Oncolytic Viruses

Subjects: Virology
Contributor: Carla Zannella

Virotherapy is a new promising approach against different types of cancers through the use of oncolytic viruses (OVs). They are naturally occurring, or genetically modified, viruses able to infect, replicate, and lyse several malignant tumor cells. Virotherapy was born in the 19th century , and in the 1950s–1970s, the first clinical trials began and live viruses were deliberately injected into patients with cancer to promote tumor regression .

Keywords: oncolytic virus ; combination treatment ; cancer ; epigenetic ; tumor resistance ; HCC ; DNA methyltransferase ; histone deacetylases ; microRNA

1. Introduction

Virotherapy is a new promising approach against different types of cancers through the use of oncolytic viruses (OVs). Virotherapy was born in the 19th century [1][2][3], and in the 1950s–1970s, the first clinical trials began and live viruses were deliberately injected into patients with cancer to promote tumor regression [1]. In the last decade, thanks to genetic engineering and the advent of in vitro experiments, the viral genome has been easily manipulated and modified to make viruses more selective for cancer cells and minimize their potential side effects [4][5][6], causing a great burst of oncolytic virotherapy. Since 2018, only three OVs have been approved for cancer therapy.

In fact, cancer cells have peculiar characteristics able to strengthen viral replication [7]: (i) they oppose apoptosis causing indefinite proliferation [8]; As a result, OVs replicate and lyse selectively tumor cells spreading viral progeny and other products of oncolysis. The release of infectious viral progeny allows oncolysis amplification also towards neighboring tumor cells. Potential adverse phenomena, such as viral encephalitis caused by HSV [9], require risk monitoring, which must be considered before treating patients.

Combinatorial treatments are required to improve the immune response and allow viral entry, replication, and diffusion between adjacent cells. In this review we discuss firstly the major viral families used in virotherapy and the clinical trials in which OVs are used; then we focus on the specific combinatorial therapies, including co-administered inhibitors of chromatin modifiers (combination strategies) and inserted target sites for miRNAs (recombination or arming strategies).

2. OVs and HDACs as Combinatorial Therapy

HDACs are epigenetic modulators that act on the epigenetic asset of the cellular system [10][11][12][13][14][15][16][17].

HDACi are anticancer agents that induce cell cycle arrest, and, additionally, they can inhibit the growth and differentiation of cancer cells [13][14][18][19][20]. , short-chain fatty acids (sodium butyrate or valproic acid), benzamide (MS-275 or entinostat), cyclic peptides (romidepsin or FR901228), and benzenesulfonamides (resminostat) [21]. In detail, SAHA is a wide HDAC class I and II inhibitor known to block the growth of cancer cells, including cutaneous T-cell lymphoma, breast, and prostate cancer. In addition, SAHA can induce cell cycle arrest in G1 phase in cancer cells through the up-regulation of cyclin-dependent kinase inhibitor p 21 [22].

To date, it has not yet received approval for clinical use, but the US FDA allowed its combinatorial treatment with exemestane for the management of advanced breast cancer [23]. It is also used in the treatment of prostate carcinoma as it is capable of preventing the development of metastases by inducing cell death [24][25] and transcriptional activation of specific genes [26]. Romidepsin (FK228 or FR90128) is a depsipeptide belonging to the group of cyclic peptides, approved by the FDA in 2009 for the anticancer treatment of cutaneous T-cell lymphoma (CTCL) and in prostatitis carcinoma [27][28]. It inhibits class I and IIb HDAC by preventing the growth of cancer cells and enhancing apoptotic processes [29][30].

To date, several oncolytic viruses have been associated with HDACi with the aim of increasing antitumor efficacy and, on the other hand, reducing the antiviral response. HSV, AdV, reovirus, VSV, vaccinia virus (VV), paramyxoviruses, and parvovirus are the most representative.

However, the effect of TSA decreased by using SN50, a NF-kB inhibitor, reducing the accumulation of p65 in the nucleus, and thus playing an important role in viral replication [31]. TSA can also up-regulate viral replication by increasing cytotoxicity [32][33]: it was able to upregulate the cyclin-dependent kinase inhibitor p21 and interrupt the cell cycle in the G0/G1 phase [34][35]. In co-treatment assay, glioma cells were treated with oHSV and VPA at the same time, meanwhile, in pre-treatment test, cellular monolayer was stimulated before with HDACi and, later, it was infected with the virus. GFP intensity resulted higher in pre-treatment than in co-treatment assay.

