Adeno-associated virus infection and its impact in human health: an overview

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Abstract
Discovered as a contaminant of adenovirus stocks in the 1960s, adeno-associated virus (AAV) is a mono-stranded DNA virus that depends on helper factors to replicate. Even though AAV is endemic in the human population (35–80%), it is remarkable that many issues concerning the natural infection by this virus remain unanswered. In this study, we reflect on the main basic aspects of AAV biology and provide an overview of the studies exploring the impact of AAV infection on human health, focusing on three major research areas including, (i) cervical and (ii) liver cancer, and (iii) reproductive system disorders. Conflicting results have been obtained into the association of AAV infection with the occurrence of adverse reproductive outcomes, such as placental complications, spontaneous abortion, and fertility disorders, or with a protective role in HPV-related cervical carcinogenesis. Noteworthy, recent reports have identified AAV insertional mutagenesis as a novel risk factor for the development of hepatocellular carcinoma. This latest finding raises concern regarding the widespread usage of AAV vectors in liver-targeted gene therapy.

Keywords  Adeno-associated virus, Pathogenesis, Tumorigenesis, Hepatocellular carcinoma, Cervical cancer, Reproductive disorders, Wildtype AAV, Gene therapy, Human health

Introduction
Adeno-associated virus
Adeno-associated virus (AAV) was discovered as a contaminant in a simian adenovirus type 15 preparation in 1965 [1, 2]. Since then, AAV has been successfully developed into therapeutic vector, with Glybera (alipogene tiparvovec) becoming the first gene therapy medication to be authorized [3]. AAV infection is asymptomatic and can last a lifetime. The lack of identifiable associated disease and the unique capacity of recombinant AAV (rAAV) vectors to transduce dividing and non-dividing cells with high efficiency, long-term transgene expression, low immunogenicity, and selective tissue tropism make AAV appealing for gene therapy [4].

Within the Parvoviridae family, AAV belongs to the Dependoparvovirus genus. There are at least 13 naturally occurring serotypes, each with a different tissue tropism [5]. AAV infects various animal species, including humans, and is found worldwide, with seroprevalence varying from roughly 35–80% in the human population, depending on the AAV serotype and cohort analyzed [6, 7].

AAV can only replicate in the presence of helper factors, which are provided by helper virus coinfections. Adenovirus type 5 (AdV5) and herpes simplex virus type 1 (HSV-1) are well-studied AAV helper viruses. Although less well studied, many other members of the herpesvirus family have been shown to support productive AAV replication, such as human cytomegalovirus (HCMV), herpes simplex virus type 2 (HSV-2), varicella
zoster virus (VZV), and human herpesvirus 6 (HHV-6) (reviewed in [8]). Recently, human bocavirus 1 was demonstrated as a helper virus during AAV replication [9]. Interestingly, AAV replication can be triggered by treating AAV-infected cells with physical or chemical carcinogens, indicating that it is not intrinsically dependent on viral coinfections but rather on a significant alteration in the cellular environment [10, 11]. Without helper factors, AAV transfers its genome into the host cell, where most copies are eliminated within a short time, but other AAV genomes stay indefinitely. Long-term persistence is thought to occur mainly in an episomal, circular form. Latent AAV reactivates after coinfection with a helper virus, resulting in the emergence of progeny virus [8].

**AAV biology**

The AAV virion is a non-enveloped icosahedral particle with a single-stranded DNA genome. The most thoroughly researched serotype of AAV is type 2 (AAV2), which serves as the AAV family’s prototype. Because most vector expertise has been gained with AAV2, we will utilize information about this serotype to explain generic AAV properties. The AAV2 virion has a diameter of around 20 nm and is made up of 60 copies of the three capsid proteins VP1, VP2, and VP3 in a 1:1:10 ratio. The VP1 and VP2 proteins share the VP3 sequence and contain extra residues at their N-termini. A conserved phospholipase A2 region found at the N-terminus of VP1 has been linked to viral escape from endosomes and is required for infectivity [12]. The VP2 protein is not required for infection or assembly [13]. The core of VP3 protein is formed by a conserved β-barrel motif consisting of antiparallel β-sheets. Other paroviruses have this pattern, but the interstrand loops are variable, and they determine receptor usage and serology [14]. Understanding the molecular interactions of virus particles has relied heavily on structural and genetic data. X-ray crystallography and cryo-electron microscopy have been used to identify the structural images of numerous AAV capsids [14–17]. At the threefold axis, where proteins join together to create three clusters of peaks on the virion’s surface structure, there are several interactions between capsid subunits [14].

The AAV genome is a 4.7 kb molecule of single-stranded DNA. The positive and negative strands are packaged in separate premade particles with equal efficiency. Inverted terminal repeats (ITRs) create T-shaped, base-paired hairpin structures at each genome end and include cis-elements necessary for replication and packaging. Four nonstructural proteins necessary for replication (Rep78, Rep68, Rep52, and Rep40) and three structural proteins that make up the capsid (VP1, VP2, and VP3) are encoded by two genes (rep and cap). The p5, p19, and p40 viral promoters are recognized by their relative map location within the viral genome. Although different AAV serotypes have varied transcription patterns, all AAV2 transcripts have a single intron [18]. Rep78 and Rep52 are encoded by unspliced RNAs, whereas Rep68 and Rep40 are expressed by spliced RNAs (Fig. 1).

After binding to a primary receptor, AAV enters the cell via endocytosis [19]. It was discovered that various serotypes attach to distinct cell receptors. AAV2, AAV3,
and AAV6 bind to heparan sulfate proteoglycan (HSPG), AAV1 to sialic acids, and AAV9 to N-linked galactose [19]. Coreceptors were proposed to have a role in AAV attachment and uptake, but further investigations failed to substantiate their importance [20–23]. Remarkably, AAV2 isolated directly from human tissues did not attach to HSPG, suggesting that lab strains have adapted to cell culture and that other AAV2 receptors exist in vivo [24]. A transmembrane protein termed AAV receptor (AAVR) was a necessary component for AAV transduction for many serotypes in a previous genetic screen [21]. In addition, GPR108 was another critical component for AAV entrance, which was suggested to function downstream in the same pathway as AAQR [25]. AAQR is found on the cell surface and is carried to the trans-Golgi network (TGN) in a retrograde endosomal way. Several endocytic routes have been proposed to play a role during the entrance. However, the clathrin-independent carrier (CLIC)/GPI-anchored protein-enriched early endosomal compartments (GEEC) pathway has been proven to be the most important endocytic route of infection [26, 27].

