A “hit-and-run” affair – A possible link for cancer progression in virally driven cancers

Author

Ferreira, Danyelle A, Tayyar, Yaman, Idris, Adi, McMillan, Nigel AJ

Published

2020

Journal Title

Biochimica et Biophysica Acta (BBA) - Reviews on Cancer

Version

Accepted Manuscript (AM)

DOI

10.1016/j.bbcan.2020.188476

A “hit-and-run” affair – A possible link for cancer progression in virally driven cancers

Danyelle A. Ferreira, Yaman Tayyar, Adi Idris*[#], Nigel A.J. McMillan[#]

Menzies Health Institute Queensland, School of Medical Science, Griffith University, Southport, Queensland, Australia

[#] Authors share senior authorship

*Address correspondence to Dr Adi Idris, a.idris@griffith.edu.au

Present address: Menzies Health Institute Queensland and School of Medical Sciences, Building G05, Room 3.37a, Gold Coast Campus, Griffith University, Queensland 4222, Australia.

Highlights:

- Viruses may cause cancers at early stages, but become dispensable over time to preserve its malignant phenotype
- Tumour viruses may tumour cells to accumulate mutations and proliferate independently of viral presence
- Viruses might be responsible for more cancers than previously thought
- Vaccination programs might prevent many cancers if the ‘hit and run’ theory is true

Abstract

Background: It is well-known that certain cancers are caused by viruses. However, viral oncogenesis is complex and only a small fraction of the infected people develop cancer. Indeed, a number of environmental factors can contribute to virally infected cells developing cancer hallmarks, promoting tumorigenesis.

Scope of review: The hit-and-run theory proposes that viruses facilitate the accumulation of mutations and promote genomic instability until the virus becomes dispensable for tumour maintenance. Indeed, several studies have reported viral genome, episome and/or oncogene loss in tumour cells without losing malignant phenotype.

Major conclusions: The current evidence supports the clear contribution of certain viruses to develop cancers. Importantly, the evidence supporting the sustained maintenance of malignancy after the loss of viral "presence" is sufficient to support the hit-and-run hypothesis of viral cancer development. Long-term tracking of vaccination outcome over the decades will test this theory.

General significance: If the hit-and-run theory is true, viruses might cause more cancers than previously thought and will have implications in the prevention of many cancers through implementing vaccination programs.

Keywords: virus, oncogene, oncogenesis, viral loss, hit-and-run theory

Abbreviations: ATL, adult T-cell leukemia; BL, Burkitt's lymphoma; BKV, BK polyomavirus; EBV, Epstein-Barr virus; HBV, Hepatitis B virus; HCC, hepatocellular carcinoma; HCV, Hepatitis C virus; HTLV-1, Human T-lymphotropic virus type 1; HD, Hodgkin's disease; HPV, Human papillomavirus; HPyVs, Human polyomaviruses; JCV, JC polyomavirus; KS, Kaposi's sarcoma; KSHV, Kaposi's sarcoma herpesvirus; MCC, Merkel cell carcinoma; MCD, multicentric Castleman's disease; MCPyV, Merkel cell polyomavirus; NMSC, non-melanoma skin cancer; NPCs, nasopharyngeal carcinomas; PEL, primary effusion lymphoma; PLC, primary liver cell carcinoma; SCC, squamous cell carcinoma; SV40, Simian virus 40; VZV, Varicella-zoster virus.

1. Viruses Associated with Human Cancer

It is estimated that 15% to 20% of all cancers are associated with viral infections. Indeed, human gammaherpesvirus 4 (Epstein-Barr virus, EBV), human gammaherpesvirus 8 (Kaposi's sarcoma herpesvirus, KSHV), hepatitis B virus (HBV), hepacivirus C (Hepatitis C virus, HCV), human T-cell leukemia virus type 1 (HTLV-1), Merkel cell polyomavirus (MCPyV) and human papillomavirus (HPV) are responsible for over 12% of all human cancers. Even though several of these viruses play a key role in the development of malignancy, none of them causes cancer in the majority of infected individuals. In fact, virus-mediated cancers development occurs in a persistent infection context and might appear years to decades after the acute infection. Viral tumours result from the interplay of multiple physiological factors together with a viral infection, including immune suppression, specific gene mutation, chronic inflammation and long-term interaction between the virus and host (reviewed in (1)). Indeed, a number of cancers are associated with viruses and viral oncogenes (Table 1). Viruses associated with human cancers have common features. For instance, they are either persistent latent or pseudo-latent infections and have their lytic replication reduced or inhibited. Is it possible that viruses might be associated with more cancers than previously thought? The hit-and-run theory proposes that viruses have a triggering role in cancer formation, where the viral genome can be entirely lost after the host cell accumulates numerous mutations (Figure 1). Therefore, relying on the presence of the virus to establish a viral aetiology may underestimate the number of cancers driven by a virus, since virus-positive cancers might represent only a fraction of all virally driven cancers. It begs the question of whether cancer malignancy absolutely requires the persistent infection of these viruses. Here, we aim to provide a concise up to date review on the commonly associated cancer-causing viruses.

Table 1 – Cancer type and oncogenes associated with viruses

VIRUS	CANCER	ONCOGENES	WHO/IARC GROUP	REFERENCE(S)
EPSTEIN-BARR VIRUS	Lymphoid and epithelial tumours, Burkitt's lymphoma, Hodgkin's lymphoma, extranodal NK T-cell lymphoma, AIDS-associated lymphomas, undifferentiated nasopharyngeal carcinoma (NPC), gastric carcinoma, carcinomas of the salivary glands, thymic carcinoma, the mesothelial tumour leiomyosarcoma, and possibly breast carcinoma	LMP1, LMP2A, LMP2B, EBNA1, EBNA2	1	(2)
KAPOSI'S SARCOMA HERPESVIRUS	Kaposi's sarcoma, multicentric Castleman disease (MCD), primary effusion lymphoma (PEL) and KSHV-associated inflammatory cytokine syndrome (KICS)	LANA-1, vCyclin, vGPCR, vBcl-2, vFLIP, vIL-6, vIRF3 and K1	1	(3)
MERKEL CELL VIRUS	Merkel cell carcinoma	T antigens, VP1	2A	(4)
BK VIRUS	Possibly Prostate and urinary tract cancer	T antigens	2B	(5)

JC VIRUS	Possibly colon, gastric, brain and esophageal cancers	T antigens	2B	(6)
SIMIAN VIRUS 40	Possibly lymphomas, malignant mesothelioma, bone and brain tumours	T antigens	3	(7)
HEPATITIS B	Hepatocellular Carcinoma	HBx	1	(8)
HEPATITIS C	Hepatocellular Carcinoma	Core, NS3, NS5A	1	(9)
HPV	Cervix, vagina, vulva, anus, penis, intraepithelial neoplasia and a subset of head and neck	E5, E6, E7	1 (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59)/ 2A (HPV 68)/ 2B (HPV 5, 8, 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, 97)	(10)
HTLV-1	Adult T-cell leukemia (ATL), cutaneous T-cell lymphoma	Tax	1	(11)

WHO/IARC group 1: Carcinogenic to humans; WHO/IARC group 2A: Probably carcinogenic to humans; WHO/IARC group 2B: Possibly carcinogenic to humans; WHO/IARC group 3: Not classifiable as to its carcinogenicity to humans.

1.1. Epstein-Barr virus

Epstein-Barr virus (EBV), an enveloped double-stranded DNA (dsDNA) virus and herpesvirus family member, is the first ever reported human tumour virus. It is estimated that 1.8% of all cancer deaths are due to EBV-associated malignancies (12), notably Hodgkin's disease (HD), Burkitt's lymphoma (BL) and B cell lymphomas. EBV encodes nearly 100 viral proteins, among which only five (i.e. oncoproteins) are necessary to transform primary B cells into uninterruptedly proliferating lymphoblastoid cell lines (13).

