

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# A Novel Therapy for Melanoma and Prostate Cancer Using a Non-Replicating Sendai Virus Particle (HVJ-E)

---

Toshihiro Nakajima, Toshimitsu Itai, Hiroshi Wada,  
Toshie Yamauchi, Eiji Kiyohara and  
Yasufumi Kaneda

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55014>

---

## 1. Introduction

The virotherapy approach to cancer therapy uses virus particles. It is based on the case reports since 1950s, which reported the regression of cancers including leukemia, Hodgkin's disease, and Burkitt's lymphoma after the infection of wild type viruses [1-11].

The earliest virotherapies involved injection of wild-type viruses and evaluation of their efficacies [1-3]. *Ex vivo* therapies using autologous irradiated tumors infected with oncolytic viruses were also investigated [12-18]. Deletion mutants of oncolytic viruses [19-24], and recombinant viruses carrying a therapeutic gene [25-32] that induce cancer apoptosis or cancer immunity have been developed and evaluated in the clinical setting.

Oncolytic viruses derived from adenovirus, poxvirus, reovirus, picornavirus, paramyxovirus, and herpes simplex virus are currently available and have been clinically evaluated [33-35]. In China, two adenovirus-based products (Gendicine and Ocorine) have been commercialized [36], while randomized phase III studies of two oncolytic viruses (reovirus and poxvirus) are ongoing in advanced countries [33-35]. Thus, virotherapy is expected to become available as a new approach for cancer treatment, and specific product approval is anticipated in the US, EU, and Japan [37].

The major drawback associated with virotherapy is safety since replicating viruses are used in this therapy. In order to reduce the toxicity to normal cells, oncolytic viruses with strict specificity for cancer cells were constructed [29, 38-42]. However, the use of these viruses is still considered to be high risk because it is theoretically possible that a virulent infection may occur after recombination with wild-type viruses [43].

An alternative option to avoid such a risk is to use a non-replicating oncolytic virus [44]. We found that a non-replicating oncolytic virus (HVJ-E: hemagglutinating virus of Japan-envelope) is able to induce cancer cell-specific apoptosis and immunity [45]. The induction of apoptosis and activation of dendritic cells *in vitro*, and anti-tumor activity *in vivo* are similar to the wild-type hemagglutinating virus of Japan (also known as Sendai virus, HVJ) [45].

The hemagglutinating virus of Japan was discovered in Sendai, Japan, in the 1950s [46]. It is a paramyxovirus with a minus-strand RNA genome. The virus has fusogenic activity [47, 48], and is used to prepare hybridoma cells for the production of monoclonal antibodies, and heterokaryons for chromosome analysis [49-51].

The hemagglutinating virus of Japan-envelope is an inactivated HVJ particle [52]. It is manufactured by a process similar to that used for whole virus particle vaccines. Good manufacturing practice (GMP)-regulated processes have been established in their production for use in preclinical and clinical studies [53].

We conducted dose-setting efficacy studies for HVJ-E in a murine cancer model in which dose-dependent anti-cancer activity was observed. We also conducted safety studies following good laboratory practices (GLP), including pharmacological safety studies and toxicokinetic (TK) studies in rats and monkeys, as part of an investigational new drug (IND) application.

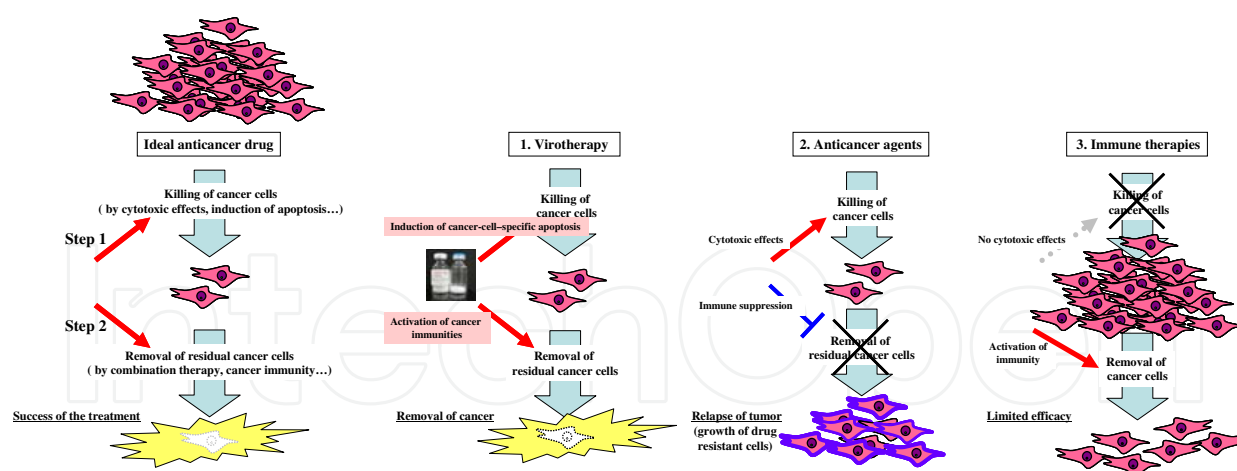
Osaka University Hospital is currently conducting two investigational clinical studies with HVJ-E for the treatment of advanced melanoma and castration-resistant prostate cancer (CRPC) [54-56]. These clinical trials are the first human studies for HVJ-E and will reveal the safety and efficacy of the non-replicating virus (HVJ-E). Virotherapy with a non-replicating oncolytic virus is a new approach that is anticipated to provide a new strategy for cancer therapy.

## 2. A new strategy for cancer therapy

Most cancers are still incurable and new approaches are required to improve the efficacy of cancer treatments. However, conventional cancer therapies are problematic.

Chemotherapy with anti-cancer agents is useful in achieving tumor regression. However, the immune system, which is important in the removal of residual cancer cells, is also suppressed by these agents (Figure 1). Therefore, surviving cancer cells and cancer stem cells (CSC) eventually acquire drug resistance, resulting in tumor relapse (Figure 1) [57]. Thus, chemotherapy with cytotoxic drugs does not generally result in the necessary eradication of cancer cells required for long-term survival.

Immune therapies for cancer offer a new approach to cancer treatment, and several products, including sipuleucel-T, are currently approved in advanced countries [58, 59]. The aim of these therapies is the removal of cancers by the immune system. Numerous cancer immune therapies are currently under evaluation in clinical studies. However, these agents are not potent because of lack of cytotoxic effect on cancer cells (Figure 1).



**Figure 1. Problems with conventional therapies and a 2-step strategy for cancer treatment.** Conventional anti-cancer therapies are problematic and a 2-step therapeutic strategy is proposed for effective cancer treatment. Virotherapy possesses ideal characteristics for such a 2-step therapy. Problems associated with conventional anti-cancer drugs and immune therapies, including cancer vaccines, are shown.

These observations suggest that a two-step strategy is necessary for the eradication of cancer cells (Figure 1).

During the first step, the direct killing of cancer cells is necessary for reduction of tumor volume. CSCs are usually resistant to conventional anti-cancer drugs and continue to proliferate during chemotherapy. Therefore, an agent that targets and kills CSCs is required for effective cancer treatment.

During the second step, the removal of residual cancer cells (and CSCs) from the body by a cancer-specific immune response is necessary to avoid relapse of the condition [57]. However, it is difficult for immune cells to recognize and remove CSCs because they exist as a minority population within the tumor, and possess a lower antigenicity than differentiated cancer cells [57]. Oncolytic viruses have the capability of both directly killing cancer cells and inducing cancer immunity.

It has been reported that several oncolytic viruses have the capability to kill CSCs [60-67]. The reovirus-based oncolytic virus [61], telomerase-specific oncolytic adenovirus [62], and herpes simplex virus-based oncolytic viruses (G47Delta and Delta 68H-6) [63, 64] reduced CSCs in murine models of breast cancer, esophageal cancer, and malignant glioma, respectively. Thus, a virotherapy approach in patients is expected to kill cancer cells and eradicate cancer cells including CSCs (Figure 1).

### 3. Non-replicating virus particles as anti-cancer agents

The use of non-replicating virus particles is a new approach in virotherapy.

A non-replicating virus particle, HVJ-E, is currently being developed as a potential new agent for the treatment of advanced melanoma and CRPC [44, 55, 56]. It is derived from HVJ, a

member of the paramyxovirus family (Figure 2). The HVJ-E particle is prepared by inactivating the wild-type virus (HVJ) by treatment with an alkylating agent and UV irradiation [52, 53]. HVJ-E was originally developed as a drug delivery system (vector) for various biopharmaceuticals such as plasmid DNAs, siRNAs, decoy oligonucleotides, antibody proteins, and anti-cancer drugs [52, 68-75].

Kurooka and Kaneda discovered that the HVJ-E particle itself displayed anti-cancer effects in a murine model of colon cancer [45]. Similar to the live (replicating) virus, HVJ-E induced maturation and differentiation of human and murine dendritic cells (DCs). It also induced infiltration of immune cells into the tumor tissue followed by activation of cancer cell-specific cytotoxic T cells. Furthermore, HVJ-E suppressed the function of regulatory T cells (Treg), which have been reported to be negative regulators of cancer immunity. Thus, HVJ-E activates cancer immunity, and simultaneously suppresses Treg [45].

### 1. Structure

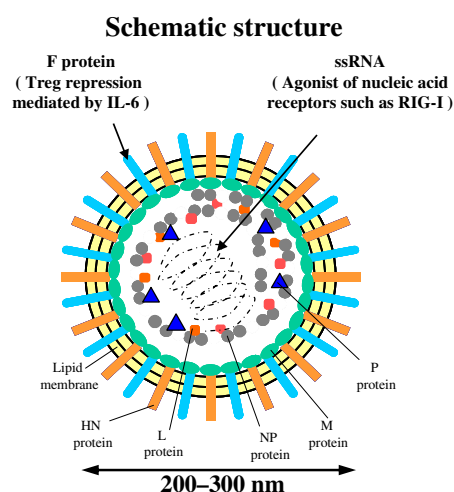
- a. Spherical particle of diameter 200–300 nm
- b. Contains single strand RNA (ssRNA)
- c. Contains proteins+lipid for delivery (functions as a natural DDS for nucleic acid)

### 2. Mode of action

- a. The RNA in the particle acts as a RIG-I agonist and induces an induces cancer cell apoptosis
- b. Activates the RIG-I/MAVS pathway and also induces anticancer immune responses

### 3. Manufacture

- a. Process adapted to GMP guidelines, and pilot plant for clinical trial is available
- b. Freeze-dried formulation is stable for over 21 months in refrigerator.