The precise method of AAV tracking from early endosomes to the cytoplasm is unknown. According to one scenario, AAV is carried from early endosomes to the TGN/Golgi apparatus, where it escapes and reaches the nucleus [28]. The transfer of AAV2 to the Golgi apparatus was revealed to be crucial for transduction, giving support to the hypothesis of retrograde endosomal transport. Retrograde transport via the endosomal system is a highly controlled and selective mechanism that allows the cell to recover and recycle proteins and lipids from the plasma membrane, allowing them to be localized to the Golgi (TGN and Golgi apparatus) and the endoplasmic reticulum (ER). Low endosomal pH and the action of proteases cause a conformational shift in the AAV capsid, exposing the N-terminal region of the major capsid protein, VP1 [29]. This so-called VP1 unique region (VP1u) comprises a phospholipase A2 domain (PLA2) and also a nuclear localization signal, enabling escape into the cytoplasm as well as nuclear import [12, 30–32]. Once within the nucleus, AAV2 was shown to accumulate in the nucleoli in an infectious form [33]. The process of AAV uncoating is poorly understood and appears to be a limiting step in AAV transduction [34, 35]. AAV may integrate into the host genome in the presence of Rep78, primarily at a locus on chromosome 19 designated AAVS1 [36, 37]. Although AAV genome integration is possible, multiple studies have demonstrated that integration inside AAVS1 and outside is rare, with just 0.1–0.5% of added infectious particles integrating [38, 39]. In cell culture and in vivo, the genomes of AAV-derived viral vectors were discovered to circulate over time, confirming the idea of a primarily circular episomal state during latency [40]. The binding of Rep78 and Rep68 to a particular region inside the p5 promoter, known as the Rep binding element (RBE), inhibits transcription during AAV latency, whereas the binding to the RBE sequence within the ITR stimulates transcription [41]. AAV enters its lytic stage after co-infection with a helper virus, which results in genome amplification and packaging.

During the construction of AAV-based vectors for gene therapy, a significant portion of the native AAV genome is deleted and the rep and cap genes are provided in trans. Briefly, rAAV vectors are commonly produced by triple plasmid transfection providing the AAV ITRs flanking a transgene cassette with a therapeutic gene of interest, the AAV rep and cap genes, and helper virus genes. The rAAV genomes are replicated and packaged into AAV capsids, which can be purified by different methods [42].

### AAV infection in humans

Even though AAV has been studied for over 50 years, little is known about the virus’s natural infection. This fact may be even more surprising given that anti-AAV antibodies are found in up to 80% of the human population [6, 7]. AAV has been found in human blood cells, cervix uteri, penis, semen, liver, epithelial cell brushings, amniotic fluid, and abortion material [43–46]. AAV may be transmitted through direct contact with an infected individual or indirect contact with the contaminated environment. Transmission routes include respiratory, gastrointestinal, and possibly sexual transmission. A concern for vertical transmission from mother to fetus also exists [46–48].

Very few studies addressing the impact of AAV infection on human health are available, and the results are conflicting. In this review, investigations on this subject were grouped into three major research topics (AAV infection and cervical cancer; AAV infection and reproductive system disorders; AAV infection and liver cancer), and were considered in the subsections below.

### AAV infection and cervical cancer

With a reported 604,127 new cases and 341,831 deaths in 2020, cervical cancer is predicted to be the fourth leading cause of cancer and the third highest cause of cancer-associated mortality in women worldwide [49].

It has been proposed that AAV can be sexually transmitted, possibly in combination with herpes or papilloma viruses [54, 57]. High-risk human papillomavirus (HPV) infection plays a critical role in the natural history of cervical cancer [50]. During HPV infection, two viral oncogenic proteins, E6 and E7, play a crucial role in the development of cervical cancer by interacting with p53 and retinoblastoma protein (pRB) to render these cellular regulatory proteins inactive [51, 52]. AAV has been demonstrated to suppress HPV-induced cell transformation in vitro, which is mediated by the AAV Rep 78 protein...
[53–55]. Furthermore, the expression of H-ras and H-fos genes along with HPV oncogenes is inhibited by Rep 78 [56]. These collective studies suggest that AAV could be directly related to the suppression of HPV infection and may be thus associated with a reduced risk for HPV-related cervical neoplasia.

In line with the idea of a protective effect, Mayor et al. (1976) demonstrated a less likelihood of cervical cancer development in HPV-infected individuals in the presence of AAV since 85% of healthy women showed to be seropositive for AAV, while in cervical cancer patients AAV antibodies could only be detected in 14% of the cases [57]. Consistent with this observation, Georg-Fries et al. (1984) showed that sera from patients with cervical carcinoma revealed average titers of AAV antibodies well below those of age-matched control groups [58]. It was shown by Coker et al. (2001) that AAV positivity was significantly associated with a decreased risk of high-grade squamous intraepithelial lesion, suggesting that AAV may play a protective or inhibitory role in late-stage cervical carcinogenesis [59]. Furthermore, Agorastos et al. (2008) found that the prevalence of AAV was significantly lower in HPV-positive than HPV-negative patients ($P=0.0009$, $P=0.00001$, and $P=0.0225$, for women with low-grade cervical lesions, high-grade cervical lesions, and cervical cancer, respectively). In contrast, no difference in the frequency of AAV DNA between HPV-positive and HPV-negative unaffected (control) women was observed [60]. More recently, Freitas et al. (2012) investigated the prevalence of AAV and HPV DNAs in cervical samples of HIV-seropositive and -seronegative women in Brazil. AAV-HPV co-infected women showed a lower rate of cervical intraepithelial neoplasia development compared with those infected only with HPV. HIV infection does not appear to influence AAV prevalence or AAV-HPV co-infection [61].

