, U2AF Spliceosome components</p>
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