**Figure 2. Characteristics and structure of HVJ-E/GEN0101.** The characteristics of HVJ-E/GEN0101 are shown on the left. A schematic structure of the particle is shown on the right.

Fujiwara and Kaneda *et al.* reported that HVJ-E induced innate immunity [76]. Intratumoral injection of HVJ-E promoted infiltration and activation of natural killer (NK) cells by the induction of C-X-C motif chemokine 10 (CXCL10) and type I interferons. When HVJ-E was injected into the tumor of a murine model of renal cell carcinoma (RCC), NK cells exhibited cytotoxic activity against the RCC cell line *in vivo* [76]. The involvement of NK cells in the anti-tumor effect was also confirmed by showing the depletion of NK cells using an asialo-GM1 antibody [76]. Activated NK cells produced interferon- $\gamma$ , which induces cancer-specific cytotoxic T cells [76]. These results indicated that HVJ-E is able to induce both innate and adaptive immunities.

In addition to the induction of cancer immunities, HVJ-E has the capability to induce cancer cell-specific apoptosis. Kawaguchi and Kaneda *et al.* reported that HVJ-E showed a dose-dependent, direct killing effect on human prostate cancer cell lines. In contrast, it showed no suppression of normal human prostate epithelium proliferation [77].

HVJ-E also induced apoptosis of prostate cancer cells *in vivo* because it showed an anti-tumor effect in severe combined immunodeficiency (SCID) mice that lack B lymphocyte- and T lymphocyte-mediated immunities [76, 77]. When NK cells were depleted from SCID mice by injection of the asialo-GM1 antibody, intratumoral injection of HVJ-E still showed an anti-tumor effect in a murine model of CRPC [77]. This result suggested that HVJ-E showed a direct killing activity *in vivo*. HVJ-E-mediated apoptosis of cancer cells was further confirmed in a murine model of prostate cancer using a NOD/SCID mouse, which lacks both innate (NK cell-mediated) and adaptive (antibody and CTL-mediated) immunities [54].

Numerous studies have revealed that the non-replicating HVJ-E particle shows anti-tumor effects in murine models of renal cell carcinoma, glioma, colon, bladder and CRPCs [45, 76-79].

In contrast, previous studies have reported that the non-replicating oncolytic Newcastle disease virus (NDV) failed to show an anti-tumor effect *in vivo* [80, 81]. These results are inconsistent with results obtained with HVJ-E [45, 76, 77, 79], and the putative reason is the difference between the number of particles used in the studies. In studies with NDV,  $5 \times 10^9$  PFU of oncolytic virus were systemically or locally administrated [81], whereas the number of HVJ-E particles administered was estimated to be higher.

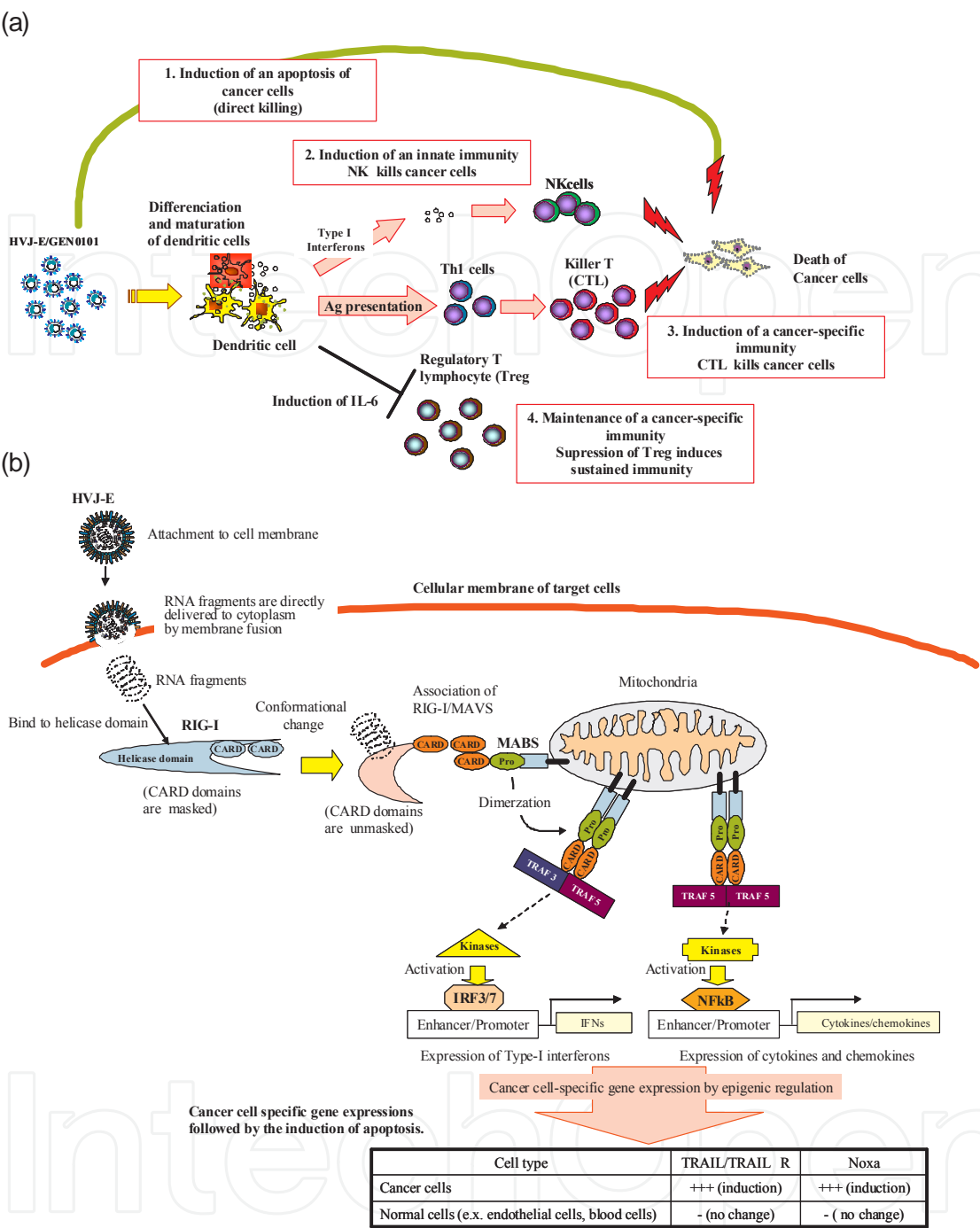
Another possibility is the difference in the capability of the virus to deliver its RNA fragments to target cancer cells. The Z strain-derived HVJ-E used in our studies has the highest level of membrane fusion activity [52]. Therefore, it is possible that HVJ-E has the ability to deliver more RNA component to the target cancer cells than the UV-inactivated NDV particle. The difference in process of inactivation may be responsible for the activity of non-replicating oncolytic viruses. The Newcastle disease virus was inactivated by UV irradiation, whereas HVJ-E was inactivated by a combination of treatment with an alkylating agent and UV irradiation [53]. Inactivation conditions affect the efficiency of delivery, and strictly regulated processes are necessary to obtain suitable performance [53].

#### 4. Mode of actions

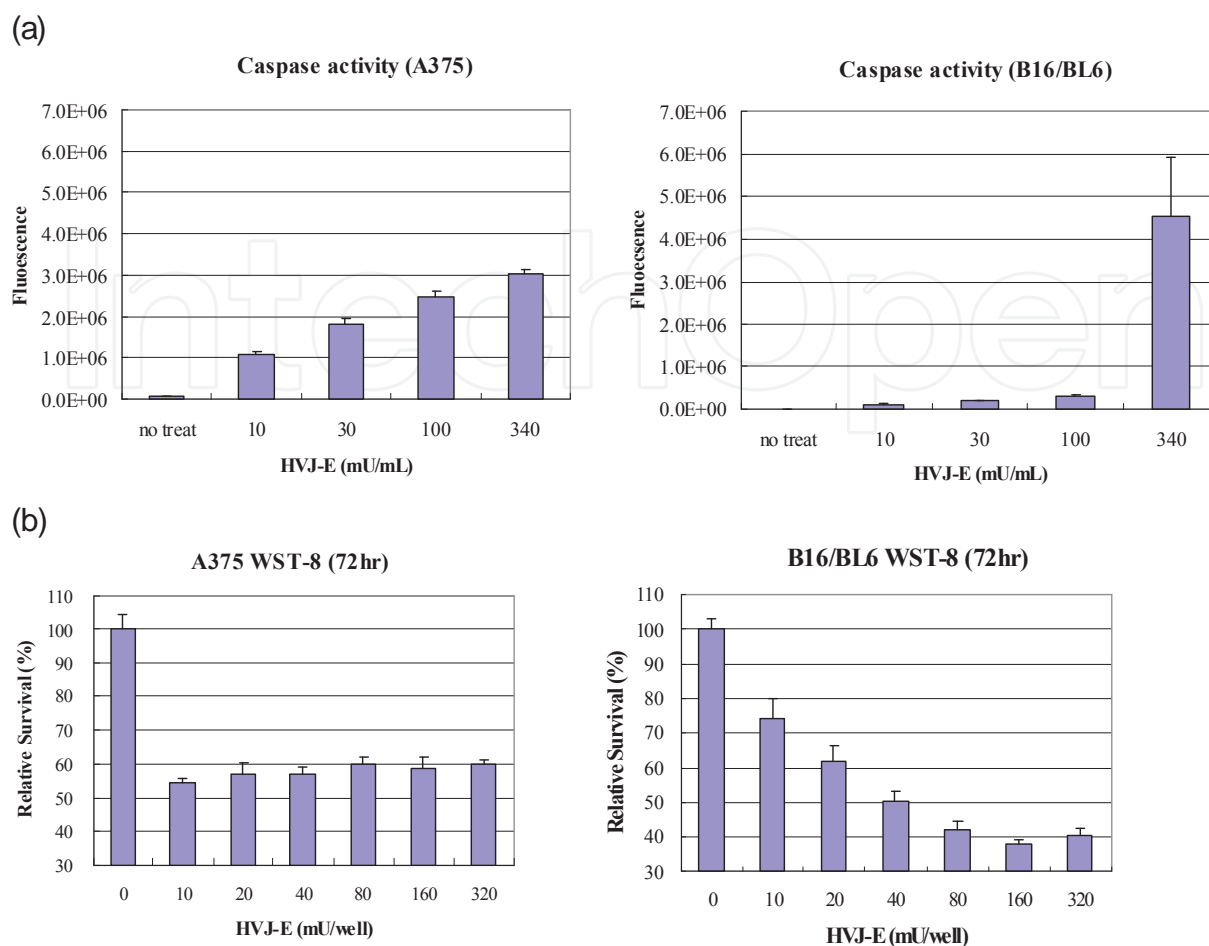
The major target cells of HVJ-E are cancer cells and dendritic cells (DCs) (Figure 3A) [44, 45, 76].