On the other hand, several studies did not support the protective role of AAV in cervical tumorigenesis. Strickler et al. (1999) found no relationship between AAV antibodies and the presence or grade of neoplasia in either Jamaican study subjects or women enrolled in a U.S. cervical cancer case [62]. In Odunsi et al. (2000), AAV DNA was not frequently present in either standard control cervical samples or cervical intraepithelial neoplasia, thus not supporting the hypothesis that AAV may be protective against cervical cancer [63]. Likewise, Ahn et al. (2003) found that AAV was not associated with any stages of cervical pre-cancer and cancer lesions by in situ hybridization and immunohistochemistry [64]. A case control study in pregnant and non-pregnant women found that AAV infection have any impact on cervical intraepithelial neoplasia development [65]. Moreover, a retrospective case control study by Zheng et al. (2006) found a low proportion of cervical cancer biopsies containing AAV genomes and no evidence that the presence of AAV in cervical smears of healthy women would be associated with a reduced risk of cervical cancer [66]. Finally, an Iranian study from 2017 detected a low proportion of cervical biopsies containing AAV genome (14.8% cervical cancer cases and 14% healthy controls) and no significant difference in correlation between HPV and cervical cancer in the presence or absence of AAV infection was found [67]. A description of the studies assessing the influence of AAV infection in cervical tumorigenesis is shown in Table 1.

### AAV infection and reproductive system disorders

Several infections have been linked to miscarriage and other adverse reproductive outcomes. Specifically, 15% of early miscarriages and 66% of late miscarriages have been attributed to infections [68]. In particular, the common occurrence of AAV DNA (and virions) in male and female genital tissues has given rise to the hypothesis that genital AAV infection may be linked to unfavorable impacts on reproduction, including placental complications, spontaneous abortion, and fertility disorders [46, 69–72]. AAV was found fatal for mouse embryos at the early stages of gestation, and transplacental infection was established [73, 74]. In humans, AAV DNA was detected by Tobiasch et al. (1994) in 40% of abortion material during the first trimester of pregnancy but not in the material of abortion from the second or third trimester. They suggested that AAV infection in the uterine mucosa and trophoblast cells may disturb placenta development and promote early miscarriage [46]. In agreement with previous results, Malhomme et al. (1997) showed the presence of AAV DNA in 69% of materials from early abortions [75]. Koi et al. (2001) hypothesized that viral infection of extravillous trophoblast cells might hinder placental penetration of the uterine wall, resulting in situations such as spontaneous miscarriage, pre-eclampsia, and premature birth [76]. In a case-control study by Arechavaleta-Velasco et al. (2006), AAV DNA was found more frequently in trophoblast cells from cases of severe pre-eclampsia (55%) than from normal term deliveries (19%, $P=0.002$) [70]. Two years later, they demonstrated that primary or reactivated AAV infection (maternal IgM seropositivity) early in pregnancy was associated with adverse reproductive outcomes linked to placental dysfunction, including pre-eclampsia, stillbirth, and spontaneous preterm delivery [69]. Kiehl et al. (2002) demonstrated AAV infection in embryo-derived tissue from Brazilian patients and further suggested a role of AAV in miscarriage and trophoblastic disease [71]. Also, in Brazil, Pereira et al. (2010) detected AAV DNA in 28.6% and 2.4% ($P<0.05$) of the spontaneous and intentional abortions, respectively, suggesting an association between AAV and spontaneous miscarriage [72].
contrast to these findings, Friedman-Einat et al. (1997) and Matovina et al. (2004) did not detect AAV DNA in any case of spontaneous abortion \[77, 78\]. Consistent with these studies, Sayyadi-Dehno et al. (2019), by analyzing the presence of AAV DNA in 81 therapeutic and

| References | Year | Country | Study population | Samples | Results | Conclusions |
|------------|------|---------|------------------|---------|---------|-------------|
| [57]       | 1976 | USA     | Cases = 120; Control = 128 | Serum | Presence of IgG antibodies to AAV type 2–3 complex in genital cancer cases was 14% and in healthy women was 85% ($P < 0.001$). | The percentage of sera with antibodies to AAV was significantly higher in the normal group than in the cancer patients. The role of AAV in possible abrogation of oncogenesis mediated through adenoviruses or herpesviruses is worthy of further investigation. |
| [58]       | 1984 | Germany | Cases = 83; Control = 50 | Serum | The control group exhibited approximately three-fold higher IgG antibodies titers against AAV type 5 than cervical cancer patients. | The data could suggest that AAV infection provides some protection against subsequent cervical cancer development. |
| [62]       | 1999 | Jamaica | Jamaican patients: Cases = 197 (CIN-1 = 105; CIN-3/CA = 92) Control = 94 U.S. patients: Cases = 74 Control = 77 | Cervical smear (Jamaica); serum (USA) | None of the 291 cervical specimens from Jamaican subjects tested positive for AAV DNA. No relationship between IgG antibodies to AAV type 2 and presence or grade of neoplasia in either the Jamaican or U.S. cervical cancer cases. | The data provide no evidence that AAV infection plays a role in cervical tumorigenesis or that AAV commonly infects cervical epithelial cells. |
| [63]       | 2000 | United Kingdom | Cases = 211 (CIN-1 = 83; CIN-3 = 128) Control = 433 | Cervical smear | 6/433 (1.4%) control cervical smears and 4/211 (1.9%) of CIN (CIN-1 = 2; CIN-3 = 2) contained AAV type 2 DNA. No correlation between AAV and any clinical feature was observed. | AAV DNA is not frequently present in either normal control cervical samples or cervical intraepithelial neoplasia. This does not support the hypothesis that AAV may be protective against cervical cancer. |
| [59]       | 2001 | USA     | Cases = 217 (HSIL = 55; LSIL = 162) Control = 96 | Cervical smear | AAV positivity was associated with a significantly reduced risk of HSIL (age and HPV-adjusted odds ratio (aOR) = 0.32) yet not with LSIL (aOR = 0.78); 53.8% of HSIL, 66.9% of LSIL, and 70.7% of controls were AAV+. | AAV may play a protective or inhibitory role in late stage cervical carcinogenesis. |
| [64]       | 2003 | South Korea | Cases = 92 (CIN-1 = 20; CIN-2 = 24; CIN-3 = 25; invasive = 23) Control (perilesional normal tissues) = 92 | Cervical tissue | AAV type 2 was detected in 55% of CIN-1, 84.5% of CIN-2, 52% of CIN-3, and 52.2% of invasive cancer cases. In perilesional normal tissues, AAV was detected in 57.6%, displaying 25% of CIN-1, 83.3% of CIN-2, 52% of CIN-3, and 65.2% of invasive cancer. | The differences in AAV prevalence are not significant between CIN and normal tissues, suggesting no significant correlation between AAV and cervical cancer. |
| [65]       | 2004 | Croatia | 165 nonpregnant and 53 pregnant women with cervical cancer. | Cervical smear | AAV type 2 DNA was found in 6% of the women. AAV infection was more frequently associated with pregnancy (17 versus 2.4%). | There is no evidence that AAV infection has any impact on cervical cancer development. These data point out that HPV could indeed be an AAV helper virus and that AAV as such can be considered sexually transmissible. |
| [66]       | 2006 | Sweden  | Cases = 104 Matched control = 104 | Cervical smear; cervical tissue | At baseline, 2% of case-women and 3% of control-women were positive for AAV type 2 DNA. At the time of cancer diagnosis, 12% of case-women and 3% of matched control-women were positive for AAV DNA. | AAV DNA was present in a low proportion of cervical cancers. No evidence that the presence of AAV in cervical smears of healthy women would be associated with reduced risk of cervical cancer. |
83 spontaneous abortions, found no statistically significant difference between the two groups [45].

