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Gene- and Viro- therapy for Hematological Malignancies

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Abstract

Treatment of the hematological malignancies has undergone recent transformation. Advances in gene therapy and molecular techniques, as well as significant gains in computational abilities have supported 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 to treat malignancies arising in cells of the blood, lymph, and marrow. We discuss the relationship of the tropism of wild-type viruses and their oncolytic behavior as well as tumoricidal and immunostimulatory properties of several attenuated and recombinant viruses in clinical development worldwide. 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. Several of the virus systems being developed for hematogic application are outlined and efficacy data is summarized and presented in the context of ongoing or future human clinical testing. Advantages and limitations of gene and virus therapy are presented to readers including challenges and opportunities to improve treatment tolerability and outcomes for patients with hematologic malignancy.

As of 2015, 1,415 cancer gene therapy clinical protocols, constituting 64% of the total number of gene therapy clinical trials, according to Journal of Gene Medicine Clinical Trial Database (http://www.abedia.com/wiley/index.html) were open and recruiting patients. In clinical practice today, gene therapy for the treatment of the hematologic cancers is still relatively uncommon. However, advancements and refinements in DNA and RNA mediated gene transfer technology continue to spur development of new potential treatments for the 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.

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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 genetic deficiencies and the treatment of hematologic malignancies such as acute leukemia. We review the most promising gene and viral therapeutic strategies currently finding their way into clinical testing, and the newest gene therapy approaches poised to have the biggest impact on the development of novel therapies for the hematologic malignancies. Notably, gene therapy techniques that focus on chimeric antigen receptor (CAR) modified T cell therapy are described in a separate dedicated chapter.

A. Gene therapy historical development

The ability of DNA to transform the physical characteristics of organisms was demonstrated in prokaryotes as early as 1944 by Avery, MacLeod and McCarty, with the report of 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 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 in 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 four-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.

1. 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 producing immunostimulatory GM-CSF, with the capability of producing excessively large quantities of GM-CSF seen in animal studies [10]. 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. 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, the expected increase GVAX tumor antigen delivery to draining lymph nodes was not observed. 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 also shown promising results in early clinical trials. In a pilot study of 19 chronic myelogenous leukemia (CML) patients receving imatinib mesylate (Gleevec) therapy in combination with a GM-CSF expressing autologous tumor cell vaccine statistically significant improvements in complete molecular remissions and deep responses were seen [11]. Patients had taken imatinib mesylate for a median of 3 years when they started 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 7 patients developed PCR undetectable disease.

A phase II trial is currently underway of K562/GM-CSF (NCT01773395) versus placebo 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 ability to activate antigen specific T cells [19]. The large, recombinant pox virus, Vaccinia 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 patient's own chronic lymphocytic leukemia (CLL) cells activates autologous T cells *in vitro*. Immune responses against allogenic CLL cells appear more potent; again highlighting 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 both 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 overshadow 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. Combination of gene and immunotherapy approaches are likely to be the most effective way to induce durable remissions for patients with hematologic malignancies.

2. 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 round worm *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 defined target sequence specificity for several distinct small dsRNAs with 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 rely on Drosha processing to be exported into the cytoplasm and exert 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 hours when exposed to serum ribonucleases [28]. Pegylated and lipid nanoparticle formulations of siRNA can now allow for 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, to silence CD40 gene expression and reduce alloimmune activation [31]. Studies in a murine xenograft model system of mantle cell lymphoma (MCL) demonstrated the ability to suppress levels of the pro-growth cyclinD1, 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 seen on the surface of CLL cells, testing of the anti-CD38 approach in CLL would be anticipated.

Immunomodulatory approaches with 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 gaining traction and targeting epigenetic and transcriptional regulation improves the potency of the 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]. Ongoing advances in delivery of RNA therapies and in the understanding diverse RNA regulatory functions will undoubtedly identify increasingly potent RNA targets for combination approaches.

3. Suicide gene therapy and GVHD

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]. 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. Unfortunately, attempts to reduce the incidence and severity of GVHD by T cell depletion increases relapse and engraftment failure [37]. Therefore, *ex vivo* manipulation and tagging of donor lymphocytes prior to infusion would allow for selective depletion of alloreactive cells *in vivo* only if the need arises. Gene transfer for induction of apoptosis (iCasp9) or conversion of prodrugs to specifically target alloreactive lymphocytes for destruction have been well studied [38]. The best studied system employs HSV-TK, the thymidine kinase from Herpes simplex virus, which preferentially phosphorylates the nucleoside analogue 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%. Among 8 evaluable patients in the phase I study, 3 patients achieved complete remission after receiving TK-modified lymphocyte infusion after a 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, though 10 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 the alloreactive lymphocytes. Binding of the AP1903 ligand to the chimeric fusion protein on modified lymphocytes leads to receptor dimerization and intracellular activation of the iCasp 9 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 also 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 patients 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 and therefore acts as marker gene and suicide gene, given its recognition by the therapeutic monoclonal antibody Rituximab [46]. Table 1 summarizes selected active gene therapy based clinical trials for treatment and management of hematological malignancy and its complications. Additional trials will certainly follow the rapid advances occurring within the 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 computational power will definitely transform how we approach 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 of the future.