Treatment of cancer cells with HVJ-E enhanced the expression and activation (cleavage) of caspases 3, 8, and 9 (Figure 4A) [77], and induced dose-dependent apoptosis of melanoma, prostate, and other cancer cell lines *in vitro* (Figure 4B) [77]. Interestingly, no apoptotic effects were observed on normal epithelial cells derived from murine prostate [54, 77]. Thus, the apoptotic activity of HVJ-E is considered to be specific to cancer cells [54, 77].





**Figure 3. The mechanism of anti-cancer effects of HVJ-E/GEN0101.** A novel type of virotherapy agent (HVJ-E/GEN0101) has a multi-mode of action that is ideal for 2-step cancer treatment. **(A)** Target cells and sequential anti-cancer effects of HVJ-E/GEN0101. **(B)** Signaling pathway induced by stimulation with HVJ-E/GEN0101. The RIG-I/MAVS pathway is the major pathway involved. RIG-I is a cytosolic receptor for nucleic acids: it usually functions as a sensor to recognize viral infection. The nucleic acids in the HVJ-E/GEN0101 particle act as an agonist for RIG-I and induce cancer cell-specific gene expression followed by the induction of apoptosis.



**Figure 4. HVJ-E/GEN0101 induces apoptosis of human and murine melanoma cells. (A)** Induction of caspase activity after treatment of melanoma cells with HVJ-E/GEN0101. Human (A375) and murine (B16/BL6) melanoma cells were treated with various amounts of HVJ-E/GEN0101, and caspase activities were measured 24 hours later. A dose-dependent activation was observed. **(B)** Survival of melanoma cells after treatment with various amounts of HVJ-E/GEN0101. Human (A375) and murine (B16/BL6) melanoma cells were treated with various amounts of HVJ-E/GEN0101 and cell survival was measured by WST-8 assay 72 hours later.

Matsushima and Kaneda *et al.* conducted an investigation to determine the active component for anti-cancer effects, and identified RNA fragments within the particle [54]. Moreover, Kaneda *et al.* analyzed the signaling pathway involved and revealed that retinoic acid inducible gene-I (RIG-I) is a key factor for signal transduction [44, 54, 77]. Retinoic acid inducible gene-I is a cytosolic nucleic acid receptor and was originally identified as a sensor that recognizes infection by single strand RNA viruses [82, 83]. Thus, RIG-I has been recognized as an inducer of immune response against infected viruses [82-84].

The RNA fragments delivered by HVJ-E bind the helicase domain of RIG-I in the cytoplasm and change the conformation to unmask the caspase activation and recruitment domain (CARD) (Figure 3B). After binding with the RNA fragments, RIG-I interacts with the mitochondrial antiviral signaling (MAVS) protein on the mitochondrial membrane (Figure 3B) [84]. The mitochondrial antiviral signaling protein forms a complex with an adaptor protein



(TRAF3/5), stimulates transcription factors (IRF3 and IRF7), and promotes the expression of interferon- $\alpha$  and  $\beta$  (Figure 3B) [85, 86]. It also stimulates kinases of regulator protein of transcription factor NK- $\kappa$ B, resulting in increased expression of cytokines, chemokines, and other genes (Figure 3B) [45, 54, 76, 77].

It has been reported that the RIG-I/MAVS pathway is activated after cancer cells are treated with HVJ-E [54]. Transfection of isolated RNA fragments from HVJ-E particle also induced apoptosis of cancer cells *in vitro* [54]. Thus, HVJ-E uses a natural oligonucleotide (RNA fragment from the inactivated virus genome) as an active ingredient, and a natural virus particle (envelope of HVJ) as a delivery system for a nucleic acid medicine (RNA fragments). The apoptosis induced by HVJ-E was cancer cell-specific because normal endothelial cells showed no apoptosis after the treatment with HVJ-E [54, 77].

Involvement of RIG-I in cancer cell-specific apoptosis has also been indicated in ovarian cancers and melanoma. Kübler and Barchet *et al.* reported that the RIG-I agonist (Poly(dAdT)) induced apoptosis and expression of MHC class I molecules in ovarian cancer cells [87, 88]. Van and Bell *et al.* also reported apoptosis of ovarian cancer cells after treatment with dsRNA [89]. Analysis by shRNA-mediated knockdown revealed that RIG-I and other receptors for dsRNA (TLR3 and MDA-5) were involved in the caspase 8/9-mediated apoptosis of cancer cells. Similar to sensitivity of HVJ-E, epithelial cells derived from ovarian surface was resistant to apoptosis mediated by RIG-I signal pathway. When combined with conventional chemotherapy (carboplatin/paclitaxel), treatment with dsRNA showed a synergistic suppression of ovarian cancer cell viability [89]. Besch *et al.* reported that stimulation of RIG-I and MDA-5 induced apoptosis of human melanoma cells [90]. The authors used pppRNA and poly(I:C) as ligands for RIG-I and MDA-5, and showed the involvement of caspase-9 and Apaf-1 during apoptosis. They also reported the reduction of lung metastasis by treatment with ligands for RIG-I and MDA-5 in the NOD/SCID mouse [90]. Details of the underlying pathways are currently being analyzed using siRNAs of apoptosis-related factors [54]. It has been suggested that differences in the expression of apoptotic genes such as Noxa, TRAIL, and TRAIL receptors in cancer cells and normal cells determine the specificity of apoptosis induced by HVJ-E (Figure 3B) [54].

HVJ-E also induced differentiation and maturation of murine and human DCs [45]. It induced the expression of surface markers on mature DCs, and the production of various cytokines and chemokines from DCs [45, 76, 77]. Activated DCs induce both innate (NK cell-mediated) and adaptive (cytotoxic T cell-mediated) immunities (Figure 3A) [45, 76]. It has been reported that an RIG-I agonist (Poly(dAdT)) induced the production of cytokines (IL-6 and TNF- $\alpha$ ) and chemokines (CXCL1 and CCL5/RANTES) [87, 88] in human ovarian cancer cells.

## 5. Efficacy in preclinical studies

The efficacy of the non-replicating oncolytic virus (HVJ-E/GEN0101) was examined in murine models of melanoma and prostate cancer (Figure 5A). GEN0101 is the identification code for the agent. The data have indicated that the anti-tumor effect is dose-depend-

ent similar to other non-viral anti-cancer agents (Figure 5B). This feature is important for the development of non-replicating oncolytic virus as a novel therapeutic agent; identification of the optimal dose for non-replicating oncolytic viruses is easier than determining the optimal dose for replicating oncolytic viruses as the latter are subject to change (increase) resulting from replication in target cancer cells. The effects of administration in a xenograft model of human CRPC were also examined (Figure 5A). Efficacy after subcutaneous administration was revealed (Figure 5C). It is known that the SCID mice lacks B lymphocyte- and T lymphocyte-mediated immunities such as antibody production, and induction of cytotoxic T cells but retains monocytes and NK cells important for innate immunity. Thus, the non-replicating virus (HVJ-E/GEN0101) is able to induce innate immunity, and show anti-cancer activity *in vivo* even in the absence of direct killing of cancer cells. Efficacy by subcutaneous administration is important for the development of a non-replicating virus because subcutaneous administration is more common than intratumoral administration. Intratumoral administration kills cancer cells directly and promotes the release of tumor antigens, which are recognized by immune cells. Subcutaneous administration is expected to enhance and sustain the immune response. Therefore, the combination of intratumoral and subcutaneous administration is suggested as a suitable regimen in the clinical setting.