Kitazono et al. evaluated the transgenic expression of adenovirus in cancer cells subjected to treatment with the HDACi romidepsin (FR901228). The authors described the treatment of malignant cells with FR901228 and the subsequent infection with Ad5 CMV-LacZ, a replication-defective type 5 adenovirus, devoid of the E1 and E3 gene. Pre-treatment caused an increase in the expression of CAR and integrin-alpha, important for mediating the attack of adenovirus on cells [36]. Effects of oncolytic virotherapy with AdV have also been observed in cervical cancer cells.

However, many cancer cells present residual innate activity that can generate resistance to viral propagation [34]. On the other hand, in vivo experiments have analyzed the combination SAHA or MS-275 and rVSV M Delta 51 in prostate, ovarian, and breast cancer xenograft models, and showed enhanced survival [37][38]. Furthermore, Muscolini et al identified SIRT1 as a probable factor limiting viral infection in prostate cancer cells. Indeed, it has been considered a restriction factor known for its importance in prostate cancer, where it acted on the permissiveness of specific tumor cells.

Combinatorial therapy between HDACi and oncolytic reoviruses has also been evaluated in patients with multiple myeloma (MM). Life expectancy is reduced: in most cases, death occurs within 5 years of diagnosis, and, in cases where the tumor is aggressive, within 24 months [39][40][41]. By performing Western blot and flow cytometry analysis, lower expression of the reovirus receptor junctional adhesion molecule 1 (JAM-1) was observed in resistant tumor cells, infected with different amounts of virus, compared to sensitive ones. In addition, Jaime-Ramirez et al. assessed the impact of the combination of oncolytic reovirus and SAHA in head and neck squamous cell carcinomas (HNSCC), demonstrating an improvement in viral replication and immune-mediated anti-cancer responses both in vitro and in vivo [42].

Important results have been obtained in cervical cancer and pancreatic duct adenocarcinoma by the combination of HDACi. It has been reported that co-treating cancer cells with VPA and H1PV, as a result the onset of oxidative stress and apoptosis of cancer cells occurred [43]. The same effects have been observed by using H1PV and NaB at sub-lethal doses. There was an increase in viral oncotoxicity determining the eradication of neoplasm, but, on the other hand, there was the regression of carcinoma [34][44].

It has been shown that under optimal conditions, when cells were infected with the P/V-CPI mutant alone, it caused an increase in the production of IFN beta, while in cells infected with the oncolytic mutant virus and treated with scriptaid, there was a reduction in the production of INF and an increase in viral propagation in cancer cells [45]. Significant progress has also been assessed in the treatment of HCC in which the oncolytic measles vaccine virus (MeV) has been associated with the oral HDACi resminostat (Res) [29]. Res-MeV co-treatment increased viral replication and apoptosis, and improved primary infections. Furthermore, Res could exert a remarkable effect on innate cellular immunity, as it could prevent the activation of genes stimulated by IFN [29].

Currently, VV is under study and their activity can be enhanced by the use of HDACi [37][46]. Among the various HDACi, TSA represents the VV enhancer both in vitro and in vivo. Indeed, TSA caused a greater effect in vitro than other inhibitors, enhancing viral replication and the killing of infection-resistant tumor cells and, on the contrary, it was able to reduce toxicity to the mice [32]. Even in vivo studies with human colon carcinoma xenografts have shown that the combinatorial treatment resulted in improved survival [34][47].

3. OVs and DNMTi as Combinatorial Therapy

and DNMT3L are not canonical demethylating enzymes, as they do not contain the catalytic activity [48]. does not methylate genomic DNA but the anticodon loop of aspartic acid transfer RNA [49]. They perform different functions by acting in particular on the remodeling of chromatin and they are responsible for the up/down expression of proteins causing the onset of different pathologies [50]. Furthermore, the role of DNA methylation in common human pathologies has also been investigated, in particular in neurological disorders [51][52] and autoimmune diseases [53][54][55].

rQNestin34.5, remarkable results were obtained both in vivo and in vitro in the treatment of glioma [56]. By treating glioma cells with oHSV and 5-aza, there was an increase in the viral replication, as reported by the high expression of some viral genes and by the increase in the number and size of infected GFP-positive glioma cells [56]. Furthermore, Okemoto et al

demonstrated that rQNestin34.5 and 5-aza can act synergistically causing apoptosis of glioma tumor cells. Monotherapy and combinatorial experiments were conducted in vitro, using cells derived from spontaneous breast fibrosarcomas (LCRT).