B. Oncolytic Viruses and Applications in Hematological Malignancies

Oncolytic viruses exploit the natural ability of viruses to kill infected cells during the process of replication [48]. Many viruses have been developed for use in various malignancies [48, 49]. One of the earliest trials assessing the use of wild type 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 rational engineering of viruses containing genetically engineered specificity elements confering 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 viruses can promote cross presentation of tumor associated antigens released during viral infection, and 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 nodules and masses. On 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 rlease, shock, and multiorgan failure upon intravascular administration. However, an understanding of viral genetics as well as 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].

1. 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. Therefore, local virus application is generally not a particularly feasible therapy for many hematological cancers, and therefore i*n vivo* applications of oncolytic viruses need to be designed with systemic administration in mind. Combination chemotherapy and HSCT is an effective therapy 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” residual cancer. As opposed to the in vivo application”, in vitro experiments can easily provide evidence supporting ex vivo clinical application in hematologic malignancies. The selective and precise killing of tumor cells with systemic in vivo application best embodies the real clinical advantage of virotherapy.

a. 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 better accessibility of the blood circulation may work out to be an advantage for optimal 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.

b. Purging—HSCT 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]. 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 are 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, the disadvantage of the approach is an absolute dependence on direct viral oncolysis for therapeutic benefit and there are no indirect immune benefits seen as with *in vivo* delivery.

2. 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 to exploit some of 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 desing 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, and the 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]. Yet, for some oncolytic viruses selectivity mechanisms are ambiguous and still to be fully defined. Some other oncolytic viruses such as vaccinia virus or adenovirus[68, 70] are designed to have selectivity by incorporation of mutation or control elements. For example, adenovirus with midkine promoter shows strong cytotoxicity in purging of pediatric malignant cells in the 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 is still 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 thof retargeted measles virus deritivatives expressing single chain antibody fragments incorporated into the viral envelope can be used, and have a more selective infection profile[75]. These selectivity strategies can be and should be combined to enhance overall targeting specificity and minimize off target viral cytotoxicity.

3. Virotherapy with Wild Type or Attenuated Viruses

Many wild type 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 protein kinase R (PKR), and other IFN-inducible genes are routinely aberrantly functioning in cancer cells [76]. Viruses showing enhanced replication in cancer cells or dependence on a gene expression signatures typical of malignant transformed cells identify promising oncolytic viruses for treatment applications in blood and marrow malignancies.

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

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

b. 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, where the FLT3-ITD leads to constitutive activation of AKT signaling [81], myxoma virus eliminates AML cells and has shown it can purge autologous stem cell transplants 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].

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

d. Vesicular Somatitis Virus (VSV)—VSV is a single strand RNA virus, belongs to 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 has 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 could induce protective immunity in 60% of animals receiving adoptively transfered splenocytes from immunized donors [90].

e. Coxsackie virus—Coxsackie viruses are small nonenveloped positive-sense single stranded RNA viruses, in the family Picornaviridae. Like poliovirus, coxackie virus is an enterovirus that naturally spreads via fecal-oral route. Coxsackievirus 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.

f. 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.

4. Virotherapy with Strategically Designed Viruses Based on Pathogenesis

Some viruses more tolerant of genentic manipulations, 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 specificity.

a. 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 with 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 transfene 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 for 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.

b. Adenovirus—Adenovirus has been used as a platform of oncolytic virus development for some 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 midline 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 of retargeting adenovirus to alternative receptors[96]. The recent advances in targeting and more regolous laternations of the capsid structure addressing afore mentioned problems are reopening a pathway for adenovirus mediated gene therapy platforms against hematologic malignancies.

c. Other viruses—Considering various other oncolytic viruses have shown promising effects in other tumor contexts (e.g. herpesvirus)[97, 98]), their potential application more broadly into hematological malignancies is expected to be explored.

5. Summary

The field of oncolytic virotherapy is in an ascending phase in its historical development. Riding on the tails of the recent FDA approval of the recombinant herpes simplex virus (T-VEC, Amgen), the field is gathering high 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 the barriers to 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., For example, we just started to see the combination therapy of oncolytic with chimeric antigen receptor T-cell therapy at Americna Society of Gene and Cell Therapy meeting in May 2016[99, 100]. and we may find it is combination gene, virus and immunotherapy approaches that will come to define the most efficacious and least toxic of the therapies for treatment of the hematologic malignancies.