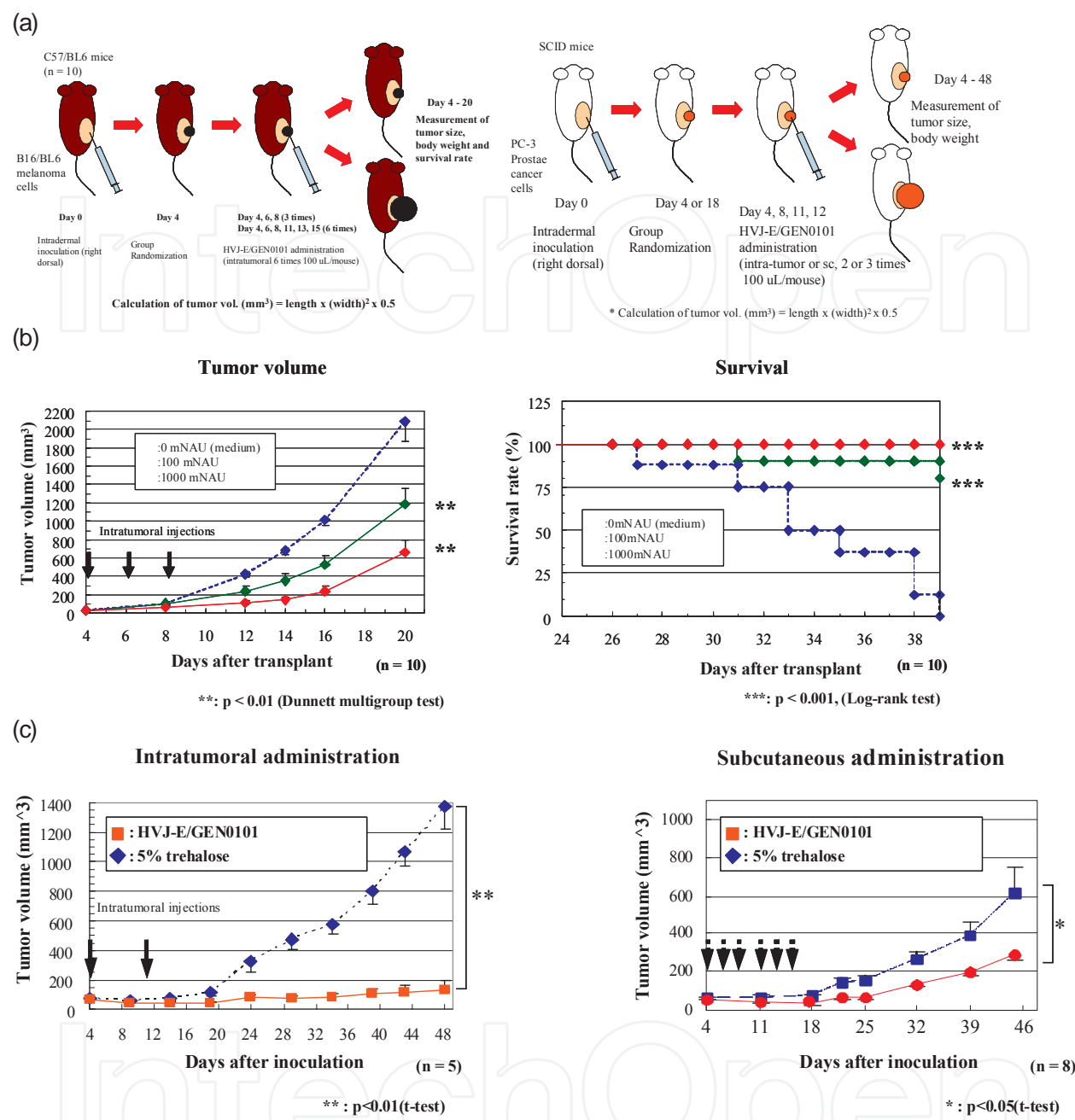
In summary, the results of efficacy studies have indicated that the anti-cancer effect of the non-replicating oncolytic virus HVJ-E/GEN0101 is dose-dependent similar to conventional anti-cancer drugs, and subcutaneous administration may be the preferred administration route for the viral and non-replicating viral agents.

## 6. Safety studies

Safety of the non-replicating oncolytic virus (HVJ-E/GEN0101) has been confirmed in non-primate (rat) and primate (Cynomolgus monkey) animals. Lists of the studies that have been conducted are shown in Table 1A and B.

Results from single dose general toxicity studies revealed that no death or severe finding was observed even in the maximum dosage groups. Similar to the single dose studies, no severe finding was observed in repeated dose, general toxicity studies (Table 1A).

Results from immunological and genetic toxicity studies in rat and monkeys revealed that no abnormal symptoms related to the test agent were observed. The levels of IL-6 and IFN- $\gamma$  in monkey serum were analyzed after subcutaneous injection of HVJ-E/GEN0101, and the levels of both cytokines were determined to be within the normal range (data not shown). A core battery of safety pharmaceutical studies was performed to determine the effects on major organs (central nervous, respiratory, and cardiovascular systems); no abnormal effect was observed with the exception of transient and non-severe pyrexia (Table 1B).



**Figure 5. Efficacy studies of HVJ-E/GEN0101 in a murine model of melanoma and prostate cancer.**(A) Protocols for the efficacy study using murine models of melanoma and prostate cancer. Left: C57/BL6 mice were transplanted with B16/BL6 cells and 3 intratumoral injections of HVJ-E/GEN0101 were administered after tumor formation (4 days after transplant). A time course study of change in tumor volume was performed, and the difference in tumor volume among the three groups was statistically analyzed at the end of the study. Significant differences were determined by Dunnett's multigroup test, and significant differences to the medium (control) group were observed ( $p < 0.01$ ). The survival rate after the injection of HVJ-E/GEN0101 was also monitored in the melanoma model. A survival study was performed and the difference among the three groups was statistically analyzed at the end of the study. Significant differences were determined by log-rank analysis. Significant differences to the medium group were observed ( $p < 0.001$ ). Right: Protocol for the efficacy study in a murine model of prostate cancer. Human CRPC (PC-3)-bearing mice were used for the study. A summary of the study protocol is shown. The anti-tumor effect of HVJ-E/GEN0101 for each administration route was examined. Severe combined immunodeficiency mice were transplanted with PC-3 cells. Two intratumoral injections or 6 subcutaneous injections of HVJ-E/GEN0101 were performed after tumor formation (4

days after transplant). A time course study of change in tumor volume was performed and differences in tumor volume between the two groups were statistically analyzed at the end of study. Significant differences were determined by t-test and significant differences to the medium were observed in both routes ( $p < 0.01$  and  $p < 0.05$ ). **(B)** Efficacy study in a murine model of melanoma. Dose-dependency of the anti-tumor effect of HVJ-E/GEN0101 was revealed. **(C)** Efficacy study in a murine model of prostate cancer. A time course study of change in tumor volume was performed and differences in tumor volume between the two groups were statistically analyzed at the end of the study. Significant differences were determined by t-test, and significant differences to the medium were observed for both administration routes ( $p < 0.01$  and  $p < 0.05$ ).

a) Toxicological study (1): General toxicity study				
Dosing Regimen	Species	Route	Dose	
Single Dose	Rat	iv	single	
	Rat	sc	single	
	Cynomolgus monkey	iv	single	
	Cynomolgus monkey	sc	single	
Repeated Dose	Rat	iv	7 days	
	Rat	sc	6 times in 2 weeks	
	Cynomolgus monkey	sc	6 times in 2 weeks	
b) Toxicological study (2): Safety pharmacology and other studies				
Study	Method	Species	Route	Dose
Safety pharmacology	Rat FOB	Rat	sc	single
	Respiratory	Rat	sc	single
	Cardiovascular	Cynomolgus monkey	sc	single
TK	Q-PCR	Rat	sc	6 times 2 weeks
Genetic toxicity	Micronucleus	Rat	sc	single
Antibody production	ELISA	Rat	sc	6 times 2 weeks

**Table 1.** Summary of toxicological studies

## 7. Clinical studies

Two clinical studies using the non-replicating oncolytic virus (HVJ-E/GEN0101) are currently being conducted in Osaka University Hospital. The target diseases are advanced melanoma (stage IIIC and stage IV) and CRPC [55, 56]. These proof-of-concept studies in melanoma and CRPC using the non-replicating oncolytic virus were initiated in July 2009 and July 2011, respectively [55, 56]. The respective summaries of both studies are shown in Table 2A and B. The primary endpoints of these studies were safety and tolerability based on the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0, whereas the secondary endpoints were efficacy and confirmation of the mode of action. The major difference between the regimens for the melanoma and CRPC studies was the route of administration and number of administrations. A combination of intratumoral and subcutaneous routes of administration (one intratumoral and three subcutaneous injections) was adopted in the CRPC study. In addition, a new injection system developed by Okayama University was used in the CRPC

study. This system permits stable refrigerated storage of the test article, and accurate injection into the prostate [91].

<b>a) Advanced melanoma</b>	
Study title	Phase I/II investigational clinical study of inactivated HVJ-E administration for advanced malignant melanoma patients
Condition	Malignant melanoma ( AJCC stage IIIC or stage IV)
Study design	Masking: Open Label
	Allocation: Non-Randomized
	Primary endpoint: Safety and tolerability
	Secondary endpoint: Anti-tumor immunity and validity
	Target sample size: 6–12 patients
Sponsor	Route: Intratumoral
	Dose: 6 times in 2 weeks/cycle, 2 cycles
URL	Osaka University Graduate School of Medicine (Osaka University Hospital)
URL	<a href="https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&amp;action=brows&amp;type=summary&amp;recptno=R000002889&amp;language=E">https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&amp;action=brows&amp;type=summary&amp;recptno=R000002889&amp;language=E</a>
<b>b) Castration-resistant prostate cancer</b>	
Study title	Phase I/II investigational clinical study to assess safety and efficacy of intratumoral and subcutaneous injection of HVJ-E to castration-resistant prostate cancer patients
Condition	Castration resistant prostate cancer (CRPC)
Study design	Masking: Open Label
	Allocation: Non-Randomized
	Primary endpoint: Safety and tolerability
	Secondary endpoint: Anti-tumor immunity and validity
	Target sample size: 6–12 patients
Sponsor	Route: Intratumoral × 1 then SC × 3
	Dose: 4 times in 2 weeks/cycle, 2 cycles
URL	Osaka University Graduate School of Medicine (Osaka University Hospital)
URL	<a href="https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&amp;action=brows&amp;type=summary&amp;recptno=R000007153&amp;language=E">https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&amp;action=brows&amp;type=summary&amp;recptno=R000007153&amp;language=E</a>

**Table 2.** Design of investigational clinical studies

8. Discussion and conclusion

The major disadvantages associated with oncolytic viruses are safety concerns because viral replication could theoretically cause the emergence of new pathogenic viruses [43]. The use of non-replicating oncolytic viruses is expected to resolve the safety issues associated with conventional oncolytic viruses because they are unable to replicate in target cells.