TMZ is mainly used for the treatment of malignant melanoma and glioma [57][58][59]; however, like the other drugs, prolonged use can induce resistance by producing the O 6- methylguanine mutagen and causing DNA damage. It has been observed that inhibition of MGMT improved the antitumor activity of the drug [57]. The combination between oncolytic adenoviruses and shRNA targeting MGMT activity could be an effective approach for fighting resistance to TMZ and for improving anticancer outcomes.

Therapeutic studies have also been conducted to enhance the treatment of onco-hematological diseases such as acute T-cell lymphocytic leukemia. Hastie et al used murine EL-4 cells from acute T-cell lymphocytic leukemia. before therapy with the DNMTi, caused tumor remission in 70% cases [60]. Cells which survived two consecutive treatments with the epigenetic modulator were more sensitive to oncolytic viral therapy, leading to durable remissions.

4. OVs and miRNA: Promising Combinatorial Treatment

In the past decade, the study of miRNAs has largely influenced the field of oncolytic virotherapy. Specific miRNAs target sequences can be integrated into the viral genome and can regulate viral proteins, improving the safety profile and strengthening the anticancer efficacy of oncolytic viruses (Table 3).

miRNAs are small non-coding RNA molecules approximately 22 nucleotides long that can negatively regulate gene expression at the post-transcriptional level [61]. In this scenario, synthetic target sequences complementary to specific miRNAs have been inserted in the UTRs of viral genes essential for replication. This approach promotes the degradation of the viral genome in healthy tissues, but not in cancer cells [62][63][64][65]. Its usefulness has been widely demonstrated and tissue specificity has been improved for many oncolytic viruses [66][64][67][68][69][70].

The results demonstrated that ICP27 protein level was higher in tumor cells than in healthy cells, indicating that this type of regulation could control HSV-1 by selectively killing NSCLC cells in vitro [71]. Generally, miRNA-21 was found to be upregulated in cancer cells [72]. An inverse miRNA control setup was created, in which miR-21 was used in cancer cells to induce, rather than repress, HSV replication. This study has shown that a viral gene under the control of miR-21 limited viral replication in healthy cells, where miR-21 was downregulated, and, at the same time, it induced a vigorous replication in cancer cells expressing miR-21 [73].

These results have prompted other studies that have combined miR-122 with miR-19, also specific for hepatocytes and downregulated in cancer cells. This modification effectively inhibited adenoviral infection in healthy pancreatic tissue and, on the contrary, it has improved the viral anti-tumor activity in pancreatic tumors [74]. The presence of viral proteins in normal tissues could create immunogenic reactions, as well as inflammation and cell death. However, other studies and clinical trials will need to be performed before the therapeutic potential of this innovative approach and its safety can be assessed in humans.

One of the first miRNA-regulated oncolytic viruses was the Coxsackievirus B3 (CVB3) characterized by the strong ability to lyse human cells of NSCLC [75][76]. The recombinant virus, called 53a-CVB, showed minimal levels of toxicity in healthy tissues and, furthermore, retained its full oncolytic activity in xenotransplant mice with human lung cancer [77]. [64] inserting target sequences complementary to miR-206 and miR-133a, specific to skeletal muscle tissue. On the contrary, the recombinant virus retained its replication ability in cancer cells, causing total regression of subcutaneous tumors, and did not replicate in healthy cells expressing complementary miRNAs, thereby reducing myotoxicity, and retaining the oncolytic potential [64].

It has shown oncolytic activity but its use can also cause side effects. In order to improve the safety profile and reduce toxicity, Ruiz et al. engineered the virus by inserting target sequences complementary to miR-124 (enriched in nerve tissue) in the 5′ UTR of the viral genome, and sequences complementary to miR-133 and miR-208 (enriched in heart tissue) were introduced in the 3′ UTR [66]. In vivo toxicity assays confirmed that miR-124, inserted within the 5′ UTR of the viral genome, suppressed viral replication in the central nervous system, while miR-133 and miR-208 inhibited viral replication in the heart tissue. This study has shown that the simultaneous use of multiple targets for miRNA reduces the saturation potential of a single miRNA.

