Int J Hematol (2016) 104:29–41
DOI 10.1007/s12185-016-2031-1

Gene and virotherapy for hematological malignancies

Evidio Domingo-Musibay[1,3] · Masato Yamamoto[2,3]

Received: 15 May 2016 / Revised: 22 May 2016 / Accepted: 24 May 2016 / Published online: 11 June 2016

Abstract Recent years have seen a transformation in the treatment of hematological malignancies. Advances in gene therapy and molecular techniques and significant gains in computational abilities have supported the rapid development of safer and better tolerated therapies for many patients with hematologic cancers. In this review, we discuss novel applications of gene therapy, including immunomodulation and gene silencing, and report on the rise of oncolytic viruses for use in the treatment of malignancies arising in cells of the blood, lymph, and marrow. We discuss the relationship of the tropism of wildtype viruses and their oncolytic behavior as well as the tumoricidal and immunostimulatory properties of a number of attenuated and recombinant viruses currently in clinical development in countries around the world. While we have focused on promising virotherapy applications for future development, we also present a historical perspective and identify areas of potential clinical and regulatory practice change. We outline several of the virus systems being developed for applications in hematology, and summarize efficacy data in the context of ongoing or future human clinical testing. We also present the advantages and limitations of gene and virus therapy, including challenges and opportunities for improved treatment tolerability and outcomes for patients with hematologic malignancies.

Keywords Gene therapy · Virotherapy · Immunovirotherapy · Purging · Clinical trials

✉ Masato Yamamoto
yamam016@umn.edu

[1] Division of Hematology, Oncology and Transplantation, Department of Medicine, University of Minnesota, Minneapolis, MN, USA

[2] Division of Basic and Translational Research, Department of Surgery, University of Minnesota, MoosT 11-210, MMC195, 515 Delaware St SE, Minneapolis, MN 55455, USA

[3] Masonic Cancer Center, University of Minnesota, Minneapolis, MN, USA

Introduction

As of 2015, there were 1415 cancer gene therapy clinical protocols open and recruiting patients, representing 64 % of the total number of gene therapy clinical trials in the *Journal of Gene Medicine* Clinical Trial Database (http://www.abedia.com/wiley/index.html). In clinical practice today, gene therapy for the treatment of the hematologic cancers is still relatively uncommon. However, advances and refinements in DNA- and RNA-mediated gene transfer technology continue to spur development of potential new treatments for hematologic malignancies. Oncolytic virotherapy, which exploits the cytotoxic effect of viruses on cells for cancer treatment, is also emerging as a viable treatment option, particularly when used in combination with other immune-based approaches. In October 2015, the FDA-approved Amgen's recombinant herpes virus expressing GM-CSF (talimogene laherparepvec) for treatment of advanced unresectable melanoma, marking a pivotal moment in the evolution of gene therapy approaches for cancer therapy.

In this review, we trace the development of the field of gene therapy from the earliest recognition of DNA's ability to transfer functional characteristics and traits between cells, to the development of gene therapy applications for the treatment of genetic deficiencies and the treatment of hematologic malignancies, such as acute leukemia. We review the most promising gene and viral therapeutic

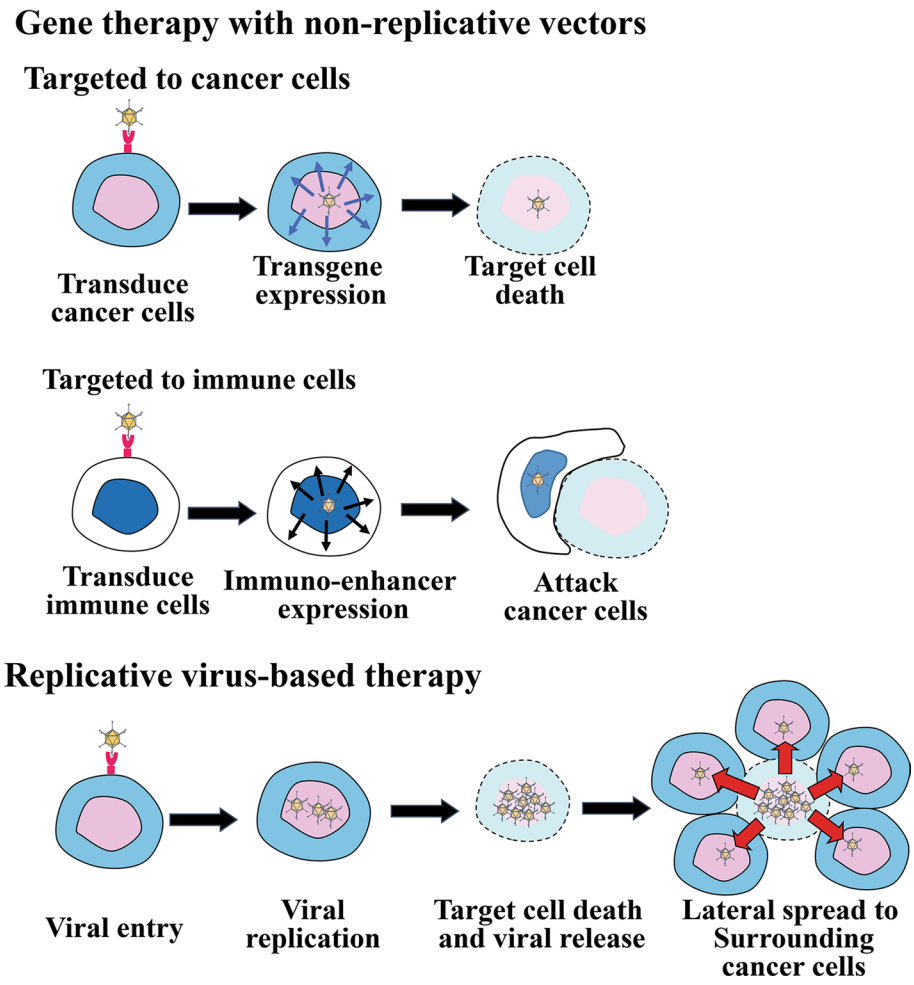

Fig. 1 Types of gene and virotherapies. Gene therapies using non-replicative vectors can be categorized into two types; one directly targets malignant cells, while the other targets the immune system and killing of cancer cells by immune cells. Virotherapy, by contrast, makes use of replicating viruses to kill malignant cells. After virus entry into target cells, the virus replicates within and kills its host. Progeny virions are released from the initially infected cells and subsequently spread and infect surrounding cells. Oncolytic viruses achieve this lateral spread in a manner specific to malignant cells

strategies now finding their way into clinical testing, and the new gene therapy approaches poised to have the greatest impact on the development of novel therapies for the hematologic malignancies (Fig. 1). Notably, gene therapy techniques that focus on chimeric antigen receptor (CAR) modified T cell therapy are described in a separate section.

Historical development of gene therapy

The ability of DNA to transform the physical characteristics of organisms was demonstrated in prokaryotes by Avery, MacLeod and McCarty as early as 1944, with their report of the conversion of unencapsulated pneumococci to fully encapsulated forms using "a highly polymerized, viscous form of desoxyribonucleic acid" [1]. Evidence of mammalian cells' ability to incorporate DNA was not experimentally demonstrated until 1961 [2]. However, it was not until 1971 that William Munyon and colleagues at Roswell Park Memorial Institute described the transfer of viral thymidine kinase enzyme activity in mammalian cells treated with UV-inactivated herpes simplex virus (HSV) [3]. This marked the first experimental demonstration of the introduction of non-native functional traits into mammalian cells through DNA transfer.