The modes of action underlying the anti-cancer effects of non-replicating virus on cancer cells and immune cells have been analyzed. One major signaling pathway is the RIG-I/MAVS pathway [54]. RIG-I is a cytosolic nucleic acid receptor that acts as a sensor that detects virus infection [82-84]. Similar to wild-type RNA viruses, the non-replicating virus (HVJ-E) is able to activate the RIG-I/MAVS pathway in DCs and induce both innate and adapted immunities. The non-replicating virus also activates the RIG-I/MAVS pathway in cancer cells and induces cancer cell-specific apoptosis. Genetic analyses suggest that differences in the expression of apoptosis-related genes define the sensitivity to the treatment with a non-replicating virus (HVJ-E) [54]. Furthermore, it is suggested that methylation of the respective enhancer/promoter regions underlies differences in transcription of apoptosis-related genes [54].

RIG-I, TLR3, and MDA-5 signaling pathways are involved in the apoptosis of ovarian cancers [87-89] and melanoma [90]. Thus, the RIG-I/MAVS pathway is likely to emerge as a new target for the development of drugs that induce cancer cell-specific apoptosis.

The non-replicating virus (HVJ-E) activates DCs to produce IL-6, which suppress the function of Treg [45]. This effect is expected to maintain the induced cancer immunity because Treg is known to be a negative regulator of immune responses [92-94]. It has been reported that cancer cells escape cancer immunity by the recruitment and activation of Treg. Therefore, it will be important to control the function of Treg for long-term effective induction and maintenance of cancer immunities. Suzuki and Kaneda *et al.* reported that the RIG-I/MAVS pathway was not required to induce the expression of IL-6 [95]. The attachment of the HVJ-E particle to the surface of DCs was sufficient for the production of IL-6, suggesting that the RNA fragments are unnecessary for the induction of this cytokine [95]. Detailed analyses identified that the F protein on the surface of HVJ-E is involved in the production of IL-6 [95]. Binding of the F protein to target cells requires expression of the HN protein [96]. Several gangliosides, such as GD1a and sialyl paragloboside, are implicated in the association of the HVJ-E particle and cancer cells because the HN protein binds the sialic acids of gangliosides. The receptor for the F protein remains unidentified to date. Taken together, the RIG-I/MAVS signal pathway, and a second pathway that induces the production of IL-6 may cooperate in the activation and sustainment of cancer immunity induced by the non-replicating virus (HVJ-E).

The development of a non-replicating oncolytic virus other than HVJ-E is possible because the manufacturing process for such a particle is similar to that of whole particle viral vaccines. In case of HVJ-E, the virus is inactivated by treatment with an alkylating agent and UV irradiation, a process used for the production of vaccines against viral diseases. Thus, the development of oncolytic viruses could be converted to the development of non-replicating oncolytic particles by similar manufacturing processes.

A disadvantage associated with non-replicating oncolytic viruses may be the defect in transmission ability. Therefore, it is possible that a greater amount of non-replicating oncolytic virus may be required for effective treatment compared with a live oncolytic virus. Alternatively, more frequent injections may be necessary for complete tumor eradication compared with the use of live oncolytic viruses. However, it is important to achieve a balance between



the risks and benefits associated with the therapy. In our opinion, repeated administration of the non-replicating virus should be tolerable because no severe finding was observed during our safety studies.

In conclusion, non-replicating virus particles such as HVJ-E may resolve the safety issue of conventional virotherapy and provide a new strategy in cancer treatment.

## 9. Future perspectives

The first non-replicating oncolytic virus (HVJ-E) is currently under evaluation in clinical studies. Proof-of-concept data for non-replicating viruses in both clinical and non-clinical studies are necessary for further development of this approach. Osaka University Hospital is currently conducting two phase I/IIa studies: one for advanced melanoma and another for CRPC [55, 56]. The results of these studies will reveal the safety, efficacy, and optimal dosage regimen necessary for phase II study or randomized, double blind phase III study.

Combination treatment may be an effective approach to increase efficacy [97]. Indeed, an increase in therapeutic efficacy has been reported for virotherapies combined with photodynamic therapy [98, 99], radiotherapy [100], chemotherapy [101, 102], or gene therapy [103]. Kiyohara and Kaneda reported that combination of the non-replicating virus (HVJ-E) and gene therapy (IL-12) increased efficacy in a murine model of melanoma [104]. Furthermore, it was reported that a combination of non-replicating virus (HVJ-E) and chemotherapy [bleomycin or cis-diamminedichloroplatinum (CDDP)] increased efficacy in murine models of colon and bladder cancers [78, 105].

Technologies for systemic administration and targeting for HVJ-E are under development. The HN protein of HVJ-E has hemagglutinating activity and causes agglutination and lysis of erythrocytes *in vitro*. Currently, inactivation of the HN protein, decreased expression of the HN protein, and “masking” with platelets is being developed for intravenous injection of HVJ-E. Targeting after the intravenous injection is also important for systemic delivery. The addition of transferrin, a single chain antibody, or platelets have been suggested as suitable modifiers for HVJ-E.

The selection of viruses, or viral strains for the preparation of non-replicating oncolytic viruses is also important for obtaining higher efficacy because the level of immune response is dependent on the selection of virus strains [106]. A number of replicating oncolytic viruses are currently under clinical development [35, 97]. Therefore, it may be feasible to select a suitable virus for therapeutic application from the oncolytic viruses that have been developed [106, 107]. The tropism and complement resistance features of each virus should be considered for targeting and stabilization in serum.

HVJ-E is the only non-replicating oncolytic virus currently undergoing clinical investigation. These studies establish a new strategy for the virotherapy and gene therapy fields. The primary goal is to provide a novel approach for improving cancer therapy.

## Summary

Conventional cancer therapies suffer from one paradox: although chemotherapeutic agents strongly kill cancer cells and decrease tumor volume, they simultaneously suppress the immune system. Chemotherapy frequently results in tumor relapse because residual cancer cells and cancer stem cells escape immune responses. In contrast, immune therapies including therapeutic cancer vaccines, effectively induce cancer immunity, but possess weak cytotoxic activity against cancer cells. Therefore, these treatments usually show weak efficacy. It has been reported that cancer is able to progress even after the activation and proliferation of cancer-specific cytotoxic T lymphocytes.

Virotherapy is predicted to become an alternative approach to obtain a model cancer therapy because it generally displays both oncolytic and immunostimulatory activities. However, the major drawback associated with current virotherapy is safety concerns. Virotherapy using a non-replicating virus is a new approach aimed at resolving safety issues. Thus, it is expected to become a novel concept for cancer therapy in the near future.

## Acknowledgements

We appreciate T. Yamazaki for helpful discussions. We also appreciate H. Ueda and S. Ishikawa for their excellent administrative supports.

This study was supported by the advanced research for medical products Mining Program of the National Institute of Biomedical Innovation (NIBIO).

## Author details

Toshihiro Nakajima<sup>1</sup>, Toshimitsu Itai<sup>1</sup>, Hiroshi Wada<sup>1</sup>, Toshie Yamauchi<sup>1</sup>, Eiji Kiyohara<sup>2,3</sup> and Yasufumi Kaneda<sup>2</sup>

<sup>1</sup> GenomIdea, Inc., Midorigaoka, Ikeda, Osaka, Japan

<sup>2</sup> Division of Gene Therapy Science, Department of Molecular Therapeutics, Graduate School of Medicine, Osaka University, Yamada-oka, Suita, Osaka, Japan

<sup>3</sup> Department of Dermatology, Graduate School of Medicine, Osaka University, Yamada-oka, Suita, Osaka, Japan

T. Nakajima is a CEO of GenomIdea. T. Itai, H. Wada, and T. Yamauchi are employees of GenomIdea. The remaining authors have no conflicts of interest.

## References

- [1] Southam CM. and Moore AE., Clinical studies of viruses as antineoplastic agents with particular reference to Egypt 101 virus., *Cancer* 1952; 5:1025-1034.
- [2] Bierman HR., Crike DM., Dod KS., Kelly KH., Petrakis NL., White LP. and Shimkin MD., Remissions in leukemia of childhood following acute infectious disease. Staphylococcus and Streptococcus, Varicella, and Feline Panleukopenia., *Cancer* 1953: 6:591-605.
- [3] Newman W and Southam CM. Virus treatment in advanced cancer; a pathological study of fifty-seven cases., *Cancer* 1954; 7:106-118.
- [4] Gallico E., The influence of viruses on the evolution of some malignant tumors., *J. Natl. Med. Assoc.* 1955 May; 47:158-161.
- [5] Barley SL., Possible effect of measles on leukemia., *Lancet* 1971 January; 1(7690) :136
- [6] Gross S., Measles and leukemia., *Lancet* 1971 February; 1(7695) :397-398.
- [7] Zygiert Z., Hodgkin's disease: remissions after measles., *Lancet.* 1971 March;1(7699): 593.
- [8] Bluming AZ, Ziegler JL., Regression of Burkitt's lymphoma in association with measles infection., *Lancet* 1971 July: 10;2(7715):105-106.
- [9] Mota HC. Infantile Hodgkin's disease: remission after measles., *Br Med J.* 1973;2: 421.
- [10] Ziegler JL., Spontaneous remission in Burkitt's lymphoma., *Natl Cancer Inst Monogr.* 1976;44: 61-65.
- [11] Taqi AM., Abdurrahman MB., Yakubu AM. and Fleming AF., Regression of Hodgkin's disease after measles., *Lancet.* 1981 May 16;1(8229):1112.
- [12] Schuepbach J. and Sauter C., Inverse correlation of antiviral antibody titers and the remission length in patients treated with viral oncolysate: a possible new prognostic sign in acute myelogenous leukemia., *Cancer.* 1981 Sep 15;48(6):1363-1367.
- [13] Wallack MK., Bash JA., McNally KR. and Leftheriotis E., Serological evaluation of melanoma patients in a phase I/II trial of vaccinia melanoma oncolysate (VMO) immunotherapy., *Cancer Detect Prev Suppl.* 1987;1:351-359.
- [14] Sauter C., Alberto P., Berchtold W., Fopp M., Gmür J., Gratwohl A., Imbach P., Maurice P., Obrecht P., Senn HJ., et al., Long-term results of two Swiss AML studies., *Haematol Blood Transfus.* 1987;30:38-44.
- [15] Cassel WA. and Murray DR., Treatment of stage II malignant melanoma patients with a Newcastle disease virus oncolysate., *Nat Immun Cell Growth Regul.* 1988;7(5-6):351-352.