Concerning to fertility factors, Rohde et al. demonstrated for the first time in 1999 the occurrence of AAV infection in human semen and suggested that sperm motility may be affected by the presence of AAV [79]. In 2001, Erles et al. showed an increased incidence of AAV infection with abnormal semen analysis, suggesting a role for AAV infection in male infertility, possibly interfering with spermatozoa development [80]. Furthermore, Mehrle et al. (2004) demonstrated that AAV DNA is integrated into testis tissue samples [81]. In contrast, Schlehofer et al. (2012) investigated AAV DNA in semen samples and endocervical material of 280 individuals of subfertile couples (146 males and 134 females), and no associations between AAV and other infectious pathogens, semen quality or subsequent fertility issues were indicated [82]. The studies investigating the association between AAV infection and adverse reproductive outcomes in humans are described in Table 2.

**AAV infection and hepatocellular carcinoma**

Liver cancer is expected to be the sixth most significant cause of cancer and the third leading cause of cancer-related mortality globally in 2020, with a reported 905,677 new cases and 830,180 deaths, posing a severe global health burden [83]. Hepatocellular carcinoma (HCC) represents 90% of all primary liver tumors [84]. HCC most commonly occurs due to chronic liver inflammation leading to liver cirrhosis, mainly caused by viral hepatitis, alcohol misuse, and nonalcoholic fatty liver disease [85]. The most common risk factor for developing HCC, accounting for around 50% of cases, is chronic infection with the hepatitis B virus (HBV) [86]. The evolution of a liver disease in HBV-infected patients (as well as for hepatitis C) is explained by the constant and unsuccessful attempts of the immune system to clear the virus, resulting in chronic liver damage [87]. However, apart from this common indirect mechanism, the development of 10 to 30% of HBV-associated HCC in a liver without cirrhosis suggests that HBV may also have direct oncogenic properties [88]. One of the genomic features of HBV-related HCC is the presence of frequent viral integrations in genes involved in carcinogenesis such as TERT, MLL4, and CCNE1 [88].

It has been demonstrated that the liver is the main site of infection of AAV [47]. Interestingly, in small sets of patients with HCC, AAV showed insertional oncogenic mutagenesis similar to HBV, with a common hot spot of viral insertion within TERT, CCNE1, and CCNA2 cancer driver genes, leading to their overexpression [44, 89, 90]. Two different mechanisms explained the oncogenic effects of AAV clonal integrations. First, the integrated AAV sequence typically comprises transcription factor binding sites as well as viral enhancers [91], which causes an oncogene to be strongly overexpressed nearby (CCNE1 or TERT) [44, 92]. Second, due to the usage of an alternate transcription start site (TSS) or the viral poly-A site, integrated viral sequences may cause the synthesis of truncated transcripts (for integrations in CCNA2 and

### Table 1 (continued)

| References | Year | Country | Study population | Samples | Results | Conclusions |
|------------|------|---------|-----------------|---------|---------|-------------|
| [60]       | 2008 | Greece  | Cases = 93      | Cervical smear | AAV infection was confirmed in 16.8% women, and AAV detection was not statistically different between HPV (–) and HPV (+) in the controls. AAV was significantly lower in the HPV (+) relative to the HPV (–) cancer patients. | HPV-infected individuals are less likely to develop cervical neoplasia if AAV is present. AAV probably demonstrates a protective role against the pathogenic consequences of HPV infection. |
| [61]       | 2012 | Brazil  | 284 women       | Cervical smear | AAV type 2 prevalence was 19.7%, with 18.7% and 20.3% in HIV-positive and -negative women, respectively. AAV was detected with higher frequency in HPV-infected women (P < 0.05). The AAV-HPV co-infected women showed a lower rate of atypical squamous cells of undetermined significance or cervical intraepithelial neoplasia development compared with those infected only with HPV. | This is the first report examining AAV in cervical samples of HIV-infected women and indicates that HIV infection does not appear to influence AAV prevalence or AAV-HPV co-infection. |
| [67]       | 2017 | Iran    | Cases = 61      | Cervical tissue | AAV type 2 DNA was detected in 7 cases (14%) of healthy controls and 9 specimens (14.8%) of case group. | A low proportion of cervical biopsies from Iranian women contained AAV genome. The correlation between HPV and cervical cancer showed no significant difference in presence or absence of AAV genome in cervix. |