In the 1980s, advances in retroviral genetics and molecular biology gave rise to the idea of using retroviral vectors to directly insert genetic material into nuclear DNA [4]. Gene transfer technology created an entirely new field of research, and ultimately led to the first successful gene therapy treatment of a 4-year-old girl for adenosine deaminase (ADA) deficiency, an autosomally recessive disorder [5]. The ease with which blood is isolated and can be manipulated makes hematopoietic cells particularly good candidates for gene therapy applications [6, 7]. Various gene therapy strategies have been developed for the treatment of hematologic cancers and associated conditions. We will review the therapeutic use of cytokine and immunostimulatory gene therapy, RNA interference (RNAi), and suicide gene-based therapies for the treatment of hematologic malignancy.

Immunomodulatory gene therapy

Gene therapy-mediated modification of immune responses to malignant diseases is an area of intense investigation. In acute myeloid leukemia, ex vivo cytokine stimulation of leukemia cells with GM-CSF, IL-4, and either TNF-alpha or CD40 ligand promotes the differentiation of AML cells into dendritic cells, which then process tumor-associated antigens and stimulate autologous anti-leukemia responses [8, 9]. Similarly, tumor cells transduced by GM-CSF-expressing viruses can generate whole-cell tumor vaccines that produce immunostimulatory GM-CSF; animal studies have shown that such viruses are capable of producing extremely large quantities of GM-CSF [10]. The use of GM-CSF-expressing bystander lymphoma cells in a BALB/c model of A20 lymphoma prevented lymphoma progression, and achieved better outcomes than an equivalent dose of autologous tumor cells alone. HLA-negative CML cells have been engineered to express GM-CSF, mixed with irradiated patient-derived CML cells, and can be given as an intradermal vaccine to maintain deep remission [11].

Combination of the GVAX (GM-CSF-producing whole tumor cell vaccine) approach with innate immune activation has also recently gained increasing attention [12]. The Toll-like receptors (TLRs) are evolutionarily conserved pattern recognition molecules capable of sensing pathogenic molecular motifs expressed on invading microorganisms [13]. Both natural (i.e., LPS, CpG DNA, dsRNA) and synthetically formulated TLR agonists induce differential gene expression programs that activate evolutionarily conserved immune effector mechanisms in neutrophils [14], mast cells [15], and NK cells [16]. In a study of GVAX combined with a novel vaccine adjuvant and TLR4 agonist, glucoyranosyl lipid A (GLA), improved responses were seen. However, against the expectation, tumor antigen delivery by GVAX was not observed in draining lymph nodes. Instead, GLA induced in situ maturation and proliferation of antigen presenting cells (APCs), which subsequently entered draining lymphatics to induce effector T cell activation [17].

The GVAX approach has shown promise in early clinical trials. In a pilot study of 19 chronic myelogenous leukemia (CML) patients receiving imatinib mesylate (Gleevec) therapy in combination with a GM-CSF-expressing autologous tumor cell vaccine there were statistically significant improvements in complete molecular remissions and deep responses [11]. Patients had taken imatinib mesylate for a median of 3 years when they began receiving vaccine therapy, yet further reductions in transcript levels were observed in 13 of 19 (68 %) patients. Of the 13 patients with transcript decreases, 12 attained the lowest levels they had yet attained, and in 7 patients disease markers were no longer detectable by PCR.

A phase II trial of K562/GM-CSF (NCT01773395) versus placebo is currently underway to assess the potential of vaccine immunotherapy after allogenic stem cell transplantation for AML. K562/GM-CSF vaccine cells are HLA-negative AML cells transduced with GM-CSF expressing adenovirus, then irradiated and returned to the patient in a series of vaccinations. The primary endpoint of the study is 18-month progression-free survival (PFS). Secondary endpoints include overall survival (OS) and the rate of development of graft-versus-host disease (GVHD).

In a slightly different approach, other groups have attempted transfer of cytokine and immune costimulatory molecules. Enveloped virus-like particles (VLPs) decorated with functionally active cytokines retain the ability to produce biologic effects similar to the native human cytokines on which they are based [18]. Interleukin-2 (hIL-2), IL-4, and granulocyte–macrophage colony stimulating factor (GM-CSF) can be fused to exterior membrane surfaces via glycosylphosphatidylinositol (GPI) anchors. Virus-like particles decorated with T cell receptor/CD3 ligands have also shown the ability to activate antigen-specific T cells [19]. Vaccinia virus, a large, recombinant pox virus, has been designed to express a triad of B7-1(CD80), ICAM-1, and LFA-3 (TRICOM) costimulatory molecules for oncolysis and antitumor vaccination. TRICOM-Vaccinia infection of a patient's own chronic lymphocytic leukemia (CLL) cells activates autologous T cells in vitro. Immune responses against allogenic CLL cells appear more potent, highlighting the potential benefits of immune activation due to minor alloantigenicity [20]. Additional costimulatory molecules tested include interleukin-12 (IL-12) and B7-1 (CD80), which, when co-expressed from tricistronic retroviral and adenoviral vectors, led to high levels of IL-12 and CD80 cell surface expression in hematologic and solid tumor models [21, 22].

Future immunomodulatory efforts are likely to identify optimal cytokine and costimulatory signals to promote the stimulation of anti-tumor responses. Blockade of immune checkpoints will be pursued further as a therapeutic strategy, and is likely to potentiate cytokine and immune costimulatory approaches. Further development and refinement of GVAX approaches are needed before the promise of in situ and whole-cell tumor vaccination can be fully realized. A combination of gene and immunotherapy approaches is likely to be the most effective means of inducing durable remission in patients with hematologic malignancies.

RNA interference (RNAi) and gene silencing

The ability of small non-coding RNA to modulate gene expression in animal cells was first demonstrated in the roundworm *Caenorhabditis elegans* [23]. Later studies revealed that double-stranded RNA (dsRNA) is the most potent and preferred guide for sequence-specific targeting via classical Watson–Crick base pairing, which defines

target sequence specificity for several distinct small dsRNAs with the ability to target specific genes for silencing [24]. Endogenous regulatory microRNAs (miRNA) measure ~22 nucleotides and are generated from processing of long primary transcripts (pri-miRNAs) into stem-loop precursors of ~70 nucleotides (pre-miRNAs) by RNase III Drosha [25]. Vector overexpression of short hairpin RNA (shRNAs) similarly relies on Drosha processing both for its export into the cytoplasm and its gene silencing effects. Exogenous delivery of short interfering RNA (siRNA) bypasses the need for transduction and nuclear processing, but rapid degradation by ribonucleases limits systemic therapy applications, resulting in slow adoption in hematological applications.