- [16] Freedman RS, Bowen JM, Atkinson EN, Wallace S, Lotzová E, Silva E, Edwards CL, Delclos L, Scott W, Patenia B, et al., Randomized comparison of viral oncolysate plus radiation and radiation alone in uterine cervix carcinoma., *Am J Clin Oncol.* 1989 Jun;12(3):244-250.
- [17] Wallack MK., Sivanandham M., Balch CM., Urist MM., Bland KI., Murray D., Robinson WA., Flaherty LE., Richards JM., Bartolucci AA., et al., A phase III randomized, double-blind multiinstitutional trial of vaccinia melanoma oncolysate-active specific immunotherapy for patients with stage II melanoma., *Cancer.* 1995 Jan 1;75(1):34-42.
- [18] Batliwalla FM., Bateman BA., Serrano D., Murray D., Macphail S., Maino VC., Ansel JC., Gregersen PK. and Armstrong CA., A 15-year follow-up of AJCC stage III malignant melanoma patients treated postsurgically with Newcastle disease virus (NDV) oncolysate and determination of alterations in the CD8 T cell repertoire., *Mol Med.* 1998 Dec;4(12):783-794.
- [19] DeWeese TL., van der Poel H., Li S, Mikhak B., Drew R., Goemann M., Hamper U., DeJong R., Detorie N., Rodriguez R., Haulk T., DeMarzo AM., Piantadosi S., Yu DC., Chen Y., Henderson DR., Carducci MA., Nelson WG. and Simons JW., A phase I trial of CV706, a replication-competent, PSA selective oncolytic adenovirus, for the treatment of locally recurrent prostate cancer following radiation therapy., *Cancer Res.* 2001 Oct 15;61(20):7464-7472.
- [20] Kemeny N., Brown K., Covey A., Kim T., Bhargava A., Brody L., Guilfoyle B., Haag NP., Karrasch M., Glasschroeder B., Knoll A., Getrajdman G., Kowal KJ., Jarnagin WR. and Fong Y., Phase I, open-label, dose-escalating study of a genetically engineered herpes simplex virus, NV1020, in subjects with metastatic colorectal carcinoma to the liver., *Hum Gene Ther.* 2006 Dec;17(12):1214-1224.
- [21] Nakao A., Takeda S., Shimoyama S., Kasuya H., Kimata H., Teshigahara O., Sawaki M., Kikumori T., Kodera Y., Nagasaka T., Goshima F., Nishiyama Y. and Imai T., Clinical experiment of mutant herpes simplex virus HF10 therapy for cancer., *Curr Cancer Drug Targets.* 2007 Mar;7(2):169-174.
- [22] Fong Y., Kim T., Bhargava A., Schwartz L., Brown K., Brody L., Covey A., Karrasch M., Getrajdman G., Mescheder A., Jarnagin W. and Kemeny N., A herpes oncolytic virus can be delivered via the vasculature to produce biologic changes in human colorectal cancer., *Mol Ther.* 2009 Feb;17(2):389-394. Epub 2008 Nov 18.
- [23] Geevarghese SK., Geller DA., de Haan HA., Hörer M., Knoll AE., Mescheder A., Nemunaitis J., Reid TR., Sze DY., Tanabe KK. and Tawfik H., Phase I/II study of oncolytic herpes simplex virus NV1020 in patients with extensively pretreated refractory colorectal cancer metastatic to the liver., *Hum Gene Ther.* 2010 Sep;21(9):1119-1128.
- [24] Nakao A., Kasuya H., Sahin TT., Nomura N., Kanzaki A., Misawa M., Shirota T., Yamada S., Fujii T., Sugimoto H., Shikano T., Nomoto S., Takeda S., Kodera Y. and Nishiyama Y., A phase I dose-escalation clinical trial of intraoperative direct intratu-

- moral injection of HF10 oncolytic virus in non-resectable patients with advanced pancreatic cancer., *Cancer Gene Ther.* 2011 Mar;18(3):167-175. Epub 2010 Nov 19.
- [25] Hu JC., Coffin RS., Davis CJ., Graham NJ., Groves N., Guest PJ., Harrington KJ., James ND., Love CA., McNeish I., Medley LC., Michael A., Nutting CM., Pandha HS., Shorrock CA., Simpson J., Steiner J., Steven NM., Wright D. and Coombes RC., A phase I study of OncoVEXGM-CSF, a second-generation oncolytic herpes simplex virus expressing granulocyte macrophage colony-stimulating factor., *Clin Cancer Res.* 2006 Nov 15;12(22):6737-6747.
- [26] Park BH., Hwang T., Liu TC., Sze DY., Kim JS., Kwon HC., Oh SY., Han SY., Yoon JH., Hong SH., Moon A., Speth K., Park C., Ahn YJ., Daneshmand M., Rhee BG., Pinedo HM., Bell JC. and Kirn DH., Use of a targeted oncolytic poxvirus, JX-594, in patients with refractory primary or metastatic liver cancer: a phase I trial., *Lancet Oncol.* 2008 Jun;9(6):533-542. Epub 2008 May 19.
- [27] Li JL., Liu HL., Zhang XR., Xu JP., Hu WK., Liang M., Chen SY., Hu F. and Chu DT., A phase I trial of intratumoral administration of recombinant oncolytic adenovirus overexpressing HSP70 in advanced solid tumor patients., *Gene Ther.* 2009 Mar;16(3):376-382. Epub 2008 Dec 25.
- [28] Kaufman HL., Kim DW., DeRaffele G., Mitcham J., Coffin RS. and Kim-Schulze S., Local and distant immunity induced by intralesional vaccination with an oncolytic herpes virus encoding GM-CSF in patients with stage IIIc and IV melanoma., *Ann Surg Oncol.* 2010 Mar;17(3):718-30.
- [29] Nemunaitis J., Tong AW., Nemunaitis M., Senzer N., Phadke AP., Bedell C., Adams N., Zhang YA., Maples PB., Chen S., Pappen B., Burke J., Ichimaru D., Urata Y. and Fujiwara T., A phase I study of telomerase-specific replication competent oncolytic adenovirus (telomelysin) for various solid tumors., *Mol Ther.* 2010 Feb;18(2):429-434. Epub 2009 Nov 24.
- [30] Galanis E., Hartmann LC., Cliby WA., Long HJ., Peethambaram PP., Barrette BA., Kaur JS., Haluska PJ Jr., Aderca I., Zollman PJ., Sloan JA., Keeney G., Atherton PJ., Podratz KC., Dowdy SC., Stanhope CR., Wilson TO., Federspiel MJ., Peng KW. and Russell SJ., Phase I trial of intraperitoneal administration of an oncolytic measles virus strain engineered to express carcinoembryonic antigen for recurrent ovarian cancer., *Cancer Res.* 2010 Feb 1;70(3):875-882. Epub 2010 Jan 26.
- [31] Harrington KJ., Hingorani M., Tanay MA., Hickey J., Bhide SA., Clarke PM., Renouf LC., Thway K., Sibtain A., McNeish IA., Newbold KL., Goldsweig H., Coffin R. and Nutting CM., Phase I/II study of oncolytic HSV GM-CSF in combination with radiotherapy and cisplatin in untreated stage III/IV squamous cell cancer of the head and neck., *Clin Cancer Res.* 2010 Aug 1;16(15):4005-4015.
- [32] Hwang TH., Moon A., Burke J., Ribas A., Stephenson J., Breitbach CJ., Daneshmand M., De Silva N., Parato K., Diallo JS., Lee YS., Liu TC., Bell JC. and Kirn DH., A mechanistic proof-of-concept clinical trial with JX-594, a targeted multi-mechanistic onco-



- lytic poxvirus, in patients with metastatic melanoma., *Mol Ther.* 2011 Oct;19(10):1913-1922. doi: 10.1038/mt.2011.132. Epub 2011 Jul 19.
- [33] Hammill AM. and Conner J, Cripe TP., Oncolytic virotherapy reaches adolescence., *Pediatr Blood Cancer.* 2010 Dec 15;55(7):1253-1263. doi: 10.1002/pbc.22724. Epub 2010 Aug 23.
- [34] Seymour LW. and Thrasher AJ., Gene therapy matures in the clinic., *Nat Biotechnol.* 2012 Jul 10;30(7):588-593. doi: 10.1038/nbt.2290.
- [35] Russell SJ., Peng KW. and Bell JC., Oncolytic virotherapy., *Nat Biotechnol.* 2012 Jul 10;30(7):658-670.
- [36] Shi J. and Zheng D., An update on gene therapy in China., *Curr Opin Mol Ther.* 2009 Oct;11(5):547-553.
- [37] Bell J., Oncolytic viruses: an approved product on the horizon?, *Mol Ther.* 2010 Feb;18(2):233-234.
- [38] Vidal L., Pandha HS., Yap TA., White CL., Twigger K., Vile RG., Melcher A., Coffey M., Harrington KJ. and DeBono JS., A phase I study of intravenous oncolytic reovirus type 3 Dearing in patients with advanced cancer., *Clin Cancer Res.* 2008 Nov 1;14(21):7127-7137.
- [39] Kimball KJ., Preuss MA., Barnes MN., Wang M., Siegal GP., Wan W., Kuo H., Saddekni S., Stockard CR., Grizzle WE., Harris RD., Aurigemma R., Curiel DT. and Alvarez RD., A phase I study of a tropism-modified conditionally replicative adenovirus for recurrent malignant gynecologic diseases., *Clin Cancer Res.* 2010 Nov 1;16(21):5277-5287. Epub 2010 Oct 26.
- [40] Rudin CM., Poirier JT., Senzer NN., Stephenson J Jr., Loesch D., Burroughs KD., Reddy PS., Hann CL. and Hallenbeck PL., Phase I clinical study of Seneca Valley Virus (SVV-001), a replication-competent picornavirus, in advanced solid tumors with neuroendocrine features., *Clin Cancer Res.* 2011 Feb 15;17(4):888-895. Epub 2011 Feb 8.
- [41] Breitbach CJ., Burke J., Jonker D., Stephenson J., Haas AR., Chow LQ., Nieva J., Hwang TH., Moon A., Patt R., Pelusio A., Le Boeuf F., Burns J., Evgin L., De Silva N., Cvancic S., Robertson T., Je JE., Lee YS., Parato K., Diallo JS., Fenster A., Daneshmand M., Bell JC. and Kim DH., Intravenous delivery of a multi-mechanistic cancer-targeted oncolytic poxvirus in humans., *Nature.* 2011 Aug 31;477(7362):99-102. doi: 10.1038/nature10358.
- [42] Pesonen S., Diaconu I., Cerullo V., Escutenaire S., Raki M., Kangasniemi L., Nokisalmi P., Dotti G., Guse K., Laasonen L., Partanen K., Karli E., Haavisto E., Oksanen M., Karioja-Kallio A., Hannuksela P., Holm SL., Kauppinen S., Joensuu T., Kanerva A. and Hemminki A., Integrin targeted oncolytic adenoviruses Ad5-D24-RGD and Ad5-RGD-D24-GMCSF for treatment of patients with advanced chemotherapy refractory