References

1. Kelly, E.; Russell, S.J. History of oncolytic viruses: Genesis to genetic engineering. Mol. Ther. 2007, 15, 651–659.
2. Hoster, H.A.; Zanes, R.P., Jr.; Von Haam, E. Studies in Hodgkin's syndrome; the association of viral hepatitis and Hodg kin's disease; a preliminary report. Cancer Res. 1949, 9, 473–480.
3. Lin, E.; Nemunaitis, J. Oncolytic viral therapies. Cancer Gene Ther. 2004, 11, 643–664.
4. Choi, J.W.; Lee, J.S.; Kim, S.W.; Yun, C.O. Evolution of oncolytic adenovirus for cancer treatment. Adv. Drug Deliv. Re v. 2012, 64, 720–729.
5. Kalyanasundram, J.; Hamid, A.; Yusoff, K.; Chia, S.L. Newcastle disease virus strain AF2240 as an oncolytic virus: A re view. Acta Trop. 2018, 183, 126–133.
6. Lin, C.Z.; Xiang, G.L.; Zhu, X.H.; Xiu, L.L.; Sun, J.X.; Zhang, X.Y. Advances in the mechanisms of action of cancer-targ eting oncolytic viruses. Oncol. Lett. 2018, 15, 4053–4060.
7. Choi, A.H.; O'Leary, M.P.; Fong, Y.; Chen, N.G. From Benchtop to Bedside: A Review of Oncolytic Virotherapy. Biomedi cines 2016, 4, 18.
8. Mohammad, R.M.; Muqbil, I.; Lowe, L.; Yedjou, C.; Hsu, H.Y.; Lin, L.T.; Siegelin, M.D.; Fimognari, C.; Kumar, N.B.; Dou, Q.P.; et al. Broad targeting of resistance to apoptosis in cancer. Semin. Cancer Biol. 2015, 35, S78–S103.
9. Sokolowski, N.A.; Rizos, H.; Diefenbach, R.J. Oncolytic virotherapy using herpes simplex virus: How far have we com e? Oncolytic Virother. 2015, 4, 207–219.
10. Li, G.; Tian, Y.; Zhu, W.G. The Roles of Histone Deacetylases and Their Inhibitors in Cancer Therapy. Front. Cell Dev. B iol. 2020, 8, 576946.
11. Verza, F.A.; Das, U.; Fachin, A.L.; Dimmock, J.R.; Marins, M. Roles of Histone Deacetylases and Inhibitors in Anticance r Therapy. Cancers 2020, 12, 1664.
12. Milazzo, G.; Mercatelli, D.; Di Muzio, G.; Triboli, L.; De Rosa, P.; Perini, G.; Giorgi, F.M. Histone Deacetylases (HDAC s): Evolution, Specificity, Role in Transcriptional Complexes, and Pharmacological Actionability. Genes 2020, 11, 556.
13. Johnstone, R.W. Histone-deacetylase inhibitors: Novel drugs for the treatment of cancer. Nat. Rev. Drug Discov. 2002, 1, 287–299.
14. Mariadason, J.M. HDACs and HDAC inhibitors in colon cancer. Epigenetics 2008, 3, 28–37.
15. Park, J.; Thomas, S.; Munster, P.N. Epigenetic modulation with histone deacetylase inhibitors in combination with immu notherapy. Epigenomics 2015, 7, 641–652.
16. Secrist, J.P.; Zhou, X.; Richon, V.M. HDAC inhibitors for the treatment of cancer. Curr. Opin. Investig. Drugs 2003, 4, 14 22–1427.
17. Nehme, Z.; Pasquereau, S.; Herbein, G. Control of viral infections by epigenetic-targeted therapy. Clin. Epigenetics 201 9, 11, 55.
18. Zhao, C.; Dong, H.; Xu, Q.; Zhang, Y. Histone deacetylase (HDAC) inhibitors in cancer: A patent review (2017-present). Expert Opin. Ther. Pat. 2020, 30, 263–274.
19. Sanaei, M.; Kavoosi, F. Histone Deacetylases and Histone Deacetylase Inhibitors: Molecular Mechanisms of Action in Various Cancers. Adv. Biomed. Res. 2019, 8, 63.
20. Hassell, K.N. Histone Deacetylases and their Inhibitors in Cancer Epigenetics. Diseases 2019, 7, 57.
21. Abbas, A.; Gupta, S. The role of histone deacetylases in prostate cancer. Epigenetics 2008, 3, 300–309.
22. Rivero-Cruz, J.F.; Lezutekong, R.; Lobo-Echeverri, T.; Ito, A.; Mi, Q.; Chai, H.B.; Soejarto, D.D.; Cordell, G.A.; Pezzuto, J.M.; Swanson, S.M. Cytotoxic constituents of the twigs of Simarouba glauca collected from a plot in Southern Florida. Phytother. Res. 2005, 19, 136–140.
23. Connolly, R.M.; Rudek, M.A.; Piekarz, R. Entinostat: A promising treatment option for patients with advanced breast ca ncer. Future Oncol. 2017, 13, 1137–1148.
24. Damaskos, C.; Garmpis, N.; Valsami, S.; Kontos, M.; Spartalis, E.; Kalampokas, T.; Kalampokas, E.; Athanasiou, A.; M oris, D.; Daskalopoulou, A.; et al. Histone Deacetylase Inhibitors: An Attractive Therapeutic Strategy Against Breast Ca ncer. Anticancer Res. 2017, 37, 35–46.
25. Schech, A.; Kazi, A.; Yu, S.; Shah, P.; Sabnis, G. Histone Deacetylase Inhibitor Entinostat Inhibits Tumor-Initiating Cells in Triple-Negative Breast Cancer Cells. Mol. Cancer Ther. 2015, 14, 1848–1857.
26. Xu, W.S.; Parmigiani, R.B.; Marks, P.A. Histone deacetylase inhibitors: Molecular mechanisms of action. Oncogene 20 07, 26, 5541–5552.

