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Oncolytic viral purging of leukemic hematopoietic stem and progenitor cells with Myxoma virus

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Abstract

High-dose chemotherapy and radiation followed by autologous blood and marrow transplantation (ABMT) has been extensively used for the treatment of certain cancers that are refractory to standard therapeutic regimes. However, a major challenge with ABMT for patients with hematologic malignancies is disease relapse, mainly due to either contamination with cancerous hematopoietic stem and progenitor cells (HSPCs) within the autograft or the persistence of residual therapy-resistant disease niches within the patient. Oncolytic viruses represent a promising therapeutic approach to prevent cancer relapse by eliminating tumor-initiating cells that contaminate the autograft. Here we summarize an ex vivo "purging" strategy with oncolytic myxoma virus (MYXV) to remove cancer-initiating cells from patient autografts prior to transplantation. MYXV, a novel oncolytic poxvirus with potent anti-cancer properties in a variety of in vivo tumor models, can specifically eliminate cancerous stem and progenitor cells from samples obtained from acute myelogenous leukemia (AML) patients, while sparing normal CD34+ hematopoietic stem and progenitor cells capable of rescuing hematopoiesis following high dose conditioning. We propose that a broader subset of patients with intractable hematologic malignancies who have failed standard therapy could become eligible for ABMT when the treatment schema is coupled with ex vivo oncolytic therapy.

Introduction

Hematologic malignancies such as acute myelogenous leukemia (AML), myelodysplastic syndromes, lymphomas and multiple myeloma together represent about 9.5% of the total new cancer cases diagnosed in the United States. High-dose chemotherapy and radiotherapy to eliminate cancer cells in the patient followed by autologous blood and marrow transplantation (ABMT) has been used as an effective therapeutic modality to treat hematologic malignancies and solid tumors including neuroblastoma (1,2). Success with ABMT relies on the autograft being free of cancer cells, to minimize the chance of disease recurrence. Generally, relapse of disease can be either caused by residual therapy-resistant cancer niches in the patient or by contaminating cancer cells within the autograft. Hematopoietic stem and progenitor cells

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(HSPCs) harvested from patients with advanced stage cancer often are contaminated with neoplastic cells, and thus can directly contribute to relapse following transplant (3).

In order to eliminate contaminating cancer cells from autografts prior to ABMT, several *ex vivo* cancer cell "purging" strategies have been tested over the last three decades. The main objective of purging is twofold: (a) to eliminate the number of contaminating cancer cells capable of initiating new tumor growth following transplant, and (b) to protect the normal HSPCs that are necessary for subsequent immune reconstitution. Different purging strategies have been evaluated, including *ex vivo* chemotherapy with drugs like mafosfamide and 4-hydroperoxycyclophosphamide (4,5), CD34+ (stem) cell enrichment using immunomagnetic selection (6), exploitation of immunotoxins or hybrid cytotoxic proteins designed to selectively kill cancer cells (7), immunomagnetic removal of tumor cells (8), and the use of oncolytic viruses (9). In many cases, these *ex vivo* purging methods were applied as combination therapy to make them more effective as an adjunct to ABMT. Examples of this include high-dose chemotherapy followed by CD34+ cell enrichment in the case of multiple myeloma (10), CD34 + cell enrichment followed by killing of residual cancer cells using immunotoxins (11), or a combination of low-dose chemotherapy and monoclonal antibodies (12). A summary of the different purging methods is presented in Table 1.

All of these purging methods have certain limitations and therefore an unmet medical need exists for effective methods that will lead to complete eradication of contaminating tumor-initiating cells within autografts prior to ABMT. For example, purging strategies that utilize high doses of chemotherapy might damage normal HSPCs, which may delay or prevent normal hematopoietic cell engraftment. On the other hand, antibody-based cell enrichment methods are technically demanding, expensive, and are frequently incomplete at cancer cell purging. The success of *ex vivo* purging methods depends on the number and type of contaminating tumor cells within the autograft, as well as the biology of the individual cancers from which they are derived (13). A successful *ex vivo* purging method has to be technically feasible for routine clinical practice and applicable for a wide variety of cancer cell types, including so-called "cancer stem cells" that might reside within the patient autograft (14). In this review, we highlight the use of oncolytic viruses, particularly Myxoma virus, as a novel *ex vivo* purging agent for hematologic malignancies.