CIN-1, low-grade cervical intraepithelial neoplasia; CIN-3/CA, CIN-3/carcinoma in situ or invasive cancer; HSIL, high-grade cervical squamous intraepithelial lesion; LSIL, low-grade cervical squamous intraepithelial lesion;
Remarkably, a region common to all inserted AAV sequences has recently been identified as a liver-specific enhancer-promoter element \([91]\). Although this region is missing in the majority of rAAV vectors currently used for gene therapy, animal studies have shown that integrated rAAV vectors can cause clonal growth and play a role in carcinogenesis \([94–96]\). On the other hand, no evidence of malignancy was observed from hundreds of normal mice treated with rAAV vectors \([97, 98]\). Likewise, big animals treated with

### Table 2: Studies on AAV infection and reproductive system disorders in humans

| References | Year | Country | Study population | Samples | Results | Conclusions |
|------------|------|---------|------------------|---------|---------|-------------|
| \[46\]     | 1994 | France  | 108 sera (24 women presenting with early miscarriage; 23 women with lesions of the cervix uteri; 61 controls). 30 biopsies of the cervix uteri (10 normal tissue; 5 lesions of the endometrium; 15 cervical cancer). 30 curettage material of spontaneous abortion. | Serum; biopsy of uterus; tissue material from spontaneous abortion | AAV type 2 DNA was amplified in histological sections of 19 of 30 biopsies of the uterine mucosa. AAV DNA was detected in abortion material during the first trimester of pregnancy (12/30 cases were positive) but not in material of abortion from the second or third trimester (9 cases). The prevalence of IgM antibodies to AAV type 2 was elevated in cases of spontaneous abortion (first trimester, 29.1%), and in women with cervical cancer (30.4%) compared to the control group (9.9%). | In view of the presence of AAV DNA and proteins in placenta tissue, serological tests might be useful to assess further the hypothesis of a possible role of AAV infection in spontaneous abortion. |
| \[75\]     | 1997 | France  | 13 histological sections of the cervix uteri; 9 endometrium biopsies; 26 samples of abortion material; 2 samples of curettage material of extrauterine gravidity; 1 sample of socially indicated abortion. | Cervical tissue; abortion material | HPV DNA was detected in approximately 60% of paraffin sections from uterus biopsies and cervical lesions containing AAV type 2 DNA and in approximately 70% of material from early miscarriage. | HPV may be a helper virus for AAV. |
| \[77\]     | 1997 | Israel  | 15 nasopharyngeal aspirates from symptomatic patients; 7 swab or fluid specimens from vesicles of patients suspected of having varicella-zoster virus infections; 21 human papilloma virus-positive genital biopsy specimens; 61 genital swab specimens from women suspected of having herpes simplex virus; 62 samples of first trimester aborted material (38 spontaneous and 24 induced abortions); 11 samples of chorionic villi; 3 lots of cultured human embryonic cells. | Different clinical samples. | AAV type 2 sequences were detected only in samples (n = 11) taken from the genital tracts of women suspected of having herpes infection and not in any of the other types of samples. | Our study demonstrates the presence of AAV in the female genital tract. However, in contrast to a previous report, we did not find solid evidence of its replication in maternal or embryonal tissues from the first trimester of pregnancy. The questions of a potential pathogenic etiology of AAV and the interaction with HSV remain open. |
| \[79\]     | 1999 | Germany | Men with diagnosed infertility = 30 Control = 8 | Semen | AAV DNA was detected in 30% (9/30) of the ejaculates from the infertile men. No AAV DNA was found in the ejaculates from the 8 control subjects. In 8 of 9 samples, AAV DNA could be found only in the spermatozoal fraction of the specimens. Seven of 9 semen specimens that contained viral DNA also demonstrated oligasthenozoospermia. Both AAV and HPV DNA was found in the spermatozoal fraction of 3 of 30 specimens. | The data demonstrate for the first time the occurrence of AAV infection in human semen. Sperm motility seems to be affected by the presence of AAV. |
### References (continued)

| References | Year | Country | Study population | Samples | Results | Conclusions |
|------------|------|---------|------------------|---------|---------|-------------|
| [80]       | 2001 | Germany | 95 men (with history of infertility = 73; without history of infertility = 22) 17 men (with malignant melanoma = 3; benign tumor = 5; stone = 1; adenoma of the prostate = 1; no diagnosis = 7) 38 azoospermic men. 57 female partners. | Semen; cervical smear; urethral smear; testicular biopsy | AAV DNA was detected in 38% (28/73) of ejaculates from men with abnormal semen analyses and in 4.6% of normal semen samples (1/22, \( P > 0.003 \)). In testes, AAV DNA was detected in 10 out of 38 biopsies from infertile men (26%), and in 2 out of 8 orchidectomy samples. | The data show an increased incidence of AAV infection with abnormal semen analysis. Detection of AAV DNA in the testes might point to a role for AAV infection in male infertility, possibly by interfering with spermatozoa development. |
| [71]       | 2002 | Brazil  | 78 paraffin-embedded tissue samples, including histologically confirmed hydatiform moles (42 complete, 4 partial, 3 invasive, 14 choriocarcinomas, and 15 materials from spontaneous abortion. | Tissue of hydatiform moles, choriocarcinomas, and spontaneous abortion. | AAV DNA was found in 43 samples (28/49 hydatiform moles, 4/14 choriocarcinomas, 11/15 miscarriage material). | These findings confirm AAV infection of embryo-derived tissue in humans and further suggest a role of AAV in miscarriage and trophoblastic disease. |
| [78]       | 2004 | Croatia | 108 women admitted to the hospital for threatened miscarriage. | Placental tissue | No detection of AAV DNA. | No influence of AAV infection in miscarriages during the first trimester of pregnancy. |
| [81]       | 2004 | Germany | 2 patients with prostate cancer. | Testis tissue | AAV DNA is present in an integrated form in testis tissue. A detailed analysis revealed integration within sequences of the so-called AAVS1 region on chromosome 19. | AAV DNA can integrate also after natural infection, and that integration occurs within the AAVS1 region, at least in some cases. |
| [70]       | 2006 | USA     | Cases of preeclampsia = 40 Control = 27 | Histological sections from the basal plate region of placentas, and trophoblast cells. | AAV type 2 DNA was found more frequently in trophoblast cells from cases of severe preeclampsia (22/40) than from normal term deliveries (5/27, \( P = 0.002 \)). | AAV infection is a previously unidentified cause of placental dysfunction. |
| [69]       | 2008 | USA     | Cases = 78 (34 spontaneous abortions; 24 spontaneous preterm deliveries; 20 women with at least one outcome usually attributed to placental dysfunction) Control = 106 | Serum | First trimester maternal IgM seropositivity to AAV type 2 was 5.6 times more prevalent among pre-eclampsia/IUGR/stillbirth cases (\( P = 0.0004 \)) and 7.6 times more prevalent among preterm deliveries (\( P < 0.0001 \)) than among controls. AAV infection (IgG seropositivity) was not associated with adverse pregnancy outcomes. | Primary or reactivated AAV infection (maternal IgM seropositivity) early in pregnancy was associated with adverse reproductive outcomes associated with placental dysfunction, including pre-eclampsia, stillbirth, and spontaneous preterm delivery. |
| [72]       | 2010 | Brazil  | Spontaneous abortion group = 68 Intentional abortion group = 13 | Decidual and chorionic tissues. | AAV type 2 was detected in 28.4% (23/81) of the abortion cases for at least one of the decidual or ovarian fragments, 32.3% (22/68) of the spontaneous and 7.7% (1/13) of intentional abortions. | The presence of AAV in decidual or trophoblastic cells in cases of abortion, as observed by in situ hybridization, implies that the virus could jeopardize the pregnancy. The significant predominance in spontaneous cases suggests possibly a causal association between AAV and abortion. |
Table 2 (continued)