Discovery of the pervasive regulatory functions of small RNA molecules in transcriptional gene silencing, epigenetic modification and chromatin structure, and chromosomal segregation provide new potential therapeutic applications for RNAi [26, 27]. Recent advances in chemical modification of RNA molecules, such as with 2′OMe RNA, extends siRNA stability from several minutes up to 24 h when exposed to serum ribonucleases [28]. Pegylated and lipid nanoparticle formulations of siRNA now enable conjugation with antibodies and targeting ligands, further improving biodistribution and tissue-targeting ability [29, 30]. Immunoliposomes coated with antibodies to dendritic cell (DC) surface antigens have been shown to effectively deliver CD40 siRNA to DCs, thereby silencing CD40 gene expression and reducing alloimmune activation [31]. Studies in a murine xenograft model system of mantle cell lymphoma (MCL) showed the ability of this approach to suppress levels of the pro-growth cyclin D1, typically overexpressed in MCL due to translocation of cyclin D1 to the immunoglobulin heavy (IgH) chain promoter. Targeting of MCL via an anti-CD38 antibody was specific for MCL cells and led to cell cycle arrest, improved survival, and bone marrow clearance [29]. Since CD38 is also present on the surface of CLL cells, we can look forward to further testing of the anti-CD38 approach in CLL.

Immunomodulatory approaches using RNAi have been studied in several cancers and inflammatory conditions, such as rheumatoid arthritis, in which silencing of TNF, IL1, IL6, and IL18 improves pathologic changes associated with the disease [32]. Broader application in hematologic and solid tumor malignancies is also gaining traction. Targeting of epigenetic and transcriptional regulation improves the potency of this approach [33]. Recently, RNAi mediated silencing of the MLL fusion protein (MLL-AF9) in precursor B cell ALL silenced the leukemogenic fusion gene and the associated downstream alterations driving the maturation arrest and malignant behavior of these cells [34]. siRNA targeting of transcription factors important in helper T cell development, such as GATA3 for Th1 cells and T-bet for Th2 cells, can also be used to correct aberrant cancer-related skewing of immune responses. Modulation of Th1 and Th2 cell subsets in mice with intraperitoneal siRNA against lineage transcription factors was shown to potentiate immune mediated tumor vaccination in vivo, independent of innate Interferon-mediated or anti-viral mechanisms [35]. Advances in the delivery of RNA therapies and in the understanding of diverse RNA regulatory functions will undoubtedly identify increasingly potent RNA targets for combination approaches.

Suicide gene therapy and GVHD

The use of therapeutic genes in hematologic malignancy has made heavy use of suicide genes to employ safety "off" switches in donor lymphocytes for stem cell transplantation of leukemia. Graft-versus-host disease (GVHD) is a serious complication of allogeneic stem cell transplantation, and the degree of HLA mismatch between donor and recipient increases risks for the disease [36]. Unfortunately, attempts to reduce the incidence and severity of GVHD by T cell depletion have increased relapse and engraftment failure [37]. Ex vivo manipulation and tagging of donor lymphocytes prior to infusion may allow for selective depletion of alloreactive cells in vivo, if the need arises. Gene transfer for induction of apoptosis (iCasp9) or conversion of prodrugs to specifically target alloreactive lymphocytes for destruction have been studied extensively [38]. The best studied system employs HSV-TK, the thymidine kinase from herpes simplex virus, which preferentially phosphorylates the nucleoside analog ganciclovir leading to DNA incorporation, interruption of cell division, and apoptosis of dividing and proliferating cells [39]. Site-directed mutagenesis of the HSV-TK active site (i.e. SR11, SR26, SR39) can increase gancyclovir and acyclovir binding affinity relative to natural thymidine substrate, reducing prodrug concentrations needed to induce suicide gene-mediated cell killing [40].

Chiara Bonini's group investigated transduction of donor lymphocytes with the retroviral vector SFCMM, expressing human low-affinity nerve growth factor (LANGF) as a fusion protein with the neomycin resistance cassette and HSV-TK (HSV-TK-NEO). Cell surface localization of the protein allows for cell sorting by LANGF, with positive selection yielding purity of preparations nearing 100 %. Of eight evaluable patients in the phase I study, three achieved complete remission on receiving TK-modified lymphocyte infusion after T cell-depleted HSCT [41]. In the follow-up TK007 phase 1/2 study, donor TK-modified lymphocytes infused after T cell-depleted HSCT led to engraftment in 22 of 28 patients with high-risk leukemia [42]. No prophylactic immunosuppression was used, although ten patients

ultimately required gancyclovir after developing GVHD symptoms. A randomized phase III study of haploidentical HSCT is currently evaluating use of HSV-TK donor lymphocyte infusion (DLI) in patients with high-risk acute leukemia (NCT00914628).

A cell cycle-independent suicide gene system called iCasp9 can similarly be introduced to express a chimeric fusion of caspase 9 (Casp9) death domain motifs fused to the human FK506-binding protein [43]. After ex vivo transduction of donor lymphocytes and infusion into the patient, signs of GVHD during the engraftment period can be treated with intravenous infusion of an inert drug (AP1903) to eliminate alloreactive lymphocytes. Binding of the AP1903 ligand to the chimeric fusion protein on modified lymphocytes leads to receptor dimerization and intracellular activation of the iCasp9 promolecule. This system has the advantage of using an otherwise bioinert molecule instead of ganciclovir, which can cause hematologic, gastrointestinal, and renal adverse effects. Since this suicide mechanism takes advantage of endogenous apoptotic signaling, and occurs throughout the cell cycle, cell killing is uniform and rapid [44]. Efficacy of DLI with iCasp9 suicide gene-modified T cells is being evaluated in a phase 1/2 trial in patients with leukemia, myelodysplastic syndrome, lymphoma, Hodgkins disease, and multiple myeloma receiving allogeneic PBSCT from HLA-matched (8/8) donors.

Another engineered system involves the truncated form of the epidermal growth factor receptor (EGFR), which can serve as a selection epitope for adoptively transferred cells, and allow for in vivo tracking and elimination of problematic cells using the therapeutic antibody Cetuximab, which results in antibody-dependent cytotoxicity and ablation of engineered cells [45]. Another promising construct, RQR8, encodes a compact 136-amino acid transmembrane protein, which can be recognized by the therapeutic monoclonal antibody Rituximab and therefore acts as marker gene and suicide gene [46]. Table 1 summarizes selected active gene therapy-based clinical trials for the treatment and management of hematological malignancy and its complications. Additional trials will follow given the rapid advances in this field.

Future suicide gene therapy applications are likely to address safety concerns of ex vivo modified adoptively transferred immune cells given potential for off-target immune toxicity. Early deaths seen with adoptive transfer of chimeric antigen receptor (CAR) T cells demonstrated the need for a safety "off" switch in misdirected cells [47]. Development of several technologies such as CRISPR- and TALEN-mediated genome editing, oncolytic virotherapy, drug delivery and increases in computational power will definitely transform the delivery of cancer treatment. Regulatory agencies will similarly need to continually reassess regulatory frameworks and requirements to keep up with emerging data, a crucial first step in promoting development of novel cancer therapies.

Oncolytic viruses and applications in hematological malignancies

Oncolytic viruses exploit the natural ability of viruses to infect and kill cells during the process of replication [48]. Many viruses have been developed for use in various malignancies [48, 49]. One of the earliest trials to assess the use of wildtype viruses for cancer in the 1950s used adenovirus for treatment of cervical cancer [50, 51]. These early efforts helped to develop adenoviruses as gene therapy vectors, and early advances in molecular biology and virology occurred in part because of the knowledge gained from this work. Later reports of paramyxoviruses causing spontaneous remissions in lymphoma patients surfaced in the 1970s and 1980s [52, 53].