27. Barbarotta, L.; Hurley, K. Romidepsin for the Treatment of Peripheral T-Cell Lymphoma. J. Adv. Pract. Oncol. 2015, 6, 22–36.

28. Suraweera, A.; O'Byrne, K.J.; Richard, D.J. Combination Therapy with Histone Deacetylase Inhibitors (HDACi) for the Treatment of Cancer: Achieving the Full Therapeutic Potential of HDACi. Front. Oncol. 2018, 8, 92.

29. Ruf, B.; Berchtold, S.; Venturelli, S.; Burkard, M.; Smirnow, I.; Prenzel, T.; Henning, S.W.; Lauer, U.M. Combination of the oral histone deacetylase inhibitor resminostat with oncolytic measles vaccine virus as a new option for epi-virotherapeutic treatment of hepatocellular carcinoma. Mol. Ther. Oncolytics 2015, 2, 15019.

30. Soukupova, J.; Bertran, E.; Penuelas-Haro, I.; Urdiroz-Urricelqui, U.; Borgman, M.; Kohlhof, H.; Fabregat, I. Resminostat induces changes in epithelial plasticity of hepatocellular carcinoma cells and sensitizes them to sorafenib-induced apoptosis. Oncotarget 2017, 8, 110367–110379.

31. Nguyen, T.L.; Wilson, M.G.; Hiscott, J. Oncolytic viruses and histone deacetylase inhibitors--a multi-pronged strategy to target tumor cells. Cytokine Growth Factor Rev. 2010, 21, 153–159.

32. Nakashima, H.; Nguyen, T.; Chiocca, E.A. Combining HDAC inhibitors with oncolytic virotherapy for cancer therapy. Oncolytic Virother. 2015, 4, 183–191.

33. Liu, T.C.; Castelo-Branco, P.; Rabkin, S.D.; Martuza, R.L. Trichostatin A and oncolytic HSV combination therapy shows enhanced antitumoral and antiangiogenic effects. Mol. Ther. 2008, 16, 1041–1047.

34. Marchini, A.; Scott, E.M.; Rommelaere, J. Overcoming Barriers in Oncolytic Virotherapy with HDAC Inhibitors and Immune Checkpoint Blockade. Viruses 2016, 8, 9.