| References | Year | Country | Study population | Samples | Results | Conclusions |
|------------|------|---------|------------------|---------|---------|-------------|
| [82]       | 2012 | Germany | 146 male and 134 female partners of asymptomatic subfertile couples | Semen samples and endocervical material | AAV DNA was detected in 20 out of 134 (14.9%) cervical swabs and in 29 out of 146 (19.9%) semen samples. 3.8% (5/133) couples were AAV DNA positive in both semen and endocervical materials. The presence of AAV DNA in semen was not significantly related to semen quality, nor was it coupled to the presence of AAV in the endocervical material of female partners. AAV DNA in endocervical material was not related to a reduced quality of cervical mucus or to other female infertility factors. | The presence of AAV DNA in semen samples or endocervical swabs showed no significant association with clinically relevant infertility factors. |

Tatsuno et al. (2019) performed virome capture sequencing using HCC and liver tissues obtained from patients with prior HBV or chronic HBV infection to investigate the integration of HBV and AAV into the human genome as a possible oncogenic event. In this study, CCNE1 and CCNA2 were transcriptionally activated by AAV in prior HBV infection, suggesting that despite the seroclearance of HBV surface antigen, such patients are at risk of developing HCC [90]. Table 3 summarizes the studies demonstrating the association between AAV infection and the development of HCC in humans.

### Conclusion

AAV-based gene therapy has significantly benefited from the fundamental knowledge developed in AAV research over the past 30 years. This progress has led to the development of several rAAV vectors, that are either undergoing clinical trials or have already received FDA approval, including Glybera, Luxturna, and Zolgensma. However, given that 35–80% of the world’s population is seropositive for neutralizing antibodies against one or more forms of AAV, it is remarkable that many issues concerning the AAV life cycle in vivo remain unanswered. Due to the intricate nature of the cause-and-effect link, it is challenging to make any firm inferences about the impact of AAV infection on human health. Although AAV is considered non-pathogenic, several reports describe AAV infection in association with adverse reproductive outcomes. The AAV link with tumor development is controversial. Some studies report an oncogenic effect of AAV infection (as in hepatocellular carcinoma), and others suggest a tumor suppressive role (as in HPV-related cervical cancer). Of note, natural infections with wild-type AAV have no demonstrable connection with the administration of current rAAV vectors since a significant portion of the native AAV genome is deleted during the construction of AAV-based vectors for gene therapy. In addition, no adverse event, including cancer of any kind, has ever been documented in clinical studies performed thus far using rAAV vectors [105,106]. Nevertheless, considering the extensive usage of rAAV vectors in liver-targeted gene therapy and its potential for insertion into the human genome, patients treated with rAAV...
vectors should be followed longitudinally to monitor long-term consequences and determine the risk of HCC development.

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TBS and NMA designed the study, searched and collected the literature, wrote, revised, and finalized the manuscript. All authors contributed to the article and approved the submitted version.

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Data availability
Not applicable.

Table 3
Studies on AAV infection and HCC development in humans

| References | Year | Country | Study population | Samples | Results | Conclusions |
|------------|------|---------|------------------|---------|---------|-------------|
| [44]       | 2015 | France  | HCC samples and corresponding non-tumor tissues = 193 | Liver tissue | Clonal integration of AAV type 2 was found in 11 of 193 HCCs. Integrations occurred in the known cancer driver genes CCNA2, (four cases), TERT (one case), CCNE1 (three cases), TNFSF10 (two cases) and KMT2B (one case), leading to overexpression of the target genes. Tumors with AAV integration mainly developed in non-cirrhotic liver (9 of 11 cases) and without known risk factors (6 of 11 cases), suggesting a pathogenic role for AAV in these patients. | AAV is a DNA virus associated with oncogenic insertional mutagenesis in human HCC. |
| [101]      | 2016 | South Korea | HCC patients = 289 (159 hepatitis-B-related cases; 16 hepatitis-C-related cases; 114 viral serology-negative cases) | HCC tissue | AAV type 2 DNA was detected in 0.7% (2/289) of the patients. AAV-related HCC showed no signs of liver cirrhosis. | AAV-associated HCC was very rare in Korean patients with HCC. This study is the first to report the clinical characteristics of Korean patients with AAV-associated HCC. These findings suggest epidemiologic differences in viral hepatocarcinogenesis between Korean and European patients. |
| [90]       | 2019 | Japan   | HCC patients = 243 (73 prior HBV without HCV infection; 81 chronic HBV; 56 prior HBV with HCV infection; 33 non-B non-C cases as negative controls) | Liver tissue | AAV type 2 was found to integrate into 3 genes in two prior HBV and 1 non-B non-C patients. CCNE1 and CCNA2 were targeted by AAV2 only in prior HBV, while SLC6A5 was integrated in a non-B non-C patient. | Despite the seroclearance of hepatitis B surface antigen, HBV or AAV integration in prior HBV was not rare; therefore, such patients are at risk of developing HCC. |
| [89]       | 2020 | France  | Patients with liver tumor = 1461 (936 HCC; 225 hepatocellular adenomas; 97 focal nodular hyperplasia; 87 hepatoblastoma or transitional tumors; 46 cholangiocarcinoma; 36 fibrolamellar carcinoma; 34 other tumors) | Liver tissue | AAV types 2 and hybrid 2/13 DNA was detected in 21% of the patients, including 6% of the tumor tissues. Episomal viral forms were found in 4% of the non-tumor tissues, frequently associated with viral RNA expression and HHV-6. In 30 HCC, clonal AAV insertions were recurrently identified in CCNA2, CCNE1, TERT, TNFSF10, KMT2B and GLI1/INHBE. AAV insertion triggered oncogenic overexpression through multiple mechanisms that differ according to the localization of the integration site. | Clonal AAV insertions were positive selected during HCC development on noncirrhotic liver challenging the notion of AAV as a nonpathogenic virus. |