Primary exposure typically occurs early in life, commonly transmitted by contact with respiratory secretions. This promotes access and entry into the oropharynx epithelium, where it probably initiates a lytic infection that leads to virus replication resulting in B cell transformation in pharyngeal lymphoid tissues. This is succeeded by a latent infection in the memory B cell pool, which provides a lifelong source of virus reactivation (14). During the latency stage, EBV persists as an episome in the nucleus of lymphocytes, epithelial cells and possibly T cells, natural killer cells, monocytes and smooth muscle cells without virion production. Here, its DNA circularizes, and a limited number of genes are expressed. This is composed of three different latency programs (I, II and III) and the virus expresses a different set of genes depending on its latency program (15). However, EBV-driven cancer development is complex and remains unclear despite the importance of the infection latency stage and its products in carcinogenesis. EBV latent membrane proteins, LMP1 and LMP2, and the Epstein-Barr nuclear antigens, EBNA1, and EBNA2, have known carcinogenic activities, though LMP1 and EBNA2 are most commonly associated in tumorigenesis (16).

LMP1 is expressed in latency II/III and is a key oncogenic factor. Indeed, tumour cells become more sensitive to chemotherapy after LMP1 inhibition (15). LMP1 stimulates the expression of vascular endothelial growth factor (VEGF), which contributes to metastatic and local invasion and contributes to immune escape and tumour progression (17). Importantly, LMP1 has

been detected in over 80% of nasopharyngeal carcinomas (NPCs), and its presence has been shown to promote an immunosuppressive microenvironment (18). LMP1 activates a plethora of signalling pathways related to p53-associated cell death inhibition, including nuclear factor-kappa B (NF-kB), c-Jun N-terminal kinase/AP-1, bcl-2, A-20 and phosphoinositide 3-kinase (PI3K)/Akt pathways (19). LMP1 can also increase aerobic glycolysis, a common cancer cell energy source, which contributes to increased cell proliferation (17). LMP1 also promotes cell survival by enhancing anti-apoptotic proteins expression (e.g. survivin and Mcl-1) and inactivating pro-apoptotic proteins (e.g. Bad and Foxo3a) (20). Contribution of LMP2 to tumour progression is also well-described (21). The expression of Bcl-xL, an anti-apoptotic protein family member, and the activation of the Ras/PI3K/Akt pathways are increased by LMP2 resulting in enhanced cell survival. LMP2 also stimulates cell cycle progression by downregulating p27, a cyclin dependent kinase inhibitor (19). LMP2 is also known to play a role in the migratory and invasive capabilities in NPCs (22). LMP2 blocks the shift to lytic infection, thereby preserving its latency state. It allows infected B cells to evade apoptosis by mimicking an activated B cell receptor (BCR) (23).

EBNA1 is expressed in all three latency stages and is responsible for viral DNA replication and integration into the host cell genome allowing virus persistency. It is the only protein found to be expressed in all EBV-related tumours (21). EBNA1 increases the ability of B cell transformation and can regulate anti-apoptotic activity, possibly by enhancing survivin expression and reducing p53 and Mdm2 expression (24). On the other hand, EBNA2 is expressed in latency stages II and III and is one of the first viral proteins produced during an EBV infection (25). EBNA2 is also important for EBV-mediated B cell transformation by activating the required viral and cellular gene transcription programs (15). Additionally, it further upregulates the expression of viral membrane proteins, LMP1 and LMP2 (23).

1.1.1. EBV and carcinogenesis – A hit-and-run affair?

In the hit-and-run theory, it is thought that the virus starts the process of cancer formation (the ‘hit’) but as mutations are accumulated over time by through carcinogenesis, the need for the viral genes is no longer there and so they are lost (the ‘run’). It is known that the EBV genome is preserved in multiple episomal copies within an infected cell in a latent stage. BL EBV-positive cell lines usually contain from 10 to 100 viral episomes per cell (26). *In vitro* studies have shown that EBV episomes are lost in the absence of selective pressure for its preservation (27). Though cells overexpressing EBNA1 under antibiotic selection will replicate, without this selection, cells lose the episome in each cell cycle until the virus is eliminated. However, the loss of EBV does not affect the malignant cell phenotype. Loss of EBNA1 expression in BL cells after cloning in soft agar has been reported, without loss of tumorigenic phenotype (28). Furthermore, the complete and spontaneous loss of EBV episome in a BL cell line, Mutu, did not compromise its tumorigenic phenotype (26). Indeed, fragments of EBV DNA have been found to be integrated into the host genome and in EBNA1-negative BL tumours lacking viral episomes (26). Gastric carcinoma and NPC cells grown in tissue culture commonly lose viral episome during passaging probably due to the loss of EBNA1, which is essential for the maintenance of the viral episome (29).

Weiss *et al.* (30) first documented EBV association with HD, and documented numerous HD tissues carrying EBV genomes. Two independent studies have reported a case of loss of EBV at relapse in HD, where EBV DNA, EBV-specific RNAs (EBER), LMP-1, and EBNA proteins were detected in the initial tumour, but were not detected in the relapse of the tumour (31, 32). Loss of EBV did not affect the pathological features or behaviour of the tumour, suggesting that EBV is not necessary for tumorigenic phenotype maintenance. In contrast to EBV loss seen in BL, where viral DNA integration in the cellular genome was observed (26), fragments of the viral genome were not detected in HD (33). These cases, where no evidence of DNA is left behind, might justify EBV-negative cases. These findings suggest that EBV has an initial role for tumorigenesis, but that viral DNA may be lost during malignant progression (27). In addition, cells which have

spontaneously lost the viral episome and preserved the malignant phenotype may have an advantage due to its capability to avoid immune surveillance (33).

Overall, there are evidences that tumour cells preserve their malignant phenotype even after losing EBV. These observations support the hypothesis that EBV may use a hit-and-run mechanism in tumorigenesis.

1.2. Kaposi's sarcoma herpesvirus

In 1872, Moritz Kaposi reported five cases of a rare cutaneous malignant tumour on the feet, and the term "Kaposi's sarcoma" (KS) was designated in 1895. But it was only in 1994 that Chang and Moore (34), using a PCR-based technique, isolated unique sequences from KS lesions in acquired immunodeficiency syndrome (AIDS) patients and identified the causative agent of KS, human herpesvirus 8 (HHV8) (also known as KS-associated herpesvirus (KSHV)). KSHV is a member of the γ-herpesvirus family and has a linear double-stranded DNA genome, enveloped in a lipid bilayer containing glycoproteins. KSHV is also aetiologically associated with two B cell lymphoproliferative conditions; primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD) (3). Moreover, KSHV has been linked to other lymphomas and diseases, including BL, angioimmunoblastic lymphoma, multiple myeloma, angiosarcomas, germinotropic lymphoproliferative disorder, primary pulmonary hypertension, and malignant skin tumours. Current evidence suggests that KSHV is transmitted mostly by saliva, and typically occurs during childhood (35), where KSHV is transmitted from mother to child and among siblings. Together with the high prevalence of Kaposi's sarcoma in human immunodeficiency virus (HIV)/AIDS patients (34), KSHV is sexually transmitted and can be found in semen. There is also a potential for transmission by blood or blood products, solid organ transplantation and drug injection (36).