- solid tumors., *Int J Cancer*. 2012 Apr 15;130(8):1937-1947. doi: 10.1002/ijc.26216. Epub 2011 Aug 8.
- [43] Peng KW., Ahmann GJ., Pham L., Greipp PR., Cattaneo R. and Russell SJ., Systemic therapy of myeloma xenografts by an attenuated measles virus., *Blood*. 2001 Oct 1;98(7):2002-7.
- [44] Kaneda Y., A non-replicating oncolytic vector as a novel therapeutic tool against cancer., *BMB Rep*. 2010 Dec;43(12):773-780.
- [45] Kurooka M. and Kaneda Y., Inactivated Sendai virus particles eradicate tumors by inducing immune responses through blocking regulatory T cells., *Cancer Res*. 2007 Jan 1;67(1):227-236.
- [46] Kuroya M., Ishida N. and Shiratori T., Newborn virus pneumonitis (type Sendai). II. The isolation of a new virus., *Tohoku J Exp Med*. 1953 Jun;58(1):62.
- [47] Okada Y. and Tadokoro J., The distribution of cell fusion capacity among several cell strains or cells caused by HVJ., *Exp Cell Res*. 1963 Dec;32:417-430.
- [48] Okada Y., Yamada K. and Tadokoro J., Effect of antiserum on the cell fusion reaction caused by HVJ., *Virology*. 1964 Mar;22:397-409.
- [49] Harris H. and Watkins JF., Hybrid cells derived from mouse and man: artificial heterokaryons of mammalian cells from different species., *Nature*. 1965 Feb 13;205:640-6..
- [50] . Harris H., Watkins JF., Campbell GL., Evans EP. and Ford CE., Mitosis in hybrid cells derived from mouse and man., *Nature*. 1965 Aug 7;207(997):606-608.
- [51] Köhler G. and Milstein C., Continuous cultures of fused cells secreting antibody of predefined specificity., *Nature*. 1975 Aug 7;256(5517):495-497.
- [52] Kaneda Y., Nakajima T., Nishikawa T., Yamamoto S., Ikegami H., Suzuki N., Nakamura H., Morishita R. and Kotani H., Hemagglutinating virus of Japan (HVJ) envelope vector as a versatile gene delivery system., *Mol Ther*. 2002 Aug;6(2):219-226.
- [53] Kaneda Y., Yamamoto S. and Nakajima T., Development of HVJ Envelope Vector and Its Application to Gene Therapy., *Adv Genet*. 2005;53PA:307-332.
- [54] Matsushima-Miyagi T., Hatano K., Nomura M., Li-Wen L., Nishikawa T., Saga K., Shimbo T. and Kaneda Y., TRAIL and Noxa are selectively up-regulated in prostate cancer cells downstream of the RIG-I/MAVS signaling pathway by non-replicating Sendai virus particles., *Clin Cancer Res*. 2012 Oct 17. [Epub ahead of print].
- [55] Phase I/II clinical trial of inactivated HVJ-E administration for advanced malignant melanoma patients., UMIN000002376 (2009/08/26 ), <https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&action=brows&type=summary&recptno=R000002889&language=E>

- [56] Phase I/II clinical trial to assess safety and efficacy of intratumoral and subcutaneous injection of HVJ-E to castration resistant prostate cancer patients. UMIN000006142(2011/09/01 ), <https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&action=brows&type=summary&recptno=R000007153&language=E>
- [57] Dey M., Ulasov IV., Tyler MA., Sonabend AM. and Lesniak MS., Cancer stem cells: the final frontier for glioma virotherapy., *Stem Cell Rev.* 2011 Mar;7(1):119-129.
- [58] Drake CG., Prostate cancer as a model for tumour immunotherapy., *Nat Rev Immunol.* 2010 Aug;10(8):580-593.
- [59] Sheikh NA., Petrylak D., Kantoff PW., Dela Rosa C., Stewart FP., Kuan LY., Whitmore JB., Trager JB., Poehlein CH., Frohlich MW. and Urdal DL., Sipuleucel-T immune parameters correlate with survival: an analysis of the randomized phase 3 clinical trials in men with castration-resistant prostate cancer., *Cancer Immunol Immunother.* 2012 Aug 3. doi 10.1007/s00262-012-1317-2
- [60] Friedman GK., Cassady KA., Beierle EA., Markert JM. and Gillespie GY., Targeting pediatric cancer stem cells with oncolytic virotherapy., *Pediatr Res.* 2012 Apr;71(4 Pt 2):500-510. doi: 10.1038/pr.2011.58. Epub 2012 Feb 15.
- [61] Marcato P., Dean CA., Giacomantonio CA. and Lee PW., Oncolytic reovirus effectively targets breast cancer stem cells., *Mol Ther.* 2009 Jun;17(6):972-979. Epub 2009 Mar 17.
- [62] Zhang X., Komaki R., Wang L., Fang B. and Chang JY., Treatment of radioresistant stem-like esophageal cancer cells by an apoptotic gene-armed, telomerase-specific oncolytic adenovirus., *Clin Cancer Res.* 2008 May 1;14(9):2813-2823.
- [63] Wakimoto H., Kesari S., Farrell CJ., Curry WT Jr., Zaupa C., Aghi M., Kuroda T., Stemmer-Rachamimov A., Shah K., Liu TC., Jeyaretna DS., Debasitis J., Pruszkak J., Martuza RL. and Rabkin SD., Human glioblastoma-derived cancer stem cells: establishment of invasive glioma models and treatment with oncolytic herpes simplex virus vectors., *Cancer Res.* 2009 Apr 15;69(8):3472-3481. Epub 2009 Apr 7.
- [64] Kanai R., Zaupa C., Sgubin D., Antoszczyk SJ., Martuza RL., Wakimoto H. and Rabkin SD., Effect of  $\gamma$ 34.5 deletions on oncolytic herpes simplex virus activity in brain tumors., *J Virol.* 2012 Apr;86(8):4420-4431. Epub 2012 Feb 15.
- [65] Alonso MM., Jiang H., Gomez-Manzano C. and Fueyo J., Targeting brain tumor stem cells with oncolytic adenoviruses., *Methods Mol Biol.* 2012;797:111-125.
- [66] Bao S., Wu Q., Li Z., Sathornsumetee S., Wang H., McLendon RE., Hjelmeland AB. and Rich JN., Targeting cancer stem cells through L1CAM suppresses glioma growth., *Cancer Res.* 2008 Aug 1;68(15):6043-6048.
- [67] Cheng L., Wu Q., Huang Z., Guryanova OA., Huang Q., Shou W., Rich JN. and Bao S., L1CAM regulates DNA damage checkpoint response of glioblastoma stem cells through NBS1., *EMBO J.* 2011 Mar 2;30(5):800-813. Epub 2011 Feb 4.

- [68] Shimamura M., Morishita R., Endoh M., Oshima K., Aoki M., Waguri S., Uchiyama Y. and Kaneda Y., HVJ-envelope vector for gene transfer into central nervous system., *Biochem Biophys Res Commun.* 2003 Jan 10;300(2):464-471.
- [69] Kaneda Y., New vector innovation for drug delivery: development of fusigenic non-viral particles., *Curr Drug Targets.* 2003 Nov;4(8):599-602.
- [70] Kotani H., Nakajima T., Lai S., Morishita R. and Kaneda Y., The HVJ-envelope as an innovative vector system for cardiovascular disease., *Curr Gene Ther.* 2004 Jun;4(2):183-194.
- [71] Kaneda Y., Applications of Hemagglutinating Virus of Japan in therapeutic delivery systems., *Expert Opin Drug Deliv.* 2008 Feb;5(2):221-233.
- [72] Kondo Y., Miyata K. and Kato F., Efficient delivery of antibody into living cells using hemagglutinating virus of Japan (HVJ) envelope., *Curr Protoc Immunol.* 2010 Apr;Chapter 2:Unit 2.16.1-12.
- [73] Kondo Y., Miyata K. and Kato F., Use of the hemagglutinating virus of Japan (HVJ) envelope as a versatile delivery system for nucleic acids and proteins to leukocytes in vitro., *Curr Protoc Immunol.* 2010 Apr;Chapter 10:Unit 10.17D.1-9.
- [74] Kaneda Y., HVJ liposomes and HVJ envelope vectors., *Cold Spring Harb Protoc.* 2011 Oct 1;2011(10):1281-1289. doi: 10.1101/pdb.prot065748.
- [75] Kaneda Y., Development of liposomes and pseudovirions with fusion activity for efficient gene delivery., *Curr Gene Ther.* 2011 Dec;11(6):434-441.
- [76] Fujihara A., Kurooka M., Miki T. and Kaneda Y., Intratumoral injection of inactivated Sendai virus particles elicits strong antitumor activity by enhancing local CXCL10 expression and systemic NK cell activation., *Cancer Immunol Immunother.* 2008 Jan;57(1):73-84. Epub 2007 Jun 30.
- [77] Kawaguchi Y., Miyamoto Y., Inoue T. and Kaneda Y., Efficient eradication of hormone-resistant human prostate cancers by inactivated Sendai virus particle., *Int J Cancer.* 2009 May 15;124(10):2478-2487.
- [78] Kawano H., Komaba S., Yamasaki T., Maeda M., Kimura Y., Maeda A. and Kaneda Y., New potential therapy for orthotopic bladder carcinoma by combining HVJ envelope with doxorubicin., *Cancer Chemother Pharmacol.* 2008 May;61(6):973-978. Epub 2007 Jul 26.
- [79] Tanaka M., Shimbo T., Kikuchi Y., Matsuda M. and Kaneda Y., Sterile alpha motif containing domain 9 is involved in death signaling of malignant glioma treated with inactivated Sendai virus particle (HVJ-E) or type I interferon., *Int J Cancer.* 2010 Apr 15;126(8):1982-1991.
- [80] Lorence RM., Reichard KW., Katubig BB., Reyes HM., Phuangsab A., Mitchell BR., Cascino CJ., Walter RJ. and Peeples ME., Complete regression of human neuroblastoma

ma xenografts in athymic mice after local Newcastle disease virus therapy., *J Natl Cancer Inst.* 1994 Aug 17;86(16):1228-1233.