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare no conflict of interest.

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References
1. Atchison RW, Casto BC, Hammon WM. Adenovirus-Associated Defective Virus Particles. Science. 1965;149:734–6.
2. Hoggan MD, Blacklow NR, Rowe WP. Studies of small DNA viruses found in various adenosine viruses: physical, biological, and immunological characteristics. Proc Natl Acad Sci U S A. 1966;55:1467–74.

3. Yla-Herttuala S. Endgame: glycerol finally recommended for approval as the first gene therapy drug in the European union. Mol Ther. 2012;20:1831–2.

4. Chandler RJ, Sands MS, Venditti CP. Reombinant Adeno-Associated Viral Integration and Genotoxicity: Insights from Animal Models. Hum Gene Ther. 2017;28:310–22.

5. Srivastava A. In virus tissue-tropism of adeno-associated viral vectors. Curr Opin Virol. 2016;21:75–80.

6. Calcedo R, Vandenberghhe LH, Gao G, Lin J, Wilson JM. Worldwide epidemiology of neutralizing antibodies to adeno-associated viruses. J Infect Dis. 2009;199:381–90.

7. Erles K, Sebokova P, Schiefler JR. Update on the prevalence of serum antibodies (IgG and IgM) to adeno-associated virus (AAV). J Med Virol. 1999;59:406–11.

8. Meier AF, Frascella F, Seyffert M. The Interplay between Adeno-Associated Virus and its Helper Viruses. Viruses 2020, 12.

9. Wang Z, Deng X, Zou W, Enghardt JF; Yan Q, Zuo J. Human Bovacavirus 1 Is a Novel Helper for Adeno-Associated Virus Replication. J Virol 2017, 91.

10. Nakamura A, Hara T, Nakahara M, Shirahata Y, Ueno T, Kamata T, et al. Detection of adeno-associated virus type 2 in synchroized cells without the addition of a helper virus. J Virol 1986;61:972–81.

11. Yalkinoglu AO, Heilbronn R, Burke A, Schiefler JR, zur Hausen H. DNA amplification of adeno-associated virus as a response to cellular genotoxic stress. Cancer Res. 1988;48:3123–9.

12. Giroud A, Wabubs CE, Zadoni Z, Reid M, Leike K, Tijssen P. Kleinschmidt JA, Hallet M. The VP1 capsid protein of adeno-associated virus type 2 is carrying a phospoholipase A2 domain required for virus infectivity. J Gen Virol. 2002;83:973–8.

13. Warrington HK Jr, Gabryatouk-Os, Harrison JK, Opie SR, Zolotukhin S, Murzynka N. Adeno-associated virus type 2 VP2 capsid protein is nonessential and can tolerate large peptide insertions at its N terminus. J Virol. 2004;78:6595–609.

14. Xie Q, Bu W, Bhatia S, Hare J, Somasundaram T, Aza A, Chapman MS. The atomic structure of adeno-associated virus (AAV-2) a, vector for human gene therapy. Proc Natl Acad Sci U S A. 2002;99:10405–10.

15. Nam HJ, Lane MD, Padrón E, Gurda B, McKenna R, Kohlbrenner E, et al. GPR108 is a Highly Con

16. Girod A, Wobus CE, Zadori Z, Ried M, Leike K, Tijssen P, Kleinschmidt JA, Hallek M. The VP1 protein acts as both a repressor and an activator to regulate AAV transcription during a productive infection. J Virol. 1997;71:1079–88.

17. Walters RW, Abgandie-Mckenna M, Bowman WD, Moninger TO, Olson NH, Seiler M, Chiorini JA, Baker TS, Agbandje-McKenna M. Structure of adeno-associated virus type 8, a gene therapy vector. J Virol. 2007;81:2269–71.

18. Padrón E, Bowman V, Kaladov N, Govindasamy L, Levy H, Nick P, McKenna R, Murzynka N, Chiorini JA, Baker TS, Agbandje-McKenna M. Structure of adeno-associated virus type 4. J Virol. 2005;79:5047–58.

19. Thomas CE, Storm TA, Huang Z, Kay MA. Rapid uncoating of vector genomes is the key to efficient liver transduction with pseudotyped adeno-associated virus vectors. J Virol. 2004;78:3110–22.

20. Cheung AK, Hoggan MD, Hauswirth WW, Bens KL. Integration of the adeno-associated virus genome into cellular DNA in latently infected humanDetroit B cells. J Virol. 1983;3:739–46.

21. Duyk J, Zabro S, Bens KL. Adeno-associated virus (AAV) site-specific integration: formation of AAV-AAV1 junctions in an in vitro system. Proc Natl Acad Sci U S A. 1999;96:12849–54.

22. Huser D, Weger S, Heilbronn R. Kinetics and frequency of adeno-associated virus site-specific integration into human chromosome 19 monitored by quantitative real-time PCR. J Virol. 2002;76:7554–9.

23. Marcy DMA, Young SM Jr, Samulski RJ. Integration of adeno-associated virus (AAV) and recombinant AAV vectors. Annu Rev Genet. 2004;38:819–49.