Development of oncolytic viruses with potent tumoricidal effects has slowly shifted towards the rational engineering of viruses containing genetically engineered specificity elements that confer safety and cancer-specific replication (e.g., vaccinia virus, adenovirus). Arming of viruses with therapeutic and imaging transgenes has also allowed for the generation of replication-competent viruses we can track in vivo and use to modulate antitumor and antiviral immunity [54, 55]. The potential of oncolytic viruses to modulate immunity against cancer stems from natural immunostimulatory effects of viruses on the human immune system. It is now clear that viruses can promote cross-presentation of tumor-associated antigens released during viral infection; these antigens may then generate tumor-specific immunity [56]. The first FDA-approved oncolytic virus-based cancer treatment, talimogene laherparepvec (T-Vec), is a recombinant, attenuated herpes simplex virus expressing GM-CSF, which was shown to be safe in early clinical testing [57], and later showed evidence of immune cell infiltration into treated tumors and durable responses in cases of advanced unresectable cutaneous melanoma [58].

Hematologic malignancies pose therapeutic challenges for virotherapeutic approaches to therapy given evolution of protective immune mechanisms to limit viral systemic dissemination. Induction of cytokine storm responses from intravascular virus delivery poses risks of systemic inflammatory response syndrome (SIRS), multiorgan failure, and even death [59]. For this reason, the development of intralesional virotherapy approaches for solid tumors has seen more progress. Local delivery and outward spread of viral progeny along membrane surfaces is more ideally suited for use disrupting adherent tumor cells forming

Table 1 Current gene therapy trials using suicide genes, immunostimulation, and RNAi for hematologic malignancies

Gene therapy application	Trial identifier	Title	Phase	Conditions	Intervention	Expected completion
RNAi	NCT01961063	Gene therapy after frontline chemotherapy in treating patients with AIDS-related non-Hodgkin lymphoma	1	AIDS-related diffuse large cell lymphoma; AIDS-related diffuse mixed cell lymphoma; AIDS-related diffuse small cleaved cell lymphoma; AIDS-related immunoblastic large cell lymphoma; AIDS-related lymphoblastic lymphoma; AIDS-related small noncleaved cell lymphoma; HIV infection	Lentivirus vector rHIV7-shI-TAR-CCR5RZ-transduced hematopoietic progenitor cells	Jun 2031
	NCT02378922	Autologous transplantation and stem cell-based gene therapy with LVsh5/C46 (CAL-1), a dual anti-HIV lentiviral vector, for the treatment of HIV-associated lymphoma	1	AIDS-related Hodgkin lymphoma; AIDS-related non-Hodgkin lymphoma	Gene-modified hematopoietic stem cells with low CCR5 via RNAi and inhibititory for HIV-1 fusion via C46 antiviral peptide	Jun 2019
	NCT02493829	B7.1/IL-2 leukaemia cell vaccine for non-transplant AML RFU-SIN2-AML2 (NTX)	1	AML	AML cell vaccine	Sep 2021
Suicide gene therapy for GVHD	NCT01875237	A phase 1/2 trial evaluating treatment of emergent graft-versus-host disease (GvHD) with AP1903 after planned donor infusions (DLIs) of T cells genetically modified with the iCasp9 suicide gene in patients with hematologic malignancies	1, 2	Leukemia; myeloma; myeloproliferative diseases	Donor lymphocyte infusion (DLI), drug: AP1903	Dec 2018
	NCT02477878	Study of gene modified donor T cell infusion in patients with recurrent disease after allogeneic transplant	1	Acute leukemia; myelodysplasia; lymphoma; multiple myeloma; other high-risk hematologic malignancies eligible for stem cell transplantation per institutional standard	BPX-501 T cell infusions; AP1903 given after BPX-501 to prevent GvHD and preserve graft-versus tumor effect	Dec 2018
	NCT01744223	A phase 1/2 dose escalation study evaluating safety and feasibility of BPX-501 T cells after partially mismatched, related, T cell-depleted HSCT (hematopoietic stem cell transplant)	1, 2	AML; ALL; lymphoma	iCasp9: BPX-501 cells and AP1903	Dec 2018

Table 1 continued

Gene therapy application	Trial identifier	Title	Phase	Conditions	Intervention	Expected completion
	NCT02487459	A phase I/II safety study of planned BPX-501 T cell infusion after partially mismatched, related, TCR αβ + T cell-depleted HSCT in adults with advanced hematologic malignances at high risk for relapse	1, 2	High-risk ALL in 1st or subsequent CR; high-risk AML in 1st or subsequent CR; CML refractory to at least 2 TKIs or resistant mutations or progression to blast phase CML; intermediate-/high-risk MDS; HL or NHL:, relapsed or refractory; other high-risk hematologic malignancies otherwise eligible for stem cell transplantation without an HLA-matched donor or in need of a fast transplant	iCasp9: BPX-501 cells and AP1903	Oct 2018
Immune modulation	NCT01773395	Randomized placebo-controlled phase II trial of irradiated, adenovirus vector transferred GM-CSF secreting autologous leukemia cell vaccination (GVAX) versus placebo vaccination in patients with advanced MDS/AML after allogeneic hematopoietic stem cell transplantation	2	AML; MDS-RAEB not in remission	Adenovirus vector transferred GM-CSF secreting autologous leukemia cell vaccination (GVAX)	Jul 2019
Transplant related infection	NCT02276820	Most closely human leukocyte antigen (HLA)-matched adenovirus-specific T lymphocytes (Viralym-A)	1	Adenovirus infection	Viralym-A	Dec 2017
	NCT02108522	Multivirus-specific T cells for the treatment of virus infections after stem cell transplant	1	Infection	Multivirus-specific T cells	Mar 2018

nodules and masses. At one extreme, there is potential for rapid clearance of virus and ineffectual dosing or on the other extreme is the possibility of excessive immune activation, cytokine release, shock, and multiorgan failure upon intravascular administration. However, our understanding of viral genetics and cellular and humoral immunity has allowed for a more nuanced approach to systemic therapy using approaches in tumor antigen-directed viral retargeting, tissue and tumor cell specific replication, and therapeutic gene expression. Using modern tools we are able to overcome the presence of neutralizing antibodies to evaluate new targets, routes of administration (IV, subQ, inhaled, intralesional), and selectivity for malignant hematologic cells [60].

Strategy of oncolytic virus-based treatment of hematologic malignancies

Compared to solid tumors, which start as localized lesions, hematologic malignancies are more often regionally and distantly distributed given involvement of the hematologic and lymphatic systems. Local virus application is thus generally not a particularly feasible therapy for many hematological cancers, and therefore in vivo applications of oncolytic viruses need to be designed with systemic administration in mind. Combinations of chemotherapy and HSCT are an effective modality for hematological malignancies, and autologous transplantation is particularly important, given widespread use in the treatment of multiple myeloma and lymphoma [61–63].

Early virotherapy applications in the hematological malignancies included the concept of stem cell graft "purging" of residual cancer, in an attempt to reduce or eliminate minimal residual disease in the autograft. As opposed to in vivo application, in vitro experimentation provided evidence to support ex vivo clinical application in the hematologic malignancies. The selective and precise killing of tumor cells with systemic in vivo application best embodies the true clinical advantages of virotherapy, so we provide a brief historical overview of early virotherapy applications of purging of minimal residual disease (MRD) in stem cell grafts and describe modern viral retargeting and selectivity engineering to achieve successful in vivo application.