KSHV enters cells mainly via the endocytic pathway. During entry, innate immune sensors activate an antiviral response allowing the virus to enter into the latency phase. KSHV has been detected in endothelial, epithelial, B cells, and monocytes *in vivo* (37), whilst, it can infect a wider range of cells *in vitro* (B cells, fibroblasts, keratinocytes, monocytes, and plasmacytoid dendritic cells) (38). Like other members of the γ-herpesvirus family, KSHV life cycle is divided into two infection phases, the lytic and latent phase (39). During latency, KSHV upregulates many cellular proteins and viral oncogenes, including latency-associated nuclear antigen (LANA), viral cyclin (vCyclin), viral G protein-coupled receptor (vGPCR), viral B-cell lymphoma 2 (vBcl-2), viral FLICE inhibitory protein (vFLIP), viral Interleukin-6 (vIL-6), viral interferon regulatory factor 7 (vIRF3), and K1 (40). One exclusive characteristic of KSHV is its ability to express viral oncogenes homologous to that of host cellular proteins (e.g. vGPCR, vCyclin, IL-6, vBcl-2 and vIRFs), to fulfil its host immune evasion properties.

1.2.1. KSHV and carcinogenesis – A hit-and-run affair?

KSHV genome loss in KS cell lines have been reported after several passages *in vitro*, suggesting that KSHV episome is not stable in KS cells and that growth can be maintained independently of KSHV (41-43). vCyclin hyper phosphorylates checkpoint molecules, such as retinoblastoma protein (RB), in infected cell lines, consequently inactivating these molecules and deregulating the host cell cycle machinery (44). vCyclin expression is highly expressed in advanced KSs (45). Kennedy *et al.* (44) found that vCyclin transcription was higher in early stages of KS. Importantly, using an *in vivo* Cre-lox model to promote virus-infected cell transformation, viral genome absence was reported in almost all emerged virus-driven tumours (46), supporting the hit-and-run theory.

1.3. *Human polyomavirus 5* (Merkel Cell Polyomavirus)

Human polyomaviruses (HPyVs) are small non-enveloped, icosahedral viruses and have a circular double-stranded DNA genome. Ludwig Gross discovered the first polyomavirus while studying leukemia induced by a murine leukemia virus (47). The second polyomavirus to be identified was *Macaca mulatta* polyomavirus 1 (Simian virus 40, SV40) in a monkey kidney cell culture used for a poliovirus vaccine study (48). In 1971, the Human polyomavirus 1 (BK polyomavirus, BKV) was isolated from a renal transplant patient urine sample (49). On the other hand, human polyomavirus 2 (JC polyomavirus, JCV) was isolated from a progressive multifocal leukoencephalopathy patient brain biopsy sample (50). In 2008, a new polyomavirus was identified to be associated with Merkel cell carcinoma (MCC), a rare malignancy of cutaneous neuroendocrine cells, so-called *Human polyomavirus 5* (MCPyV) (51). Since the discovery of mouse polyomaviruses (MuPyV) in 1953, polyomaviruses have been associated as potential aetiologic agents in several human cancers. Indeed, studies show that 69 to 85% of MCC tumours presented MCPyV DNA (52). MCPyV transmission occurs in early childhood via respiratory, fecal-oral or cutaneous routes, culminating in asymptomatic primary infection. The virus is preserved as an encapsidated virion mostly in the skin as part of the normal microbiota. The virus can reactivate as a result of UV radiation, immune system suppression, AIDS, or natural aging (53).

1.3.1. MCPyV and carcinogenesis – A hit-and-run affair?

It is known that MCPyV is present prior to clonal expansion of tumour cells and that the virus is neither a passenger nor an incidental infection. Numerous studies have reported that between 15% to 30% of MCC patients had no detectable MCPyV DNA in tumour biopsies (52). Interestingly, 23% of MCC patients negative for MCPyV DNA were seropositive for antibodies against MCV viral protein, VP1 (4). This suggests that MCPyV may be important for early cancer initiation, but not for malignant phenotype preservation in some MCC patients. It has been proposed that there exist two different subgroups of MCC (54). The first group is a less aggressive type linked to MCPyV infection and is presented with a high viral load, the presence of the T

antigen in the tumour site and high anti-MCPyV antibody titres. The T antigen is the major MCPyV oncogene that mediates tumour development. The more aggressive type, however, is not linked to MCPyV infection and is characterised with low MCPyV copy number per cell, lack of the T antigen and low MCPyV antibody levels. It is reasonable to hypothesise that the loss of viral oncogene addiction is the first step for the hit-and-run mechanism, where the virus infection stimulates cell transformation, but some transformed cells can overcome the virus loss by accumulating mutations and become more aggressive. Indeed, knockdown of the T antigen had no effect on the growth of the MCPyV-positive cell line, LoKe (55). This cell line may have acquired additional mutations, thereby permitting cells to continue growing without the need of the T antigen. Alternatively, MCPyV could be required for early tumour development and as the tumour progresses, additional genomic aberrations contribute to the loss of MCPyV.

1.4. Hepatitis viruses

Hepatitis B virus (HBV) is a small partially double-stranded DNA virus belonging to the *Hepadnaviridae* family. HBV transmission occurs through blood or body fluid contact during sexual intercourse and could also be vertically transmitted from mother to child. Though HBV persists as an episome in the infected cell, viral DNA integration into the host genome is not crucial for its life cycle (56). HBV is the causative agent for acute and chronic hepatitis and hepatocellular carcinoma (HCC).

1.4.1. Hepatitis B and C Virus and malignancies – A hit-and-run affair?

A model, developed by Hessein *et al.* (57), could explain how the virus acts in a hit-and-run fashion. The model suggests this happens through the acquisition and loss of HBV-DNA integrations during the tumour formation. In this model, progressive HBV DNA integration in the host cell followed by allele loss in clonally related regenerative nodules was observed in different liver segments. Genetic instability in HBV DNA during HCC development has been reported,

possibly due to viral DNA integration near a fragile site (specific chromosomal loci that is vulnerable to breakage) (58). In such cases, the persistence of the viral genome would be achieved through integration in stronger sites (regions not susceptible to breakage). Indeed, the viral allelic loss is more frequent in some chromosomes than in others (59). Though HBV is shown to be crucial for initiating the malignant transformation in primary liver cell carcinoma (PLC), it may not be essential for maintaining its malignant state. HBV DNA have not been detected tumours of most PLC cases (60) and in about 50% of hepatitis B viral protein (HBe)-positive patients with hepatoma and 11% of anti-HBe antibody-positive patients (61). HBV DNA integration detected in some PLC patients represents a possible preceding integration, which was expanded during clonal growth of tumour cells (61).

Hepatitis B or C (HCV) chronic infection is the main risk factor for HCC development (62). Hepatitis C virus, a member of the Flaviviridae family, is a single-stranded RNA enveloped virus. Although HCV RNA failed to be detected in HCV-associated hepatocellular carcinoma (HCC), it is highly detectable in non-cancerous liver cells (63, 64). Taking into account that HCV is known to promote genomic mutations, it is possible that HCV-induced tumour formation could occur via hit-and-run mechanism, where the transformed cell accumulates mutations and no longer require the viral oncogenes.