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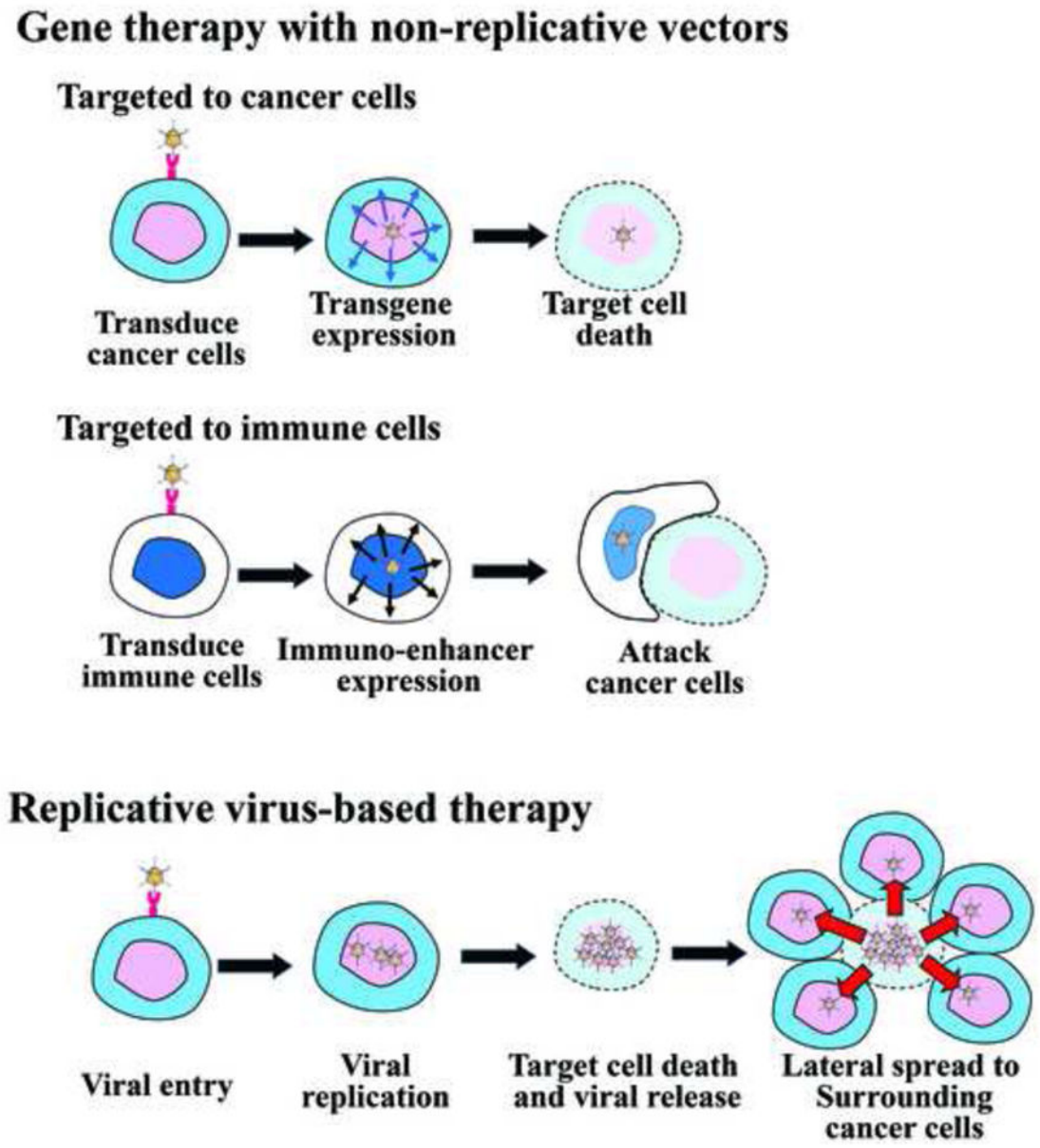

Figure 1. Types of Gene- and Viro- Therapies

Gene therapy with non-replicative vectors can be categorized into two types; one is to directly target malignant cells to kill them, and the other is to target the immune system to stimulate immune cell killing of cancer cells. Virotherapy is the technology of using replicatiing 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 initialy infected cells and subsequently spread and infect surrounding cells. Oncolytic viruses achieve this lateral spread in a manner specific to malignant cells.

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	n/a
	NCT02493829	B7.1/IL-2 Leukaemia Cell Vaccine for Non-Transplant AML RFUSIN2-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	n/a
	NCT00710892	CASPALLO: A Phase I Study Evaluating the Use of Allodepleted T Cells Transduced With Inducible Caspase 9 Suicide Gene After Haploidentical Stem Cell Transplantation	1	ALL; NHL; MDS; CML	Alloreactive T cell depletion with RFT5-dgA; iCasp9: AP1903	Jul 2026
	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
	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	iCasp9: BPX-501 cells and AP1903	

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Gene Therapy Application	Trial Identifier	Title	Phase	Conditions	Intervention	Expected Completion
				high-risk hematologic malignancies otherwise eligible for stem cell transplantation without an HLA matched donor or in need of a fast transplant		
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)	Jan 2033
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