In vivo application of oncolytic viruses

In vivo applications are the most straightforward way to apply oncolytic viruses to hematological diseases. Hematologic diseases require systemic therapy, and the ease of access to peripheral blood may be an advantage in optimizing the functionality of systemically injected therapeutics. Historically, disease regression after naturally acquired viral infection has been reported in some hematological malignancies (e.g., regression of Hodgkin's lymphoma after measles [52]), and vaccine strains of these viruses have been tested in the clinic [64, 65]. The design of novel genetically engineered viruses is being pursued therapeutically in many fields, including the hematological malignancies. The biggest challenge underlying the use of gene and viral therapy in malignant hematology is the simultaneous achievement of two rather conflicting goals: (i) acquiring sufficient delivery of the therapeutics to the target malignant cells, and (ii) avoiding toxicity of the virus to non-target cells.

Purging

Hematopoietic stem cell transplantation combined with chemotherapy has been performed for hematological malignancies, and autologous HSCT is frequently performed in certain diseases with efficacy and safety, including no risk of graft-versus-host disease (GVHD) [61–63]. However, one potential drawback of using autologous stem cells is the risk of contamination of the stem cell graft with malignant cells [66], though most agree incomplete tumor eradication causes most if not all relapse. Ex vivo applications, such as purging, bypass the barrier of specific delivery of therapeutic viruses to intended target cells and mitigates potential in vivo toxicity after systemic administration. While autologous stem cell transplantation for AML has not been shown efficacious, autologous stem cell grafts can be purged of AML cells while leaving function and differentiation of $CD34^+$ HSCs intact [67]. Adenoviruses designed to express genes under control of the midkine promoter induce tumor-specific oncolysis of metastatic tumor cells within pediatric bone marrow stem cell grafts without harming normal hematopoietic cells [68]. However, a disadvantage of this approach is its absolute dependence on direct viral oncolysis for therapeutic benefit, and there are no indirect immune benefits, as seen with in vivo delivery.

Design for cancer selectivity

Replication of oncolytic viruses is ideally limited exclusively to the malignant cell; however, this requires sufficient contrast between target cells and bystander normal cells for the selective killing of tumor cells. In general, there are two major strategies for exploiting these inherent differences. One is selectivity of viral replication, and the other is selectivity of infection/binding.

Replication selectivity of the oncolytic viruses is based on either natural viral tropism or the design for preferential replication. In more detail, the interaction of viral

replication mechanism and the altered signaling in malignant cell results in inherent cancer tropism of the viruses. Incorporation of extrinsic regulatory elements into virus genomic organization, such as with use of tumor-specific promoters or viral mutations, allow for targeting based on distinct cellular differences between normal and malignant cells. Myxoma virus, for example, shows intrinsic selectivity for malignant cells primarily on the basis of constitutive activation of AKT signaling within malignant cells [69]. For some oncolytic viruses, however, selectivity mechanisms are still ambiguous and not fully defined. Other oncolytic viruses, such as vaccinia virus or adenovirus [68, 70] may be designed to have selectivity by incorporation of mutation or control elements. For example, adenovirus with midkine promoter elements shows strong cytotoxicity in purging of pediatric malignant cells in bone marrow, leaving normal cells intact [68].

Selectivity of viral infection/binding has great potential for increasing specificity of the cytocidal effects on malignant cell targets, but as a modality it remains underdeveloped. In theory, viral infection starts with the binding of the virus to its host receptor on the cell surface. For example, infection of oncolytic measles virus occurs through CD46 [71], which is overexpressed in solid and hematological malignancies [72, 73] including lymphoma [74]. However, since normal cells express low levels of CD46, genetic engineering has been used to create retargeted measles virus derivatives expressing single chain antibody fragments incorporated into the viral envelope to achieve a more selective infection profile [75]. These and other selectivity strategies can be and should be combined to enhance overall targeting specificity and minimize off-target viral cytotoxicity.

Virotherapy with wildtype or attenuated viruses

Many wildtype and attenuated viruses demonstrate intrinsic preferences for malignant cells. This reflects the compromised tumor cell's loss of normal innate defenses against viruses. Innate antiviral gene expression and cell signaling programs involving Interferon (IFN), dsRNA-dependent protein kinase (PKR), and other IFN-inducible genes are routinely aberrant in cancer cells [76]. Viruses showing enhanced replication in cancer cells or dependence on gene expression signatures typical of malignant transformed cells identify promising oncolytic viruses for treatment applications in blood and marrow malignancies.

Measles virus

Wildtype measles virus exhibits natural tropism for lymphocytes, macrophages, and dendritic cells, and binds via its cellular receptor, signaling lymphocyte activation molecule (SLAM), a membrane glycoprotein [77]. EBV-transformed B- cell lines have shown susceptibility to Measles, and complete regression of Burkitt's lymphoma with wildtype measles infection [52] has been reported.

The Edmonston vaccine strain of measles virus showing infection via CD46 expressed on the cell surface has been modified and several derivatives have been tested in human clinical trials [78]. In recent clinical trial testing for multiple myeloma, intravenous infusion of a measles derivative expressing the sodium iodide symporter (NIS) gene led to the first documented complete remission using this approach [79]. In this sense, measles virus is an interesting and promising candidate for applications in hematological malignancies, and refinements in virus retargeting using single chain antibody fragments against selectivity markers such as CD38 and EGFR [75] may increase specificity and efficacy without need of massive dose intensification.

Myxoma virus

Myxoma virus is a poxviridae virus, which causes myxomatosis in rabbits. Its replication cycle involves the AKT pathway and overactivation turns on viral replication [80]. Myxoma virus therefore has the ability to target a diversity of cancer cells dependent on AKT induced growth signaling, as has been shown in models of acute myeloid leukemia (AML) and multiple myeloma (MM). In AML, in which the FLT3-ITD leads to constitutive activation of AKT signaling [81], myxoma virus eliminates AML cells and has shown it can purge without affecting the CD34+ hematopoietic stem cell graft [60, 67, 82]. In multiple myeloma, myxoma virus was similarly effective in an ex vivo treatment model [83].

Reovirus

Reovirus is a double-strand RNA virus, the replication of which depends on activation of the dsRNA-dependent protein kinase (PKR), which is activated as a downstream event of K-RAS constitutive activation [84]. This virus also shows activity in ex vivo purging applications [85, 86].

Vesicular somatitis virus (VSV)

Vesicular somatitis virus is a single-strand RNA virus, belonging to the bullet-shaped family of rhabdoviridae. This virus attaches via the low density lipoprotein (LDL) cell surface receptor, though it has a natural preference for insects and domestic livestock. The virus is sensitive to the effects of IFN, and is highly dependent on defective type-I interferon (IFN) signaling for its replication, which is frequently observed in cancer cells [87].

Replication-competent VSV has potent cytocidal effects on acute leukemia cell lines [88], and UV-inactivated non-replicating VSV retains cytocidal properties and induces immunogenic cell death in multiple acute leukemia models [89]. In immunocompetent syngeneic mouse models of ALL, vaccination with an irradiated preparation of ex vivo rhabdovirus-infected leukemia cells induced protective immunity in 60 % of animals receiving adoptively transferred splenocytes from immunized donors [90].

Coxsackie virus

Coxsackie viruses are small non-enveloped positive-sense single stranded RNA viruses, in the family Picornaviridae. Like poliovirus, coxsackie virus is an enterovirus that naturally spreads via fecal-oral route. Coxsackie virus A21 (CVA21) shows strong cytocidal effect and selectivity for multiple myeloma cells [91], presumably due to host cell expression of the intracellular adhesion molecule, ICAM-I.