24. Sun X, Lu Y, Bish LT, Calcedo R, Wilson JM, Gao G. Molecular analysis of vector genome structures after liver transduction by conventional and self-complementary adeno-associated viral serotype vectors in murine and nonhuman primate models. Hum Gene Ther. 2010;21:750–61.

25. Pereira DJ, McCarty DM, Muzyczka N. The adeno-associated virus (AAV) Rep protein acts both as a repressor and an activator to regulate AAV transcription during a productive infection. J Virol. 1997;71:1079–88.

26. Zolotukhin S. Production of recombinant adeno-associated virus vectors. Hum Gene Ther. 2005;16:651–7.

27. Grossman Z, Mendelson E, Brok-Simoni F, Mileuir F, Leitner Y, Rechavi G, Ramot B. Detection of adeno-associated virus type 2 in human peripheral blood cells. J Gen Virol. 1993;74:1:981–6.

28. Nault JC, Datta S, Imbeaud S, Francon I, Mallet M, Couchy G, Letouze E, Pilati C, Verret BL, et al. Recurrent AAV-related insertional mutagenesis in human hepatocellular carcinomas. Nat Genet. 2015;47:1187–93.

29. Sayadzadi-Dehno Z, Seyyed Khorrami SM, Ghavami N, Ghotbi-Zadeh F, Khushi M, Hosseini M, Malekshahi SS, Shafiei-Jandaghi NZ. Molecular Detection of Adeno-Associated Virus DNA in Cases of Spontaneous and Therapeutic Abortion. Fetal Pediatr Pathol. 2019;38:206–14.

30. Tobiasch E, Rabreau M, Geletney K, Larue-Charlus S, Severin F, Becker N, Schiefler JR. Detection of adeno-associated virus DNA in human genital tissue and in material from spontaneous abortion. J Med Virol. 1994;44:215–22.

31. Gao G, Vandenberghhe LH, Alivra MR, Lu Y, Calcedo R, Zhou K, Wilson JM. Clades of Adeno-associated viruses are widely disseminated in human tissue. J Virol. 2004;78:6381–8.

32. Walz CM, Anisi TR, Schlehofer JR, Gissmann L, Schneider A, Muller M. Detection of infectious adeno-associated virus particles in human cervical biopsies. J Virol. 2004;78:8101–5.

33. Bublitz B, Abele-Meyer I, Kersten S, Seiler M, Schiefler JR. Detection of adeno-associated virus DNA in human genital tissue and in material from spontaneous abortion. J Med Virol. 1994;44:215–22.

34. Gao G, Vandenberghhe LH, Alivra MR, Lu Y, Calcedo R, Zhou K, Wilson JM. Clades of Adeno-associated viruses are widely disseminated in human tissues. J Virol. 2004;78:6381–8.

35. Chen CL, Jensen RL, Schnpee BC, Connell MJ, Shell R, Sfera TB, Bartlett JS, Clark KR, Johnson PR. Molecular characterization of adeno-associated viruses infecting children. J Virol. 2005;79:14781–92.

36. Dudek AM, Zabaleta N, Zinn E, Pillay S, Zengel J, Porter C, Franceschini JS, Estelren R, Carette JZ, Zhou G, Vandenbergh N. GP108 is a Highly Conserved AAV Entry Factor. Mol Ther. 2020;28:967–83.

37. Bartlett JS, Wilcher R, Samulski RJ. Infectious entry pathway of adeno-associated virus and adeno-associated virus vectors. J Virol. 2000;74:2777–85.

38. Nonnenmacher ME, Citrano J, Gillett D, Weber T. Syntaxin 5-dependent retrograde transport to the trans-Golgi network is required for adeno-associated virus infection. J Virol. 2015;89:1673–87.
53. Hermonat PL. Adeno-associated virus inhibits human papillomavirus type 16: a viral interaction implicated in cervical cancer. Cancer Res. 1997;57:5227–81.
54. Hermonat PL, Platt RT, Santin AD, Parmam GP, Flick JT. Adeno-associated virus Rep78 inhibits oncogenic transformation of primary human keratinoocytes by a human papillomavirus type 16-ras chimeric. Gynecol Oncol. 1997;66:487–94.
55. Wacz CM, Corea-Ochoa MW, Muller M, Schlehofer JR. Adeno-associated virus type 2-induced inhibition of the human papillomavirus type 18 promoter in transgenic mice. Virology. 2002;303:172–81.
56. Hermonat PL. Down-regulation of the human c-fos and c-myc proto-oncogene promoters by adeno-associated virus Rep78. Cancer Lett. 1994;81:129–36.
57. Mayor HD, Drake S, Stahmann J, Muford DM. Antibodies to adeno-associated satellite virus and herpes simplex in sera from cancer patients and normal adults. Am J Obstet Gynecol. 1976;126:100–4.
58. Georg-Fries B, Biederlack S, Wolf J, zur Hausen H. Analysis of proteins, helper dependence, and seroepidemiology of a new human parvovirus. Virology. 1984;134:644–71.
59. Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S, Coker AL, Russell RB, Bond SM, Pirisi L, Liu Y, Mane M, Kokorina N, Gerasimova S.
98. Li H, Malani N, Hamilton SR, Schlachterman A, Bussadori G, Edmonson SE, Shah R, Arruda VR, Mingozzi F, Wright JF, et al. Assessing the potential for AAV vector genotoxicity in a murine model. Blood. 2011;117:3311–9.

99. Li S, Ling C, Zhong L, Li M, Su Q, He R, Tang Q, Greiner DL, Shultz LD, Brehm MA, et al. Efficient and Targeted Transduction of Nonhuman Primate Liver With Systemically Delivered Optimized AAV3B Vectors. Mol Ther. 2015;23:1867–76.

100. Nichols TC, Whitford MH, Arruda VR, Stedman HH, Kay MA, High KA. Translational data from adenovirus-associated virus-mediated gene therapy of hemophilia B in dogs. Hum Gene Ther Clin Dev. 2015;26:5–14.

101. Park KJ, Lee J, Park JH, Joo JW, Kwon CH, Kim JW. Adeno-Associated Virus 2-Mediated Hepatocellular Carcinoma is Very Rare in Korean Patients. Ann Lab Med. 2016;36:469–74.

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