35. Katsura, T.; Iwai, S.; Ota, Y.; Shimizu, H.; Ikuta, K.; Yura, Y. The effects of trichostatin A on the oncolytic ability of herpes simplex virus for oral squamous cell carcinoma cells. Cancer Gene Ther. 2009, 16, 237–245.

36. Kitazono, M.; Goldsmith, M.E.; Aikou, T.; Bates, S.; Fojo, T. Enhanced adenovirus transgene expression in malignant cells treated with the histone deacetylase inhibitor FR901228. Cancer Res. 2001, 61, 6328–6330.

37. Eckschlager, T.; Plch, J.; Stiborova, M.; Hrabeta, J. Histone Deacetylase Inhibitors as Anticancer Drugs. Int. J. Mol. Sci 2017, 18, 1414.

38. Thurn, K.T.; Thomas, S.; Moore, A.; Munster, P.N. Rational therapeutic combinations with histone deacetylase inhibitors for the treatment of cancer. Future Oncol. 2011, 7, 263–283.

39. Kapoor, P.; Kumar, S.; Fonseca, R.; Lacy, M.Q.; Witzig, T.E.; Hayman, S.R.; Dispenzieri, A.; Buadi, F.; Bergsagel, P.L.; Gertz, M.A.; et al. Impact of risk stratification on outcome among patients with multiple myeloma receiving initial therapy with lenalidomide and dexamethasone. Blood 2009, 114, 518–521.

40. Siegel, R.; Ma, J.; Zou, Z.; Jemal, A. Cancer statistics, 2014. CA Cancer J. Clin. 2014, 64, 9–29.

41. Cai, W.; Chen, G.; Luo, Q.; Liu, J.; Guo, X.; Zhang, T.; Ma, F.; Yuan, L.; Li, B.; Cai, J. PMP22 regulates self-renewal and chemoresistance of gastric cancer cells. Mol. Cancer Ther. 2017, 16, 1187–1198.

42. Jaime-Ramirez, A.C.; Yu, J.G.; Caserta, E.; Yoo, J.Y.; Zhang, J.; Lee, T.J.; Hofmeister, C.; Lee, J.H.; Kumar, B.; Pan, Q.; et al. Reolysin and Histone Deacetylase Inhibition in the Treatment of Head and Neck Squamous Cell Carcinoma. Mol. Ther. Oncolytics 2017, 5, 87–96.

43. Bretscher, C.; Marchini, A. H−1 Parvovirus as a Cancer-Killing Agent: Past, Present, and Future. Viruses 2019, 11, 562.

44. Li, J.; Bonifati, S.; Hristov, G.; Marttila, T.; Valmary-Degano, S.; Stanzel, S.; Schnolzer, M.; Mougin, C.; Aprahamian, M.; Grekova, S.P.; et al. Synergistic combination of valproic acid and oncolytic parvovirus H−1PV as a potential therapy against cervical and pancreatic carcinomas. EMBO Mol. Med. 2013, 5, 1537–1555.

45. Fox, C.R.; Parks, G.D. Histone Deacetylase Inhibitors Enhance Cell Killing and Block Interferon-Beta Synthesis Elicited by Infection with an Oncolytic Parainfluenza Virus. Viruses 2019, 11, 431.

46. Li, Y.; Seto, E. HDACs and HDAC Inhibitors in Cancer Development and Therapy. Cold Spring Harb. Perspect. Med. 2016, 6, 218.

47. MacTavish, H.; Diallo, J.S.; Huang, B.; Stanford, M.; Le Boeuf, F.; De Silva, N.; Cox, J.; Simmons, J.G.; Guimond, T.; Falls, T.; et al. Enhancement of vaccinia virus based oncolysis with histone deacetylase inhibitors. PLoS ONE 2010, 5, e14462.

48. Lyko, F. The DNA methyltransferase family: A versatile toolkit for epigenetic regulation. Nat. Rev. Genet. 2018, 19, 81–92.

49. Goll, M.G.; Kirpekar, F.; Maggert, K.A.; Yoder, J.A.; Hsieh, C.L.; Zhang, X.; Golic, K.G.; Jacobsen, S.E.; Bestor, T.H. Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2. Science 2006, 311, 395–398.

50. Gnyszka, A.; Jastrzebski, Z.; Flis, S. DNA methyltransferase inhibitors and their emerging role in epigenetic therapy of c ancer. Anticancer Res. 2013, 33, 2989–2996.