1.5. Human papillomavirus

Human papillomavirus (HPV) is a non-enveloped virus with a double-stranded DNA belonging to the *Papillomaviridae* family, which includes 16 different genera. Of these there are five major genera, alpha, beta, gamma, mu and nu. The genus Alphapapillomavirus is associated with mucosal tumour development in humans, while Betapapillomavirus is associated with cutaneous tumour. The genus beta was initially associated with skin cancer when HPV 5 and 8 were isolated in patients with an autosomal recessive disorder, called epidermodysplasia verruciformis (EV), which provides high susceptibility to beta HPV infection and, consequently,

non-melanoma skin cancer (NMSC) development (65). HPV is broadly divided into two groups; low and the high-risk HPV types. Over 200 types have been described based on their capsid protein L1; however, only the mucosal high-risk HPV types have been clearly linked to oncogenesis. Indeed, high-risk HPVs are associated with intraepithelial neoplasias, head and neck, cervical, anogenital cancers (10) and possibly breast cancer (66). HPV oncogenes associated with cellular transformation are E5, E6 and E7 (67). HPV is transmitted by contact within infected areas when the virus gains access to the basal epithelial layer through micro-lesion of the skin or mucosa and enters the cell through a cell surface receptor. HPV can persist as an episome, depending on the host cell replication for encoding important genes for the HPV life cycle. In this stage, the expression of E6 and E7 is necessary to maintain the cell being mitotically active; moreover, E1, E2, E4 and E5 gene expression promote the amplification of HPV (68). When the virus enters the basal epithelial layer, it activates the late viral genes leading to capsid protein formation and viral DNA replication. Once the viral particle is produced, the particles are released, exposing other cells to a possible infection. During the normal viral life cycle, HPV replicates extrachromosomally, but eventually integrates into the host genome in the E1/E2 region, which together with hypermethylation mechanisms might disrupt the E2 gene (68), which has a role for down-regulating E6 and E7 oncogenes. This leads to E6 and E7 overexpression and consequently the ability to progress to cancer. The changes caused by the virus integration might eventually cause genomic instability due to cell cycle disruption.

1.5.1. HPV and carcinogenesis – A hit-and-run affair?

It was long believed that HPV cancers are “oncogene addicted”. That is the expression of E6 and E7 is always required for these cells to grow. Indeed, this has been shown for cervical and head and neck cancers (69). However, it has long been speculated that this might not always be the case for all HPV cancers and that a “hit-and-run” event might be occurring. The idea is that E6 and E7 oncogenes start the process of cancer formation but as mutations are accumulated

over time through carcinogenesis, the need for the viral genes is no longer there and so they are lost. This was thought to explain the presence of HPV DNA in a range of other cancers such as breast cancer and prostate cancer (70, 71). Indeed, no HPV DNA sequences were detected in two out of eleven high-risk HPV 18-induced transformed cell lines, but these cells maintained the ability to induce tumour growth in mice after transplantation (72). In another study, higher HPV genome copy numbers were detected in pre-malignant actinic keratosis lesion rather than in malignant squamous cell carcinoma (SCC) lesions (73). Hasche *et al.* (75) used the mouse papillomavirus 1 (MnPV1)/mouse model to study the role of cutaneous papillomaviruses in NMSC development. This model simulates UV-induced NMSC formation in humans and the results demonstrated that MnPV1-positive mice exposed to UV developed frequently more skin tumours than virus-free mice exposed to UV or MnPV1-positive unirradiated mice. Importantly, seminal work by Viarisio *et al.* (76) have shown that an accumulation of UV-induced mutations are promoted when transgenic mice expressing β-HPV type 38 E6 and E7 oncogenes are exposed to chronic UV irradiation. Consequently, the mice develop severe skin lesions; whereas, the wild-type mice exposed to the same UV radiation do not develop pre-malignant lesions. These findings indicate that HPV have an initial role in tumour formation, promoting cellular transformation and may be dispensable for preserve the transforming state after full establishment of non-melanoma skin cancer.

1.6. Human T cell lymphotropic virus type 1 and malignancies – A hit-and-run affair?

Human T-cell leukemia virus type 1 (HTLV-1) is a single-stranded RNA retrovirus, which causes adult T-cell leukemia (ATL) and tropical spastic paraparesis/HTLV-associated myelopathy (77). It mainly infects T lymphocytes, but it also infects B lymphocytes, endothelial cells, myeloid cells, fibroblast, dendritic cells, plasmacytoid dendritic cells and monocytes (78, 79). Virus transmission occurs vertically, from mother to child through breast milk, and infected T cells in blood and semen (80). HTLV-1 encodes the oncoprotein Tax, which plays a central role in the T-

cell transformation. Tax is crucial for HTLV-1 replication and gene expression (80). Many cellular and viral genes are regulated by Tax, some resulting in growth and cell cycle control disruption (81). Loss of Tax gene in ATL patients have been reported (82, 83). It is possible that Tax is playing a role in the initial stages of cell proliferation and immortalization and that only infected cells that escape the immune system are maintained and can accumulate additional genetic alterations to result in acute ATL. For this purpose, the Tax expression loss allows ATL cells to survive via a possible hit-and-run mechanism since it permits the cell to escape from cytotoxic T-lymphocytes (84).

1.7. Other cancer-causing viruses – Possible hit-and-run mechanisms?

1.7.1. Human polyomaviruses

Among all polyomaviruses, only MCPyV is clearly associated with oncogenesis and having a putative role in the onset of carcinogenesis. Though there is little evidence to prove the association of Human polyomavirus 1 (BKV) and Human polyomavirus 2 (JCV) with human cancers, *in vitro* and *in vivo* investigations have reported that both polyomaviruses direct promote tumour formation via persistent expression of T-antigen oncoproteins (85). As a result of sufficient evidence in animals and the difficulty in replicate in humans to prove their direct role in human cancers, they were classified as possibly carcinogenic to human (group 2B) by the International Agency for Research on Cancer (86).

1.7.1.1. Human polyomavirus 1 (BKV)

BKV primary infection is asymptomatic and it is during primary infection that the virus disseminates to various organs, especially the kidney, where it is maintained in a latent state (87). There are two BKV proteins associated with oncogenesis: the small t antigen (tag) and the large T antigen (Tag). Tag binds and blocks the functions of tumour suppressor proteins, p53 protein and RB, while tag cooperates with Tag and inactivates the protein phosphatase 2A (PP2A),

another tumour suppressor protein (87). BKV oncogenicity is also associated with its ability to cause chromosomal damage (88). Tognon *et al* (87) hypothesized that low viral load and low reported cases of BKV Tag-positive cells in human tumours are justified for the hit-and-run mechanism, by which BKV stimulates chromosomal mutations in the cells. Once chromosomal aberrations are consolidated in host cells, the viral sequence might be dispensable to preserve the malignant phenotype. There is evidence that viral DNA and viral products are present in the prostate pre-cancerous lesion. It is also speculated that BKV has a co-factorial role in prostate cancer development, where the virus could promote cancer development in the initial phase, and then, disappear or act as a bystander, which explains the sporadic virus detection in advanced tumours (89).

1.7.1.2. Human polyomavirus 2 (JCV)

JCV is the opportunistic aetiologic agent of progressive multifocal leukoencephalopathy. It was speculated that approximately 80% of the human population are infected with both JCV and BKV in early childhood and may establish a subacute chronic infection in the kidney (6). Additionally, several others have proposed that JCV could be associated with human malignancies as a consequence of the transforming potential of Tag (6, 90). Furthermore, the presence of JCV DNA have been detected in colon, gastric, brain and esophageal cancers (91-93). Importantly, numerous studies have proposed that colorectal cancer could be associated with JCV infection (94). JCV Tag expression loss was reported in a neuroectodermal tumour cell line after several passages *in vitro*, and the oncogenic phenotype preserved (6, 95, 96). Furthermore, the detection of viral DNA is higher than Tag expression in the central nervous system and colonic tumours, possibly as a result of the loss of viral genome sequences in advanced tumours. Evidence of extensive Tag expression in the initial stage of carcinogenesis rather than in already established tumours supports this hypothesis (96). Therefore, Tag may be necessary in the early stages of tumorigenesis, promoting genetic mutations, cell-cycle shifts, and chromosomal

instability; these modifications in the cell might promote the deletion of the viral DNA and T-antigen. The presence of JCV DNA has been reported in colorectal adenocarcinomas but was absent in normal surrounding tissue (97). Moreover, JCV DNA detection was significantly higher in poorly/moderately differentiated adenocarcinoma than in polyps in advanced stages of tumorigenesis. This finding suggests that JCV is important in early phases of tumorigenesis and is associated with premalignant lesions.