- [81] Phuangsab A., Lorence RM., Reichard KW., Peebles ME. and Walter RJ., Newcastle disease virus therapy of human tumor xenografts: antitumor effects of local or systemic administration., *Cancer Lett.* 2001 Oct 22;172(1):27-36.
- [82] Kawai T and Akira S., Innate immune recognition of viral infection., *Nat Immunol.* 2006 Feb;7(2):131-137.
- [83] Takeuchi O. and Akira S., Innate immunity to virus infection., *Immunol Rev.* 2009 Jan;227(1):75-86.
- [84] Seth RB., Sun L. and Chen ZJ., Antiviral innate immunity pathways., *Cell Res.* 2006 Feb;16(2):141-147.
- [85] Saha SK. and Cheng G., TRAF3: a new regulator of type I interferons., *Cell Cycle.* 2006 Apr;5(8):804-807. Epub 2006 Apr 17.
- [86] Tang ED. and Wang CY., TRAF5 is a downstream target of MAVS in antiviral innate immune signaling., *PLoS One.* 2010 Feb 11;5(2):e9172.
- [87] Kübler K., Gehrke N., Riemann S., Böhnert V., Zillinger T., Hartmann E., Pölcher M., Rudlowski C., Kuhn W., Hartmann G. and Barchet W., Targeted activation of RNA helicase retinoic acid-inducible gene-I induces proimmunogenic apoptosis of human ovarian cancer cells., *Cancer Res.* 2010 Jul 1;70(13):5293-5304. Epub 2010 Jun 15.
- [88] Kübler K., tho Pesch C., Gehrke N., Riemann S., Dassler J., Coch C., Landsberg J., Wimmenauer V., Pölcher M., Rudlowski C., Tüting T., Kuhn W., Hartmann G. and Barchet W., Immunogenic cell death of human ovarian cancer cells induced by cytosolic poly(I:C) leads to myeloid cell maturation and activates NK cells., *Eur J Immunol.* 2011 Oct;41(10):3028-3039. doi: 10.1002/eji.201141555. Epub 2011 Aug 30.
- [89] Van DN., Roberts CF., Marion JD., Lépine S., Harikumar KB., Schreiter J., Dumur CI., Fang X., Spiegel S. and Bell JK., Innate immune agonist, dsRNA, induces apoptosis in ovarian cancer cells and enhances the potency of cytotoxic chemotherapeutics., *FASEB J.* 2012 Aug;26(8):3188-3198. Epub 2012 Apr 24.
- [90] Besch R., Poeck H., Hohenauer T., Senft D., Häcker G., Berking C., Hornung V., Endres S., Ruzicka T., Rothenfusser S. and Hartmann G., Proapoptotic signaling induced by RIG-I and MDA-5 results in type I interferon-independent apoptosis in human melanoma cells., *J Clin Invest.* 2009 Aug;119(8):2399-2411. doi: 10.1172/JCI37155. Epub 2009 Jul 20.
- [91] Nasu Y., Saika T., Ebara S., Kusaka N., Kaku H., Abarzua F., Manabe D., Thompson TC. and Kumon H., Suicide gene therapy with adenoviral delivery of HSV-tK gene for patients with local recurrence of prostate cancer after hormonal therapy., *Mol Ther.* 2007 Apr;15(4):834-840. Epub 2007 Feb 27.

- [92] Nishikawa H. and Sakaguchi S., Regulatory T cells in tumor immunity., *Int J Cancer*. 2010 Aug 15;127(4):759-767.
- [93] Jacobs JF., Nierkens S., Figdor CG., de Vries IJ., Adema GJ., Regulatory T cells in melanoma: the final hurdle towards effective immunotherapy?, *Lancet Oncol*. 2012 Jan; 13(1):e32-42.
- [94] Le DT. and Jaffee EM., Regulatory T-cell modulation using cyclophosphamide in vaccine approaches: a current perspective., *Cancer Res*. 2012 Jul 15;72(14):3439-3444. Epub 2012 Jul 3.
- [95] Suzuki H., Kurooka M., Hiroaki Y., Fujiyoshi Y., Kaneda Y., Sendai virus F glycoprotein induces IL-6 production in dendritic cells in a fusion-independent manner., *FEBS Lett*. 2008 Apr 16;582(9):1325-1329. Epub 2008 Mar 20.
- [96] Chang A. and Dutch RE., Paramyxovirus fusion and entry: multiple paths to a common end., *Viruses*. 2012 Apr;4(4):613-636. Epub 2012 Apr 19.
- [97] Galanis E., Cancer: Tumour-fighting virus homes in., *Nature*. 2011 Aug 31;477(7362):40-41. doi: 10.1038/477040a
- [98] Fujii H., Matsuyama A., Komoda H., Sasai M., Suzuki M., Asano T., Doki Y., Kirihaata M., Ono K., Tabata Y., Kaneda Y., Sawa Y. and Lee CM., Cationized gelatin-HVJ envelope with sodium borocaptate improved the BNCT efficacy for liver tumors in vivo., *Radiat Oncol*. 2011 Jan 20;6:8.
- [99] Sakai M., Fujimoto N., Ishii K., Nakamura H., Kaneda Y. and Awazu K., In vitro investigation of efficient photodynamic therapy using a nonviral vector; hemagglutinating virus of Japan envelope., *J Biomed Opt*. 2012 Jul;17(7):078002.S
- [100] Harrington KJ., Karapanagiotou EM., Roulstone V., Twigger KR., White CL., Vidal L., Beirne D., Prestwich R., Newbold K., Ahmed M., Thway K., Nutting CM., Coffey M., Harris D., Vile RG., Pandha HS., Debono JS. and Melcher AA., Two-stage phase I dose-escalation study of intratumoral reovirus type 3 dearing and palliative radiotherapy in patients with advanced cancers., *Clin Cancer Res*. 2010 Jun 1;16(11):3067-3077. Epub 2010 May 18.
- [101] Lolkema MP., Arkenau HT., Harrington K., Roxburgh P., Morrison R., Roulstone V., Twigger K., Coffey M., Mettinger K., Gill G., Evans TR. and de Bono JS., A phase I study of the combination of intravenous reovirus type 3 Dearing and gemcitabine in patients with advanced cancer., *Clin Cancer Res*. 2011 Feb 1;17(3):581-588. Epub 2010 Nov 24.
- [102] Kanai R., Rabkin SD., Yip S., Sgubin D., Zaupa CM., Hirose Y., Louis DN., Wakimoto H. and Martuza RL., Oncolytic virus-mediated manipulation of DNA damage responses: synergy with chemotherapy in killing glioblastoma stem cells. *J Natl Cancer Inst*. 2012 Jan 4;104(1):42-55. Epub 2011 Dec 15.



- [103] Hwang TH., Moon A., Burke J., Ribas A., Stephenson J., Breitbach CJ., Daneshmand M., De Silva N., Parato K., Diallo JS., Lee YS., Liu TC., Bell JC. and Kirn DH., A mechanistic proof-of-concept clinical trial with JX-594, a targeted multi-mechanistic oncolytic poxvirus, in patients with metastatic melanoma., *Mol Ther.* 2011 Oct;19(10):1913-1922. doi: 10.1038/mt.2011.132. Epub 2011 Jul 19.
- [104] Kaneda Y., Kiyohara E., Nakajima T. and Itai T., Gene Therapy of Melanoma Using Inactivated Sendai Virus Envelope Vector (HVJ-E) with Intrinsic Anti-Tumor Activities., *Viral Gene Therapy*, Ke Xu (Ed.), 2011, ISBN: 978-953-307-539-6 doi: 10.5772/17492, InTech, Available from: <http://www.intechopen.com/books/viral-gene-therapy/gene-therapy-of-melanoma-using-inactivated-sendai-virus-envelope-vector-hvj-e-with-intrinsic-anti-tu>
- [105] Kawano H., Komaba S., Kanamori T. and Kaneda Y., A new therapy for highly effective tumor eradication using HVJ-E combined with chemotherapy., *BMC Med.* 2007 Sep 21;5:28.
- [106] Apostolidis L., Schirmacher V. and Fournier P., Host mediated anti-tumor effect of oncolytic Newcastle disease virus after locoregional application., *Int J Oncol.* 2007 Nov;31(5):1009-1019.
- [107] Prestwich RJ., Ilett EJ., Errington F., Diaz RM., Steele LP., Kottke T., Thompson J., Galivo F., Harrington KJ., Pandha HS., Selby PJ., Vile RG.. and Melcher AA., Immune-mediated antitumor activity of reovirus is required for therapy and is independent of direct viral oncolysis and replication., *Clin Cancer Res.* 2009 Jul 1;15(13):4374-4381. Epub 2009 Jun 9.

IntechOpen