Other viruses

Parvovirus B19 [92] and sindbis [93] viruses have also been reported to exhibit oncolytic effects in hematologic malignancies, and further analyses for their clinical potential is needed.

Virotherapy with strategically designed viruses based on pathogenesis

Some viruses are more tolerant of genetic manipulations, and can be designed to incorporate a wide range of regulatory components in order to confer multiple layers of specificity and allow maximum safety and tailored selectivity.

Vaccinia virus

Vaccinia virus (VV) has been known as a very safe vaccine for small pox. Interestingly, the AS strain of vaccinia was applied in treatment of IgA multiple myeloma in a Japanese patient and exhibited remarkable IgA reduction without detectable adverse effect [64]. More recently, genetically engineered vaccinia, JX-594 (Jennerex Biotherapeutics) was generated by deleting the viral thymidine kinase for selective replication in high TK expressing cells, and expression of GM-CSF transgene for immunostimulation. This virus is reported to show very nice antitumoral effects after systemic injection [94], and it is now in worldwide phase III clinical trial testing for intratumoral delivery in hepatocellular carcinoma (clinicaltrials.gov). In this sense, VV can be genetically modified for target selectivity and therapeutic potency, and therefore has high potential for future development of in vivo approaches for treatment of hematological malignancies.

Adenovirus

Adenovirus has been used as a platform of oncolytic virus development for many years. Actually, this virus is one of the earliest viruses tested in humans as a cancer therapeutic and overall safety and tolerability was demonstrated in clinical trials for cervical cancer in the 1950s [50, 51]. Potent antitumor activity has been documented in vitro [95], as well as in studies of midkine promoter driven oncolytic adenoviruses for the eradication of metastatic cancer cells in bone marrow stem cell preparations [68]. Systemic delivery applications, and by extension use in the hematologic malignancies, has been impeded by the neutralizing antibodies and vector sequestration by the liver and reticuloendothelial system. Furthermore, hematopoietic cancer cells do not express the coxsackie adenovirus receptor (CAR). Recently, however, we have developed novel methods for retargeting adenovirus to alternative receptors [96]. The recent advances in targeting and more regolous laternations of the capsid structure (including hexon and penton-base modification) addressing the aforementioned problems are reopening a pathway for adenovirus-mediated gene therapy platforms against hematologic malignancies.

Other viruses

Given various other oncolytic viruses have shown promising effects in other tumor contexts (e.g., herpesvirus) [97, 98]), we expect their potential application more broadly into hematological malignancies will continue to be explored.

Summary

The field of oncolytic virotherapy is in an ascending phase in its historical development. Following the recent FDA approval of the recombinant herpes simplex virus (T-VEC, Amgen), the field is attracting increased attention. Amongst a variety of oncolytic viruses, successful application in the hematological malignancies has been limited. Recent advancements in vectorology have mitigated early difficulties with specific targeting for in vivo applications, but barriers to the systemic administration of gene and viral therapies remain, and have blunted the development of gene and viral therapy applications for hematologic cancers. The immunotherapeutic potential of oncolytic virotherapy applications, however, is only now beginning to be fully explored. We have only now begun to see the first combination therapies using oncolytics with chimeric antigen receptor T cell

therapy at American Society of Gene and Cell Therapy meeting in May 2016 [99, 100]. We may find that a combination of gene, virus and immunotherapy approaches comes to define the most efficacious and least toxic of the therapies for treatment of the hematologic malignancies.

Acknowledgments We thank Drs. Shernan Holtan and Daniel Weisdorf for their valuable input.

Compliance with ethical standards

Conflict of interest Dr. Yamamoto reports Grants from NIH R01CA196215 R01CA168448, during the conduct of the study; In addition, he has two patents pending related to the content of this article. Dr. Domingo-Musibay has nothing to disclose.

References

1. Avery OT, Macleod CM, McCarty M. Studies on the chemical nature of the substance inducing transformation of pneumococcal types: induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. J Exp Med. 1944;79(2):137–58.
2. Kay ER. Incorporation of deoxyribonucleic acid by mammalian cells in vitro. Nature. 1961;191:387–8.
3. Munyon W, et al. Transfer of thymidine kinase to thymidine kinaseless L cells by infection with ultraviolet-irradiated herpes simplex virus. J Virol. 1971;7(6):813–20.
4. Cone RD, Mulligan RC. High-efficiency gene transfer into mammalian cells: generation of helper-free recombinant retrovirus with broad mammalian host range. Proc Natl Acad Sci USA. 1984;81(20):6349–53.
5. Blaese RM, et al. T lymphocyte-directed gene therapy for ADA-SCID: initial trial results after 4 years. Science. 1995;270(5235):475–80.
6. Karlsson S. Treatment of genetic defects in hematopoietic cell function by gene transfer. Blood. 1991;78(10):2481–92.
7. Bregni M, et al. Human peripheral blood hematopoietic progenitors are optimal targets of retroviral-mediated gene transfer. Blood. 1992;80(6):1418–22.
8. Choudhury BA, et al. Dendritic cells derived in vitro from acute myelogenous leukemia cells stimulate autologous, antileukemic T-cell responses. Blood. 1999;93(3):780–6.
9. Cignetti A, et al. CD34(+) acute myeloid and lymphoid leukemic blasts can be induced to differentiate into dendritic cells. Blood. 1999;94(6):2048–55.
10. Serafini P, et al. High-dose granulocyte-macrophage colony-stimulating factor-producing vaccines impair the immune response through the recruitment of myeloid suppressor cells. Cancer Res. 2004;64(17):6337–43.
11. Smith BD, et al. *K562/GM-CSF* immunotherapy reduces tumor burden in chronic myeloid leukemia patients with residual disease on imatinib mesylate. Clin Cancer Res. 2010;16(1):338–47.
12. Kawai T, Akira S. Innate immune recognition of viral infection. Nat Immunol. 2006;7(2):131–7.
13. Medzhitov Ruslan AI. Toll-like receptor control of the adaptive immune responses. Nat Immun. 2004;5(10):987–95.
14. Hayashi F, Means TK, Luster AD. Toll-like receptors stimulate human neutrophil function. Blood. 2003;102(7):2660–9
15. Supajatura V, Ushio H, Nakao A, Akira S, Okumura K, Ra C, Ogawa H. Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity. J Clin Invest. 2002;109(10):1351–9
16. Brackett CM, et al. Toll-like receptor-5 agonist, entolimod, suppresses metastasis and induces immunity by stimulating an NK-dendritic-CD8+ T-cell axis. Proc Natl Acad Sci USA. 2016;113(7):E874–83.
17. Kadayakkara DK, Korrer MJ, Bulte JW, Levitsky HI. Paradoxical decrease in the capture and lymph node delivery of cancer vaccine antigen induced by a TLR4 agonist as visualized by dual-mode imaging. Cancer Res. 2015;75(1):51–61.
18. Kueng HJ, Leb VM, Haiderer D, Raposo G, Thery C, Derdak SV, Schmetterer KG, Neunkirchner A, Sillaber C, Seed B, Pickl WF. General strategy for decoration of enveloped viruses with functionally active lipid-modified cytokines. J Virol. 2007;81(16):8666–76
19. Derdak SV, et al. Direct stimulation of T lymphocytes by immunosomes: virus-like particles decorated with T cell receptor/CD3 ligands plus costimulatory molecules. Proc Natl Acad Sci USA. 2006;103(35):13144–9.
20. Palena C, et al. Potential approach to immunotherapy of chronic lymphocytic leukemia (CLL): enhanced immunogenicity of CLL cells via infection with vectors encoding for multiple costimulatory molecules. Blood. 2005;106(10):3515–23.
21. Wen XY, et al. Tricistronic viral vectors co-expressing interleukin-12 (1L-12) and CD80 (B7-1) for the immunotherapy of cancer: preclinical studies in myeloma. Cancer Gene Ther. 2001;8(5):361–70.
22. Lee YS, Kim JH, Choi KJ, Choi IK, Kim H, Cho S, Cho BC, Yun CO. Enhanced antitumor effect of oncolytic adenovirus expressing interleukin-12 and B7–1 in an immunocompetent murine model. Clin Cancer Res. 2006;12(19):5859–68
23. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993;75(5):843–54.
24. Fire A, et al. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature. 1998;391(6669):806–11.
25. Cleary DS, et al. Synthetic shRNAs as potent RNAi triggers. Nat Biotechnol. 2004;23(2):227–31.
26. Morris KV, et al. Small interfering RNA-induced transcriptional gene silencing in human cells. Science. 2004;305(5688):1289–92.
27. Weinberg MS, Morris KV. Transcriptional gene silencing in humans. Nucleic Acids Res. 2016. doi:10.1093/nar/gkw139
28. Choung S, et al. Chemical modification of siRNAs to improve serum stability without loss of efficacy. Biochem Biophys Res Commun. 2006;342(3):919–27.
29. Weinstein S, et al. Harnessing RNAi-based nanomedicines for therapeutic gene silencing in B-cell malignancies. Proc Natl Acad Sci USA. 2016;113(1):E16–22.
30. Sato Y, et al. Resolution of liver cirrhosis using vitamin A-coupled liposomes to deliver siRNA against a collagen-specific chaperone. Nat Biotechnol. 2008;26(4):431–42.
31. Zheng X, Vladau C, Zhang X, Suzuki M, Ichim TE, Zhang ZX, Li M, Carrier E, Garcia B, Jevnikar AM, Min WP. A novel in vivo siRNA delivery system specifically targeting dendritic cells and silencing CD40 genes for immunomodulation. Blood. 2009;113(12):2646–54
32. Khoury M, et al. Efficient new cationic liposome formulation for systemic delivery of small interfering RNA silencing tumor necrosis factor alpha in experimental arthritis. Arthritis Rheum. 2006;54(6):1867–77.
33. Han J, Kim D, Morris KV. Promoter-associated RNA is required for RNA-directed transcriptional gene silencing in human cells. Proc Natl Acad Sci USA. 2007;104(30):12422–7.