51. Andrews, S.V.; Ellis, S.E.; Bakulski, K.M.; Sheppard, B.; Croen, L.A.; Hertz-Picciotto, I.; Newschaffer, C.J.; Feinberg, A. P.; Arking, D.E.; Ladd-Acosta, C.; et al. Cross-tissue integration of genetic and epigenetic data offers insight into autism spectrum disorder. Nat. Commun. 2017, 8, 1011.

52. Cuddapah, V.A.; Pillai, R.B.; Shekar, K.V.; Lane, J.B.; Motil, K.J.; Skinner, S.A.; Tarquinio, D.C.; Glaze, D.G.; McGwin, G.; Kaufmann, W.E.; et al. Methyl-CpG-binding protein 2 (MECP2) mutation type is associated with disease severity in Rett syndrome. J. Med. Genet. 2014, 51, 152–158.

53. Jin, Z.; Liu, Y. DNA methylation in human diseases. Genes Dis. 2018, 5, 1–8.

54. Liu, Y.; Aryee, M.J.; Padyukov, L.; Fallin, M.D.; Hesselberg, E.; Runarsson, A.; Reinius, L.; Acevedo, N.; Taub, M.; Ronn inger, M.; et al. Epigenome-wide association data implicate DNA methylation as an intermediary of genetic risk in rheu matoid arthritis. Nat. Biotechnol. 2013, 31, 142–147.

55. Sun, Z.H.; Liu, Y.H.; Liu, J.D.; Xu, D.D.; Li, X.F.; Meng, X.M.; Ma, T.T.; Huang, C.; Li, J. MeCP2 Regulates PTCH1 Expr ession Through DNA Methylation in Rheumatoid Arthritis. Inflammation 2017, 40, 1497–1508.

56. Okemoto, K.; Kasai, K.; Wagner, B.; Haseley, A.; Meisen, H.; Bolyard, C.; Mo, X.; Wehr, A.; Lehman, A.; Fernandez, S.; et al. DNA demethylating agents synergize with oncolytic HSV1 against malignant gliomas. Clin. Cancer Res. 2013, 19, 5952–5959.

57. Chen, X.J.; Zhang, K.; Xin, Y.; Jiang, G. Oncolytic adenovirus-expressed RNA interference of O(6)-methylguanine DNA methyltransferase activity may enhance the antitumor effects of temozolomide. Oncol. Lett. 2014, 8, 2201–2202.

58. Bleehen, N.M.; Newlands, E.S.; Lee, S.M.; Thatcher, N.; Selby, P.; Calvert, A.H.; Rustin, G.J.; Brampton, M.; Stevens, M.F. Cancer Research Campaign phase II trial of temozolomide in metastatic melanoma. J. Clin. Oncol. 1995, 13, 910–913.

59. O'Reilly, S.M.; Newlands, E.S.; Glaser, M.G.; Brampton, M.; Rice-Edwards, J.M.; Illingworth, R.D.; Richards, P.G.; Ken nard, C.; Colquhoun, I.R.; Lewis, P.; et al. Temozolomide: A new oral cytotoxic chemotherapeutic agent with promising activity against primary brain tumours. Eur. J. Cancer 1993, 29A, 940–942.

60. Shi, T.; Song, X.; Wang, Y.; Liu, F.; Wei, J. Combining Oncolytic Viruses with Cancer Immunotherapy: Establishing a Ne w Generation of Cancer Treatment. Front. Immunol. 2020, 11, 2076.

61. Friedman, R.C.; Farh, K.K.; Burge, C.B.; Bartel, D.P. Most mammalian mRNAs are conserved targets of microRNAs. G enome Res. 2009, 19, 92–105.

62. Vojtechova, Z.; Tachezy, R. The Role of miRNAs in Virus-Mediated Oncogenesis. Int. J. Mol. Sci. 2018, 19, 1217.

63. Gu, S.; Jin, L.; Zhang, F.; Sarnow, P.; Kay, M.A. Biological basis for restriction of microRNA targets to the 3′ untranslate d region in mammalian mRNAs. Nat. Struct. Mol. Biol. 2009, 16, 144–150.

64. Kelly, E.J.; Hadac, E.M.; Greiner, S.; Russell, S.J. Engineering microRNA responsiveness to decrease virus pathogenic ity. Nat. Med. 2008, 14, 1278–1283.