1.7.2. *Macaca mulatta* polyomavirus 1 (Simian Virus 40)

Simian Virus 40 (SV40) can infect and replicate in humans, even though it is a natural monkey virus. SV40 has a tropism for the gastrointestinal tract. Notably, SV40 is suggested to be an oncogenic virus. Indeed, several studies have reported the SV40 transforming potential *in vitro* and its ability to induce tumours in hamsters (98). Moreover, SV40 have been identified in human tumours, such as lymphomas, malignant mesothelioma, bone and brain tumours (86). However, given the lack of evidence that SV40 naturally infects human and that it is transmitted from human to human, SV40 is classified as non-carcinogenic to humans (group 3). The role of SV40 in some tumours have been explored. In fact, it is suggested that this virus acts as a cofactor in tumorigenesis (7). SV40 T antigen, Tag, loss after passages *in vitro* have been reported (96, 99). Likewise, transformed cells losing Tag expression over time have been documented in transgenic mice (100, 101). It is possible that SV40 viral replication is not necessary for tumour preservation, the entire viral genome is often eliminated. However, in some cases, SV40 episomal DNA may persist (96, 102).

1.7.3. Adenovirus

Adenovirus, a member of the *Adenoviridae* family, is a non-enveloped double-stranded DNA. This virus is linked with a variety of clinical disorders in humans. With over 50 distinct serotypes, a portion of them have been reported to promote tumour transformation (103). The

persistent expression of adenovirus early proteins, E1A and E1B, transform cells into an oncogenic phenotype. These oncoproteins are known to inactivate RB and p53 (103). The complete loss of integrated adenovirus DNA after numerous passages *in vitro* have been reported and the oncogenic phenotype was not affected as it continues to induce tumours into hamsters after reinjection (104-106). Furthermore, adenovirus E1A proteins have been reported to associate with cytomegalovirus IE1 and IE2 proteins to promote oncogenic transformation in primary rat cells (107). Cytomegalovirus DNA loss occurred after oncogenic progression. Additionally, the presence of these oncoproteins are temporary during tumour development since mutations are accumulated in the host cell, and the continued accumulation of mutation impairs cell survival.

1.7.4. Human alphaherpesvirus 3 (Varicella-zoster Virus)

Varicella-zoster virus (VZV), a double-stranded DNA α-herpesvirus, is the etiological agent of generalized vesicular skin rash (varicella), and localized dermatomal skin rash (herpes zoster). Even though herpes zoster is associated with cancer mostly in immunosuppressed patients, numerous population-based studies have proposed that VZV infection increases cancer risk (108, 109). Moreover, it has been demonstrated that VZV can promote mammalian cell transformation *in vitro* (110, 111). Gelb and Dohner (112) demonstrated that VZV is present at an early stage and induces *in vitro* cell transformation; even so, transformed cells do not exhibit virus DNA traces after transformation, supporting the hit-and-run theory.

2. Discussion

In this review, we have presented an up to date summary of the evidence on viruses' association with various cancer types. Many viruses, such as Epstein-Barr virus, Merkel Cell Virus, and HPV, to name a few, were found to closely associate with a number of cancer types. However, the majority of the available data has analysed causality based on the presence of the

virus, or its genetic material, in the sampled tumours. While this approach is a great tool as a starting point to identify possible links, it does not take into account the early stages of tumorigenesis, nor the possibility of loss of the viral genetic material during the development of the tumour. Although some cancers were found to be addicted to the continued expression of viral oncogenes, this might not be the case for all virally initiated cancers. Here, we hypothesize that the absence of viral genes from the tumour does not necessarily exclude the viral aetiology. The hit-and-run theory suggests that the viruses have a triggering role in the cancer formation, and the viral genome can be entirely lost after the host cell accumulates numerous mutations (Figure 1). If this mechanism occurs, it would imply that many cancers not associated with viruses might, in fact, have arisen firstly as virus-positive cancers that have lost viral DNA. If this is true, a transitional genotype would be detected, where both virus and key mutations are detectable.

A recent Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium studied the viral load of 2,658 cancers and identified more virus-positive samples than previously reported, suggesting that currently implemented methods may be insufficient for detecting viral traces (113). The analysis also revealed a transitional stage during which the tumour loses dependency on the viral oncogenes by implementing driver mutations within the host cells' genome, such as p53 and PIK3CA. This was evident by the presence of three subgroups of carcinogenesis; viral, genomic mutations, and a combination of both, resembling the 'transitional' state. In this proposed 'transitional' stage, the presence of a virus will result in the accumulation of mutations in the host cell caused by the cell's natural defence mechanism. This may be driven by the instability of the integrated viral genes and the documented significant abundance of T-cell and M1 macrophage expression (113), which will aggressively attempt to expel foreign viral nucleic acids. The result of this transformation is the loss of tumour dependency on the viral oncogenes to survive. However, the rate of this transformation may be diverse based on the infection location and the integrated viral genes among other factors. Hence, the transformation in some cancer types, for

instance HPV-positive cervical cancers, may require a longer period than practically possible to detect, while other, such as HPV-positive head and neck cancers, which occur on regions of high mutagenic load and environmental exposure, are more prone to malignant transformation. More studies are required to further understand the possible roles of viruses in the establishment and progression of cancers.

The difficulty to prove that the virus contributed to the tumour development and then disappeared is notable. Though, to test this theory, it is necessary to monitor the outcomes of national vaccination campaigns against viruses. Furthermore, the development of reliable models using gene editing tools *in vivo* (e.g. CRISPR, Cre-Lox recombination, TALENs and ZFN) to delete viral oncogenes and evaluate the progression of tumour growth would aid to test this theory, as supported by Viarisio *et al.* (2018) study (76). Ultimately, we might be underestimating the tumour virus burden and if this theory is proven true. It would significantly contribute to our understanding of viruses and cancer association and will have momentous implications in the early detection, prophylaxis and therapy of many cancer types.