34. Fleischmann KK, et al. RNAi-mediated silencing of MLL-AF9 reveals leukemia-associated downstream targets and processes. Mol Cancer. 2014;13:27.
35. Tesniere A, et al. In vivo depletion of T lymphocyte-specific transcription factors by RNA interference. Cell Cycle. 2010;9(14):2830–5.
36. Loiseau P, et al. HLA association with hematopoietic stem cell transplantation outcome: the number of mismatches at HLA-A, -B, -C, -DRB1, or -DQB1 is strongly associated with overall survival. Biol Blood Marrow Transplant. 2007;13(8):965–74.
37. Marmont AM, et al. T-cell depletion of HLA-identical transplants in leukemia. Blood. 1991;78(8):2120–30.
38. Georgoudaki AM, Sutlu T, Alici E. Suicide gene therapy for graft-versus-host disease. Immunotherapy. 2010;2(4):521–37.
39. Borrelli E, et al. Targeting of an inducible toxic phenotype in animal cells. Proc Natl Acad Sci USA. 1988;85(20):7572–6.
40. Black ME, Kokoris MS, Sabo P. Herpes simplex virus-1 thymidine kinase mutants created by semi-random sequence mutagenesis improve prodrug-mediated tumor cell killing. Cancer Res. 2001;61(7):3022–6.
41. Bonini C, et al. HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. Science. 1997;276(5319):1719–24.
42. Fabio C, et al. Infusion of suicide-gene-engineered donor lymphocytes after family haploidentical haemopoietic stem-cell transplantation for leukaemia (the TK007 trial): a non-randomised phase I–II study. Lancet Oncol. 2009;10(5):489–500.
43. Straathof KC, Pulè MA, Yotnda P, Dotti G, Vanin EF, Brenner MK, Heslop HE, Spencer DM, Rooney CM. An inducible caspase 9 safety switch for T-cell therapy. Blood. 2005;105(11):4247–54
44. Di Stasi A, et al. Inducible apoptosis as a safety switch for adoptive cell therapy. N Engl J Med. 2011;365(18):1673–83.
45. Wang X, et al. A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells. Blood. 2011;118(5):1255–63.
46. Introna M, et al. Genetic modification of human T cells with CD20: a strategy to purify and lyse transduced cells with anti-CD20 antibodies. Hum Gene Ther. 2000;11(4):611–20.
47. Ertl HC, Zaia J, Rosenberg SA, June CH, Dotti G, Kahn J, Cooper LJ, Corrigan-Curay J, Strome SE. Considerations for the clinical application of chimeric antigen receptor T cells: observations from a recombinant DNA advisory committee symposium held 15 June 2010. Cancer Res. 2011;71(9):3175–81
48. Chiocca EA. Oncolytic viruses. Nat Rev Cancer. 2002;2(12):938–50.
49. Kelly E, Russell SJ. History of oncolytic viruses: genesis to genetic engineering. Mol Ther. 2007;15(4):651–9.
50. Huebner RJ, et al. Studies on the use of viruses in the treatment of carcinoma of the cervix. Cancer. 1956;9(6):1211–8.
51. Huebner RJ, et al. Studies of adenoidal–pharyngeal–conjunctival vaccines in volunteers. J Am Med Assoc. 1955;159(10):986–9.
52. Bluming AZ, Ziegler JL. Regression of Burkitt's lymphoma in association with measles infection. Lancet. 1971;2(7715):105–6.
53. Taqi AM, et al. Regression of Hodgkin's disease after measles. Lancet. 1981;1(8229):1112.
54. Shashkova EV, et al. Targeting interferon-alpha increases antitumor efficacy and reduces hepatotoxicity of E1A-mutated spread-enhanced oncolytic adenovirus. Mol Ther. 2007;15(3):598–607.
55. Kishimoto H, et al. In vivo imaging of lymph node metastasis with telomerase-specific replication-selective adenovirus. Nat Med. 2006;12(10):1213–9.
56. Prestwich RJ et al. Tumor infection by oncolytic reovirus primes adaptive antitumor immunity. 2008.
57. Bahlis NJ, et al. CD28-mediated regulation of multiple myeloma cell proliferation and survival. Blood. 2007;109(11):5002–10.
58. Andtbacka RH, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. J Clin Oncol. 2015;33(25):2780–8.
59. Marshall E. Gene therapy death prompts review of adenovirus vector. Science. 1999;286(5448):2244–5.
60. Bais S, et al. Oncolytic virotherapy for hematological malignancies. Adv Virol. 2012;2012:186512.
61. Dicke KA, et al. Autologous bone marrow transplantation in acute leukemia. Surv Synth Pathol Res. 1985;4(1):84–96.
62. Breems DA, Lowenberg B. Acute myeloid leukemia and the position of autologous stem cell transplantation. Semin Hematol. 2007;44(4):259–66.
63. Spellberg B, Schiller GJ. Autologous bone marrow transplantation in acute leukemia. Hematol Oncol Clin N Am. 1999;13(5):919–38.
64. Kawa A, Arakawa S. The effect of attenuated vaccinia virus AS strain on multiple myeloma; a case report. Jpn J Exp Med. 1987;57(1):79–81.
65. Lech PJ, Russell SJ. Use of attenuated paramyxoviruses for cancer therapy. Expert Rev Vaccines. 2010;9(11):1275–302.
66. Billett AL, Sallan SE. Autologous bone marrow transplantation in childhood acute lymphoid leukemia with use of purging. Am J Pediatr Hematol Oncol. 1993;15(2):162–8.
67. Kim M, et al. Myxoma virus targets primary human leukemic stem and progenitor cells while sparing normal hematopoietic stem and progenitor cells. Leukemia. 2009;23(12):2313–7.
68. Adachi Y, et al. A midkine promoter-based conditionally replicative adenovirus for treatment of pediatric solid tumors and bone marrow tumor purging. Cancer Res. 2001;61(21):7882–8.
69. Wang G, et al. Infection of human cancer cells with myxoma virus requires Akt activation via interaction with a viral ankyrin-repeat host range factor. Proc Natl Acad Sci USA. 2006;103(12):4640–5.
70. Yamamoto M, et al. Infectivity enhanced, cyclooxygenase-2 promoter-based conditionally replicative adenovirus for pancreatic cancer. Gastroenterology. 2003;125(4):1203–18.
71. Naniche D, et al. Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. J Virol. 1993;67(10):6025–32.
72. Domingo-Musibay E, et al. Measles Edmonston vaccine strain derivatives have potent oncolytic activity against osteosarcoma. Cancer Gene Ther. 2014;21(11):483–90.
73. Msaouel P, et al. Oncolytic measles virus strains as novel anticancer agents. Expert Opin Biol Ther. 2013;13(4):483–502.
74. Seya T, et al. Distribution of C3-step regulatory proteins of the complement system, CD35 (CR1), CD46 (MCP), and CD55 (DAF), in hematological malignancies. Leuk Lymphoma. 1994;12(5–6):395–400.
75. Nakamura T, et al. Rescue and propagation of fully retargeted oncolytic measles viruses. Nat Biotechnol. 2005;23(2):209–14.
76. Critchley-Thorne RJ, et al. Impaired interferon signaling is a common immune defect in human cancer. Proc Natl Acad Sci. 2009;106(22):9010–5.
77. Hashimoto K, Ono N, Tatsuo H, Minagawa H, Takeda M, Takeuchi K, Yanagi Y. SLAM (CD150)-independent measles virus entry as revealed by recombinant virus expressing green fluorescent protein. J Virol. 2002;76(13):6743–9
78. Russell SJ, Peng KW. Measles virus for cancer therapy. Curr Top Microbiol Immunol. 2009;330:213–41.
79. Arney K. Could measles cure cancer? Uh, not exactly…. UK: Cancer Research; 2014.