65. Ylosmaki, E.; Hakkarainen, T.; Hemminki, A.; Visakorpi, T.; Andino, R.; Saksela, K. Generation of a conditionally replicat ing adenovirus based on targeted destruction of E1A mRNA by a cell type-specific MicroRNA. J. Virol. 2008, 82, 11009 –11015.

66. Ruiz, A.J.; Hadac, E.M.; Nace, R.A.; Russell, S.J. MicroRNA-Detargeted Mengovirus for Oncolytic Virotherapy. J. Virol. 2016, 90, 4078–4092.

67. Leber, M.F.; Bossow, S.; Leonard, V.H.; Zaoui, K.; Grossardt, C.; Frenzke, M.; Miest, T.; Sawall, S.; Cattaneo, R.; von K alle, C.; et al. MicroRNA-sensitive oncolytic measles viruses for cancer-specific vector tropism. Mol. Ther. 2011, 19, 10 97–1106.

68. Shayestehpour, M.; Moghim, S.; Salimi, V.; Jalilvand, S.; Yavarian, J.; Romani, B.; Mokhtari-Azad, T. Targeting human b reast cancer cells by an oncolytic adenovirus using microRNA-targeting strategy. Virus Res. 2017, 240, 207–214.

69. Santella, B.; Pignataro, D.; Lavano, M.A.; Rinaldi, M.; Galdiero, F. Comment on: Expressions of MiR−132 in patients wit h chronic hepatitis B, posthepatitic cirrhosis and hepatitis B virus-related hepatocellular carcinoma. Eur. Rev. Med. Phar macol. Sci. 2019, 23, 1384–1385.

70. Santella, B.; Folliero, V.; Pirofalo, G.M.; Serretiello, E.; Zannella, C.; Moccia, G.; Santoro, E.; Sanna, G.; Motta, O.; De Caro, F.; et al. Sepsis-A Retrospective Cohort Study of Bloodstream Infections. Antibiotics 2020, 9, 851.

71. Li, J.M.; Kao, K.C.; Li, L.F.; Yang, T.M.; Wu, C.P.; Horng, Y.M.; Jia, W.W.; Yang, C.T. MicroRNA−145 regulates oncolytic herpes simplex virus−1 for selective killing of human non-small cell lung cancer cells. Virol. J. 2013, 10, 241.

72. Krichevsky, A.M.; Gabriely, G. miR−21: A small multi-faceted RNA. J. Cell Mol. Med. 2009, 13, 39–53.

73. Marzulli, M.; Mazzacurati, L.; Zhang, M.; Goins, W.F.; Hatley, M.E.; Glorioso, J.C.; Cohen, J.B. A Novel Oncolytic Herpes Simplex Virus Design based on the Common Overexpression of microRNA−21 in Tumors. J. Gene Ther. 2018, 3, 2060.
74. Bofill-De Ros, X.; Gironella, M.; Fillat, C. miR−148a- and miR−216a-regulated oncolytic adenoviruses targeting pancreatic tumors attenuate tissue damage without perturbation of miRNA activity. Mol. Ther. 2014, 22, 1665–1677.
75. Miyamoto, S.; Inoue, H.; Nakamura, T.; Yamada, M.; Sakamoto, C.; Urata, Y.; Okazaki, T.; Marumoto, T.; Takahashi, A.; Takayama, K.; et al. Coxsackievirus B3 is an oncolytic virus with immunostimulatory properties that is active against lung adenocarcinoma. Cancer Res. 2012, 72, 2609–2621.
76. Shafren, D.R.; Williams, D.T.; Barry, R.D. A decay-accelerating factor-binding strain of coxsackievirus B3 requires the coxsackievirus-adenovirus receptor protein to mediate lytic infection of rhabdomyosarcoma cells. J. Virol. 1997, 71, 9844–9848.
77. Jia, Y.; Miyamoto, S.; Soda, Y.; Takishima, Y.; Sagara, M.; Liao, J.; Hirose, L.; Hijikata, Y.; Miura, Y.; Hara, K.; et al. Extremely Low Organ Toxicity and Strong Antitumor Activity of miR−34-Regulated Oncolytic Coxsackievirus B3. Mol. Ther. Oncolytics 2019, 12, 246–258.