Acknowledgements

Not applicable.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Reference

1. McLaughlin-Drubin ME, Munger K. Viruses associated with human cancer. Biochimica et biophysica acta. 2008;1782(3):127-50.
2. Niller HH, Wolf H, Minarovits J. Viral hit and run-oncogenesis: Genetic and epigenetic scenarios. Cancer Letters. 2011;305(02):200-17.
3. Schulz TF, Cesarman E. Kaposi Sarcoma-associated Herpesvirus: mechanisms of oncogenesis. Current Opinion in Virology. 2015;14:116-28.
4. Carter JJ, Paulson KG, Wipf GC, Miranda D, Madeleine MM, Johnson LG, et al. Association of Merkel Cell Polyomavirus–Specific Antibodies With Merkel Cell Carcinoma. JNCI: Journal of the National Cancer Institute. 2009;101(21):1510-22.
5. Abend JR, Jiang M, Imperiale MJ. BK virus and human cancer: Innocent until proven guilty. Seminars in Cancer Biology. 2009;19(4):252-60.
6. Khalili K, Del Valle L, Otte J, Weaver M, Gordon J. Human neurotropic polyomavirus, JCV, and its role in carcinogenesis. Oncogene. 2003;22:5181.
7. Qi F, Carbone M, Yang H, Gaudino G. Simian virus 40 transformation, malignant mesothelioma and brain tumors. Expert Rev Respir Med. 2011;5(5):683-97.
8. Neuveut C, Wei Y, Buendia MA. Mechanisms of HBV-related hepatocarcinogenesis. Journal of Hepatology. 2010;52(4):594-604.
9. Jin DY. Molecular pathogenesis of hepatitis C virus-associated hepatocellular carcinoma. Front Biosci. 2007;12:222-33.
10. Vonsky M, Shabaeva M, Runov A, Lebedeva N, Chowdhury S, Palefsky JM, et al. Carcinogenesis Associated with Human Papillomavirus Infection. Mechanisms and Potential for Immunotherapy. 2019;84(7):782-99.
11. Takachi T, Takahashi M, Takahashi-Yoshita M, Higuchi M, Obata M, Mishima Y, et al. Human T-cell leukemia virus type 1 Tax oncoprotein represses the expression of the BCL11B tumor suppressor in T-cells. Cancer Sci. 2015;106(4):461-5.
12. Khan G, Hashim MJ. Global burden of deaths from Epstein-Barr virus attributable malignancies 1990-2010. Infectious agents and cancer. 2014;9(1):38-.
13. Wang LW, Jiang S, Gewurz BE. Epstein-Barr Virus LMP1-Mediated Oncogenicity. Journal of virology. 2017;91(21):e01718-16.
14. Thorley-Lawson DA, Hawkins JB, Tracy SI, Shapiro M. The pathogenesis of Epstein-Barr virus persistent infection. Current opinion in virology. 2013;3(3):227-32.
15. Ali AS, Al-Shraim M, Al-Hakami AM, Jones IM. Epstein- Barr Virus: Clinical and Epidemiological Revisits and Genetic Basis of Oncogenesis. The open virology journal. 2015;9:7-28.
16. Young LS, Rickinson AB. Epstein–Barr virus: 40 years on. Nature Reviews Cancer. 2004;4(10):757-68.
17. Piccaluga PP, Weber A, Ambrosio MR, Ahmed Y, Leoncini L. Epstein-Barr Virus-Induced Metabolic Rearrangements in Human B-Cell Lymphomas. Frontiers in microbiology. 2018;9:1233-.
18. Gonzalez-Moles MA, Gutierrez J, Rodriguez MJ, Ruiz-Avila I, Rodriguez-Archilla A. Epstein-Barr Virus Latent Membrane Protein-1 (LMP-1) Expression in Oral Squamous Cell Carcinoma. 2002;112(3):482-7.
19. Ok CY, Li L, Young KH. EBV-driven B-cell lymphoproliferative disorders: from biology, classification and differential diagnosis to clinical management. Experimental &Amp; Molecular Medicine. 2015;47:e132.
20. Tsao S-W, Tsang CM, To K-F, Lo K-W. The role of Epstein-Barr virus in epithelial malignancies. The Journal of pathology. 2015;235(2):323-33.

21. El-Sharkawy A, Al Zaidan L, Malki A. Epstein-Barr Virus-Associated Malignancies: Roles of Viral Oncoproteins in Carcinogenesis. Frontiers in oncology. 2018;8:265-.
22. Gulley ML, Pulitzer DR, Eagan PA, Schneider BG. Epstein-barr virus infection is an early event in gastric carcinogenesis and is independent of bcl-2 expression and p53 accumulation. Human Pathology. 1996;27(1):20-7.
23. Hardie DR. Human γ-herpesviruses: A review of 2 divergent paths to oncogenesis. Transfusion and Apheresis Science. 2010;42(2):177-83.
24. Saha A, Robertson ES. Mechanisms of B-cell oncogenesis induced by Epstein-Barr virus. 2019:JVI.00238-19.
25. Thompson MP, Kurzrock R. Epstein-Barr Virus and Cancer. 2004;10(3):803-21.
26. Srinivas SK, Sample JT, Sixbey JW. Spontaneous Loss of Viral Episomes Accompanying Epstein-Barr Virus Reactivation in a Burkitt's Lymphoma Cell Line. The Journal of Infectious Diseases. 1998;177(6):1705-9.
27. Razzouk BI, Sample CE, Sixbey JW, Srinivas S, Singh V. Epstein-Barr Virus DNA Recombination and Loss in Sporadic Burkitt's Lymphoma. The Journal of Infectious Diseases. 1996;173(3):529-35.
28. Shimizu N, Tanabe-Tochikura A, Kuroiwa Y, Takada K. Isolation of Epstein-Barr virus (EBV)-negative cell clones from the EBV-positive Burkitt's lymphoma (BL) line Akata: malignant phenotypes of BL cells are dependent on EBV. Journal of virology. 1994;68(9):6069-73.
29. Chen H, Lee JM, Wang Y, Huang DP, Ambinder RF, Hayward SD. The Epstein-Barr virus latency BamHI-Q promoter is positively regulated by STATs and Zta interference with JAK/STAT activation leads to loss of BamHI-Q promoter activity. Proceedings of the National Academy of Sciences of the United States of America. 1999;96(16):9339-44.
30. Weiss LM, Strickler JG, Warnke RA, Purtilo DT, Sklar J. Epstein-Barr viral DNA in tissues of Hodgkin's disease. The American journal of pathology. 1987;129(1):86-91.
31. Delecluse H-J, Marafioti T, Hummel M, Dallenbach F, Anagnostopoulos I, Stein H. Disappearance of the Epstein–Barr virus in a relapse of Hodgkin's disease. 1997;182(4):475-9.
32. Nerurkar AY, Soman CS, Vijayan P, Srinivas V, Naresh KN, Dinshaw KA, et al. Discrepancies in Epstein—Barr virus association at presentation and relapse of classical Hodgkin's disease: Impact on pathogenesis. Annals of Oncology. 2000;11(4):475-8.
33. Ambinder RF. Gammaherpesviruses and "Hit-and-Run" oncogenesis. The American journal of pathology. 2000;156(1):1-3.
34. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science. 1994;266(5192):1865-9.
35. Hussein HAM, Okafor IB, Walker LR, Abdel-Raouf UM, Akula SMJAoV. Cellular and viral oncogenes: the key to unlocking unknowns of Kaposi’s sarcoma-associated herpesvirus pathogenesis. 2018;163(10):2633-43.
36. Mariggiò G, Koch S, Schulz TF. Kaposi sarcoma herpesvirus pathogenesis. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 2017;372(1732):20160275.
37. Dittmer DP, Damania B. Kaposi sarcoma-associated herpesvirus: immunobiology, oncogenesis, and therapy. The Journal of clinical investigation. 2016;126(9):3165-75.
38. Giffin L, Damania B. KSHV: pathways to tumorigenesis and persistent infection. Adv Virus Res. 2014;88:111-59.
39. Purushothaman P, Dabral P, Gupta N, Sarkar R, Verma SC. KSHV Genome Replication and Maintenance. Frontiers in microbiology. 2016;7:54-.
40. Cavallin LE, Goldschmidt-Clermont P, Mesri EA. Molecular and Cellular Mechanisms of KSHV Oncogenesis of Kaposi's Sarcoma Associated with HIV/AIDS. PLOS Pathogens. 2014;10(7):e1004154.