80. Chan WM, Rahman MM, McFadden G. Oncolytic myxoma virus: the path to clinic. Vaccine. 2013;31(39):4252–8.
81. Brandts CH, Sargin B, Rode M, Biermann C, Lindtner B, Schwäble J, Buerger H, Müller-Tidow C, Choudhary C, McMahon M, Berdel WE, Serve H. Constitutive activation of Akt by Flt3 internal tandem duplications is necessary for increased survival, proliferation, and myeloid transformation. Cancer Res. 2005;65(21):9643–50.
82. Rahman MM, et al. Oncolytic viral purging of leukemic hematopoietic stem and progenitor cells with Myxoma virus. Cytokine Growth Factor Rev. 2010;21(2–3):169–75.
83. Bartee E, et al. Selective purging of human multiple myeloma cells from autologous stem cell transplantation grafts using oncolytic myxoma virus. Biol Blood Marrow Transplant. 2012;18(10):1540–51.
84. Strong JE, et al. The molecular basis of viral oncolysis: usurpation of the Ras signaling pathway by reovirus. EMBO J. 1998;17(12):3351–62.
85. Thirukkumaran CM, et al. Reovirus as a successful ex vivo purging modality for multiple myeloma. Bone Marrow Transplant. 2014;49(1):80–6.
86. Thirukkumaran CM, et al. Reovirus oncolysis as a novel purging strategy for autologous stem cell transplantation. Blood. 2003;102(1):377–87.
87. Stojdl DF, et al. Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus. Nat Med. 2000;6(7):821–5.
88. Lichty BD, et al. Vesicular stomatitis virus: a potential therapeutic virus for the treatment of hematologic malignancy. Hum Gene Ther. 2004;15(9):821–31.
89. Batenchuk C, et al. Non-replicating rhabdovirus-derived particles (NRRPs) eradicate acute leukemia by direct cytolysis and induction of antitumor immunity. Blood Cancer J. 2013;3:e123.
90. Conrad DP, et al. Leukemia cell-rhabdovirus vaccine: personalized immunotherapy for acute lymphoblastic leukemia. Clin Cancer Res. 2013;19(14):3832–43.
91. Au GG, et al. Oncolytic Coxsackievirus A21 as a novel therapy for multiple myeloma. Br J Haematol. 2007;137(2):133–41.
92. Kishore J, Kishor D. Can Parvovirus B19 infection be naturally oncolytic: clinical findings raise such a possibility in leukaemic children. Indian J Med Res. 2014;139(6):952–3.
93. Suzme R, et al. Sindbis viral vectors target hematopoietic malignant cells. Cancer Gene Ther. 2012;19(11):757–66.
94. Kim JH, et al. Systemic armed oncolytic and immunologic therapy for cancer with JX-594, a targeted poxvirus expressing GM-CSF. Mol Ther. 2006;14(3):361–70.
95. Wei X, et al. Potent antitumor activity of the Ad5/11 chimeric oncolytic adenovirus combined with interleukin-24 for acute myeloid leukemia via induction of apoptosis. Oncol Rep. 2015;33(1):111–8.
96. Miura Y, et al. Infectivity-selective oncolytic adenovirus developed by high-throughput screening of adenovirus-formatted library. Mol Ther. 2013;21(1):139–48.
97. Hu JC, et al. A phase I study of OncoVEXGM-CSF, a second-generation oncolytic herpes simplex virus expressing granulocyte macrophage colony-stimulating factor. Clin Cancer Res. 2006;12(22):6737–47.
98. Esfandyari T, et al. Transcription factors down-stream of Ras as molecular indicators for targeting malignancies with oncolytic herpes virus. Mol Oncol. 2009;3(5–6):464–8.
99. Watanabe K, et al. Oncolytic adenovirus armed with cytokines enhances CAR-T cell efficacy in pancreatic tumor model. Mol Ther. 2016;24(S1):S205.
100. Shaw AR, et al. Armed-ad gene therapy expressing PDL1 minibody enhances the anti-tumor effect of adoptively transferred chimeric antigen receptor T-cells for solid tumor treatment. Mol Ther. 2016;24(S1):P204.