41. Benelli R, Albini A, Parravicini C, Carlone S, Repetto L, Tambussi G, et al. Isolation of spindle-shaped cell populations from primary cultures of Kaposi's sarcoma of different stage. Cancer Letters. 1996;100(1):125-32.
42. Lunardi-Iskandar Y, Gill P, Lam VH, Zeman RA, Michaels F, Mann DL, et al. Isolation and Characterization of an Immortal Neoplastic Cell Line (KS Y-1) From AIDS-Associated Kaposi's Sarcoma. JNCI: Journal of the National Cancer Institute. 1995;87(13):974-81.
43. Davis MA, Opalenik SR, Browning PJ, Stürzl M, Blasig C, Schreier A, et al. Expression of Human Herpesvirus 8-Encoded Cyclin D in Kaposi's Sarcoma Spindle Cells. JNCI: Journal of the National Cancer Institute. 1997;89(24):1868-74.
44. Kennedy MM, Biddolph S, Lucas SB, Howells DD, Picton S, McGee JO, et al. Cyclin D1 expression and HHV8 in Kaposi sarcoma. 1999;52(8):569-73.
45. Horenstein MG, Cesarman E, Wang X, Linkov I, Prieto VG, Louie DC. Cyclin D1 and retinoblastoma protein expression in Kaposi's sarcoma. J Cutan Pathol. 1997;24(10):585-9.
46. Stevenson PG, May JS, Connor V, Efstathiou S. Vaccination against a hit-and-run viral cancer. The Journal of general virology. 2010;91(Pt 9):2176-85.
47. Gross L. A filterable agent, recovered from Ak leukemic extracts, causing salivary gland carcinomas in C3H mice. Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine (New York, NY). 1953;83(2):414-21.
48. Sweet BH, Hilleman MR. The vacuolating virus, S.V. 40. Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine (New York, NY). 1960;105:420-7.
49. Gardner SD, Field AM, Coleman DV, Hulme B. New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet (London, England). 1971;1(7712):1253-7.
50. Padgett BL, Walker DL, ZuRhein GM, Eckroade RJ, Dessel BH. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. Lancet (London, England). 1971;1(7712):1257-60.
51. Feng H, Shuda M, Chang Y, Moore PS. Clonal Integration of a Polyomavirus in Human Merkel Cell Carcinoma. 2008;319(5866):1096-100.
52. Houben R, Shuda M, Weinkam R, Schrama D, Feng H, Chang Y, et al. Merkel cell polyomavirus-infected Merkel cell carcinoma cells require expression of viral T antigens. Journal of virology. 2010;84(14):7064-72.
53. Spurgeon ME, Lambert PF. Merkel cell polyomavirus: a newly discovered human virus with oncogenic potential. Virology. 2013;435(1):118-30.
54. Touzé A, Bidre EL, Laude H, Fleury MJJ, Cazal R, Arnold F, et al. High Levels of Antibodies Against Merkel Cell Polyomavirus Identify a Subset of Patients With Merkel Cell Carcinoma With Better Clinical Outcome. Journal of Clinical Oncology. 2011;29(12):1612-9.
55. Houben R, Grimm J, Willmes C, Weinkam R, Becker JC, Schrama D. Merkel Cell Carcinoma and Merkel Cell Polyomavirus: Evidence for Hit-and-Run Oncogenesis. Journal of Investigative Dermatology. 2012;132(1):254-6.
56. Ringelhan M, McKeating JA, Protzer U. Viral hepatitis and liver cancer. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 2017;372(1732):20160274.
57. Hessein M, Saad EG, Mohamed AA, Kamel EAM, Abdel Hady A, Amina M, et al. Hit-And-Run Mechanism of HBV-Mediated Progression to Hepatocellular Carcinoma. 2005;91(3):241-7.
58. Feitelson MA, Lee J. Hepatitis B virus integration, fragile sites, and hepatocarcinogenesis. Cancer Letters. 2007;252(2):157-70.
59. Rogler C, Sherman M, Su C, Shafritz D, Summers J, Shows T, et al. Deletion in chromosome 11p associated with a hepatitis B integration site in hepatocellular carcinoma. 1985;230(4723):319-22.

60. Shafritz DA, Shouval D, Sherman HI, Hadziyannis SJ, Kew MC. Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. Studies in percutaneous liver biopsies and post-mortem tissue specimens. N Engl J Med. 1981;305(18):1067-73.
61. Fowler MJF, Greenfield C, Chu CM, Karayiannis P, Dunk A, Lok ASF, et al. Integration of HBV-DNA may not be a prerequisite for the maintenance of the state of malignant transformation: An analysis of 110 liver biopsies. Journal of Hepatology. 1986;2(2):218-29.
62. Sukowati C, Reyes P, Tell G, Tiribelli C. Oncogenicity of viral hepatitis B and C in the initiation of hepatic cancer stem cells. Hepatoma Research. 2019;2019.
63. Takeda S, Shibata M, Morishima T, Harada A, Nakao A, Takagi H, et al. Hepatitis C virus infection in hepatocellular carcinoma. Detection of plus-strand and minus-strand viral RNA. 1992;70(9):2255-9.
64. Ferri C, Caracciolo F, Zignego AL, Civita LL, Monti M, Longombardo G, et al. Hepatitis C virus infection in patients with non-Hodgkin's lymphoma. 1994;88(2):392-4.
65. Tommasino M. The human papillomavirus family and its role in carcinogenesis. Seminars in Cancer Biology. 2014;26:13-21.
66. Lawson JS, Salmons B, Glenn WK. Oncogenic Viruses and Breast Cancer: Mouse Mammary Tumor Virus (MMTV), Bovine Leukemia Virus (BLV), Human Papilloma Virus (HPV), and Epstein-Barr Virus (EBV). Frontiers in oncology. 2018;8:1-.
67. Tumban E. A Current Update on Human Papillomavirus-Associated Head and Neck Cancers. 2019;11(10):922.
68. Estêvão D, Costa NR, Gil da Costa RM, Medeiros R. Hallmarks of HPV carcinogenesis: The role of E6, E7 and E5 oncoproteins in cellular malignancy. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms. 2019;1862(2):153-62.
69. Jubair L, Fallaha S, McMillan NAJ. Systemic Delivery of CRISPR/Cas9 Targeting HPV Oncogenes Is Effective at Eliminating Established Tumors. Molecular Therapy. 2019.
70. Chen AC-H, Waterboer T, Keleher A, Morrison B, Jindal S, McMillan D, et al. Human Papillomavirus in Benign Prostatic Hyperplasia and Prostatic Adenocarcinoma Patients. 2011;17(3):613-7.
71. Antonsson A, Spurr TP, Chen AC, Francis GD, McMillan NAJ, Saunders NA, et al. High prevalence of human papillomaviruses in fresh frozen breast cancer samples. 2011;83(12):2157-63.
72. Iwasaka T, Hayashi Y, Yokoyama M, Hara K, Matsuo N, Sugimori H. 'Hit and run' oncogenesis by human papillomavirus type 18 DNA. Acta Obstetricia et Gynecologica Scandinavica. 1992;71(3):219-23.
73. Weissenborn SJ, Nindl I, Purdie K, Harwood C, Proby C, Breuer J, et al. Human Papillomavirus-DNA Loads in Actinic Keratoses Exceed those in Non-Melanoma Skin Cancers. Journal of Investigative Dermatology. 2005;125(1):93-7.
74. Ngan C, Lawson JS, Clay R, Delprado W, Whitaker NJ, Glenn WK. Early Human Papilloma Virus (HPV) Oncogenic Influences in Breast Cancer. Breast Cancer (Auckl). 2015;9:93-7.
75. Hasche D, Stephan S, Braspenning-Wesch I, Mikulec J, Niebler M, Gröne H-J, et al. The interplay of UV and cutaneous papillomavirus infection in skin cancer development. PLOS Pathogens. 2017;13(11):e1006723.
76. Viarisio D, Müller-Decker K, Accardi R, Robitaille A, Dürst M, Beer K, et al. Beta HPV38 oncoproteins act with a hit-and-run mechanism in ultraviolet radiation-induced skin carcinogenesis in mice. PLoS pathogens. 2018;14(1):e1006783-e.
77. Gessain A, Mahieux R. Tropical spastic paraparesis and HTLV-1 associated myelopathy: clinical, epidemiological, virological and therapeutic aspects. Rev Neurol (Paris). 2012;168(3):257-69.
78. Gross C, Thoma-Kress AK. Molecular Mechanisms of HTLV-1 Cell-to-Cell Transmission. Viruses. 2016;8(3):74.
79. Zhang L-l, Wei J-y, Wang L, Huang S-l, Chen J-l. Human T-cell lymphotropic virus type 1 and its oncogenesis. Acta Pharmacologica Sinica. 2017;38(8):1093-103.

80. Yoshida M. Multiple Viral Strategies of HTLV-1 for Dysregulation of Cell Growth Control. 2001;19(1):475-96.
81. Nakase K, Cheng J, Zhu Q, Marasco WA. Mechanisms of SHP-1 P2 promoter regulation in hematopoietic cells and its silencing in HTLV-1-transformed T cells. J Leukoc Biol. 2009;85(1):165-74.
82. Matsuoka M. Human T-cell leukemia virus type I (HTLV-I) infection and the onset of adult T-cell leukemia (ATL). Retrovirology. 2005;2:27-.
83. Takeda S, Maeda M, Morikawa S, Taniguchi Y, Yasunaga J-i, Nosaka K, et al. Genetic and epigenetic inactivation of tax gene in adult T-cell leukemia cells. 2004;109(4):559-67.
84. Boxus M, Twizere J-C, Legros S, Kettmann R, Willems L. Interaction of HTLV-1 Tax with minichromosome maintenance proteins accelerates the replication timing program. 2012;119(1):151-60.
85. White MK, Khalili K. Polyomaviruses and human cancer: molecular mechanisms underlying patterns of tumorigenesis. Virology. 2004;324(1):1-16.
86. Bouvard V, Baan RA, Grosse Y, Lauby-Secretan B, El Ghissassi F, Benbrahim-Tallaa L, et al. Carcinogenicity of malaria and of some polyomaviruses. Lancet Oncol. 2012;13(4):339-40.
87. Tognon M, Corallini A, Martini F, Negrini M, Barbanti-Brodano G. Oncogenic transformation by BK virus and association with human tumors. Oncogene. 2003;22:5192.
88. Trabanelli C, Corallini A, Gruppioni R, Sensi A, Bonfatti A, Campioni D, et al. Chromosomal Aberrations Induced by BK Virus T Antigen in Human Fibroblasts. Virology. 1998;243(2):492-6.
89. Keller EX, Delbue S, Tognon M, Provenzano M. Polyomavirus BK and prostate cancer: a complex interaction of potential clinical relevance. 2015;25(6):366-78.
90. Nosho K, Shima K, Kure S, Irahara N, Baba Y, Chen L, et al. JC virus T-antigen in colorectal cancer is associated with p53 expression and chromosomal instability, independent of CpG island methylator phenotype. Neoplasia. 2009;11(1):87-95.
91. Del Valle L, White MK, Enam S, Oviedo SP, Bromer MQ, Thomas RM, et al. Detection of JC virus DNA sequences and expression of viral T antigen and agnoprotein in esophageal carcinoma. 2005;103(3):516-27.
92. Del Valle L, Gordon J, Ferrante P, Khalili KJHPM, Clinical Perspectives KK, pp GS. JC virus in experimental and clinical brain tumorigenesis. 2001:409-30.
93. Ksiaa F, Ziadi S, Mokni M, Korbi S, Trimeche M. The presence of JC virus in gastric carcinomas correlates with patient's age, intestinal histological type and aberrant methylation of tumor suppressor genes. Modern Pathology. 2010;23(4):522-30.
94. Coelho TR, Almeida L, Lazo PA. JC virus in the pathogenesis of colorectal cancer, an etiological agent or another component in a multistep process? Virol J. 2010;7:42-.
95. Hori R, Murai Y, Tsuneyama K, Abdel-Aziz HO, Nomoto K, Takahashi H, et al. Detection of JC virus DNA sequences in colorectal cancers in Japan. 2005;447(4):723-30.
96. Ricciardiello L, Baglioni M, Giovannini C, Pariali M, Cenacchi G, Ripalti A, et al. Induction of Chromosomal Instability in Colonic Cells by the Human Polyomavirus JC Virus. 2003;63(21):7256-62.
97. Toumi W, Ripalti A, Ricciardiello L, Cherif A, Gargouri D, Bouhafa A, et al. Detection of a new JCV strain of genotype A in a subpopulation of colorectal adenocarcinomas in Tunisia. The new microbiologica. 2017;40(2):99-106.
98. Rotondo JC, Mazzoni E, Bononi I, Tognon M, Martini F. Association Between Simian Virus 40 and Human Tumors. Frontiers in oncology. 2019;9:670-.
99. Ray FA, Peabody DS, Cooper JL, Cram LS, Kraemer PM. SV40 T antigen alone drives karyotype instability that precedes neoplastic transformation of human diploid fibroblasts. Journal of cellular biochemistry. 1990;42(1):13-31.
100. Sepulveda AR, Finegold MJ, Smith B, Slagle BL, DeMayo JL, Shen R-F, et al. Development of a transgenic mouse system for the analysis of stages in liver carcinogenesis using tissue-specific expression

of SV40 large T-antigen controlled by regulatory elements of the human α-1-antitrypsin gene. 1989;49(21):6108-17.
101. Ewald D, Li M, Efrat S, Auer G, Wall RJ, Furth PA, et al. Time-sensitive reversal of hyperplasia in transgenic mice expressing SV40 T antigen. 1996;273(5280):1384-6.
102. Butel JS. Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease. Carcinogenesis. 2000;21(3):405-26.
103. Täuber B, Dobner T. Adenovirus early E4 genes in viral oncogenesis. Oncogene. 2001;20(54):7847-54.
104. Kuhlmann I, Achten S, Rudolph R, Doerfler W. Tumor induction by human adenovirus type 12 in hamsters: loss of the viral genome from adenovirus type 12-induced tumor cells is compatible with tumor formation. The EMBO journal. 1982;1(1):79-86.
105. Pfeffer A, Schubbert R, Orend G, Hilger-Eversheim K, Doerfler W. Integrated viral genomes can be lost from adenovirus type 12-induced hamster tumor cells in a clone-specific, multistep process with retention of the oncogenic phenotype. Virus Research. 1999;59(1):113-27.
106. Nevels M, Täuber B, Spruss T, Wolf H, Dobner T. “Hit-and-Run” Transformation by Adenovirus Oncogenes. Journal of Virology. 2001;75(7):3089-94.
107. Shen Y, Zhu H, Shenk T. Human cytomegalovirus IE1 and IE2 proteins are mutagenic and mediate “hit-and-run” oncogenic transformation in cooperation with the adenovirus E1A proteins. 1997;94(7):3341-5.
108. Schmidt SAJ, Mor A, Schønheyder HC, Sørensen HT, Dekkers OM, Cronin-Fenton D. Herpes zoster as a marker of occult cancer: A systematic review and meta-analysis. Journal of Infection. 2017;74(3):215-35.
109. Cotton SJ, Belcher J, Rose P, K Jagadeesan S, Neal RD. The risk of a subsequent cancer diagnosis after herpes zoster infection: primary care database study. British Journal Of Cancer. 2013;108:721.
110. Gelb LD, Huang JJ, Wellinghoff WJ. Varicella-Zoster Virus Transformation of Hamster Embryo Cells. 1980;51(1):171-7.
111. Wlodarski K, Ostrowski KJMmPPjom, pharmacy. Investigations on the properties of established cell lines of human and animal origin to induce cartilage and bone and properties of human fibroblasts transformed by varicella virus. 1975;7(1):3.
112. Gelb L, Dohner D. Varicella-Zoster Virus-Induced Transformation of Mammalian Cells in Vitro. Journal of Investigative Dermatology. 1984;83(1, Supplement):S77-S81.
113. Zapatka M, Borozan I, Brewer DS, Iskar M, Grundhoff A, Alawi M, et al. The landscape of viral associations in human cancers. Nature Genetics. 2020;52(3):320-30.

Figure legends

Figure 1 Schematic representation of how viruses might contribute to human carcinogenesis through hit-and-run mechanism.