Virus-mediated oncolysis and cancer therapy

The use of viruses to target and kill cancer cells dates back almost a century (15). However, the real potential of oncolytic virotherapy for different cancers has gained particular attention over the past 15 years, as some of the candidate oncolytic viruses have entered clinical trials, or, in the case of one derivative of adenovirus, have reached clinical practice. The major oncolytic viruses include adenovirus, Herpes simplex virus (HSV), measles, vaccinia, reovirus and Newcastle disease virus (NDV) (16). In some cases, oncolytic viruses have been tested in combination with other anti-cancer drugs. For example, one Adenovirus construct (ONYX-015) was more effective when combined with cisplatin and 5-fluorouracil chemotherapy for treatment of head and neck cancer (17). Eventually, the E1B gene-deleted variant of adenovirus H101 was approved in China for clinical use, after a phase III study (18). These experiences provide a clinical paradigm for other oncolytic viruses for their progression from preclinical animal models, through clinical trials, to approved usage for cancer treatment in patients. However, in many cases the efficacy in patients has been limited and there have been continued concerns about safety in some patients, particularly those that are immunosuppressed.

An oncolytic virus candidate must meet several criteria to become an effective cancer therapy (19). The virus should preferentially replicate within cancer cells, spread within tumor tissues,

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avoid infecting normal cells and tissues, be amenable for genetic manipulation to improve its safety and efficacy, and be nonpathogenic to humans and preferably other animal hosts as well. In some cases, oncolytic viruses have been genetically engineered to express immune stimulatory molecules within the tumor, for example Vaccinia JX-594 encodes human GM-CSF (20), or to express gene products that will enhance tumor cell killing, for example adenovirus Ad5-CD/TKrep-encoding a cytosine deaminase/thymine kinase (CD/TK) fusion gene (21). However, these genetic alterations in viruses should not result in toxic effects on normal somatic cells or tissues. Virus-mediated oncolysis as an *ex vivo* cancer cell purging strategy

Oncolytic viruses, with or without genetic modifications, have been tested for *ex vivo* elimination of cancer cells, as previous studies have indicated that normal bone marrow cells are particularly resistant to infection by many oncolytic virus candidates. A recombinant HSV-1 vector (JS1/34.5-/47-) with deletions of the ICP34.5 and ICP47 genes, permitting tumor-selective replication and enhanced antigen presentation in HSV-infected cells, was reported to successfully infect breast cancer cells, without affecting normal bone marrow cells *in vitro* (22). Similar results were found using another recombinant HSV-1 (HSV-1/G207) in an *in vitro* infection model (23). However, neither virus has been tested *in vivo* to evaluate the actual purging efficiency for ABMT. The purging potential of vesicular stomatitis virus (VSV), a negative strand RNA virus, was tested using interferon-sensitive VSV variants AV1, AV2 and a heat-resistant strain of VSV. These viruses were able to selectively eliminate a leukemic cell line with only minimal effects on the colony-forming ability of normal peripheral blood progenitor cells (PBPCs) (24). Another potential oncolytic virus, reovirus, a double-stranded RNA virus, has also been tested for its purging ability of cancer cells. In an *in vitro* purging strategy, reovirus eliminated myeloma cells or breast cancer cells without affecting the subsequent colony-forming potential of normal CD34+ stem cells (25,26). All of these oncolytic viruses have thus shown promise in the *in vitro* purging of tumor cells with minimal toxicity on normal CD34+ stem cells. However, none of these studies directly demonstrated *ex vivo* cancer cell purging, because the virus-treated cells were never tested for their *in vivo* engraftment potential in immunocompromised hosts.

One oncolytic DNA virus, an adenovirus derivative, and its genetically engineered variants, such as virus expressing cytosine deaminase (Ad-CD) or carrying wild-type p53 as a transgene, have been tested in several *ex vivo* purging models of breast, myeloma and neuroblastoma tumor cells. In addition to *ex vivo* purging, adenovirus had no toxic effects on engraftment of normal human hematopoietic cells when the virus-purged cells were subsequently injected in a non-obese diabetic/severe combined immunodeficient (NOD/scid) immunocompromised mouse model (27,28). Adenovirus has also been tested for *ex vivo* elimination of cancer cells in combination with other treatment methods. For example, breast cancer cells treated with Ad-CD and 5-fluorocytosine (5-FC) were unable to form tumors after transplantation in mice (29). Although these viruses were effective at *ex vivo* purging of several human cancer cell lines, they have not been tested for *ex vivo* purging of primary human cancer cells derived directly from patients.

Oncolytic poxviruses: Myxoma virus, a new oncolytic virus candidate

Poxviruses are all large, double-stranded DNA viruses that infect either invertebrates (*Entomopoxvirinae*) or vertebrates (*Chordopoxvirinae*) and replicate exclusively in the cytoplasm of infected cells. Vaccinia virus, the prototypic member of the genus *orthopoxvirus* and used as the vaccine to eradicate smallpox, has been tested in various cancer models, and attenuated variants are currently in different phases of clinical trial (30-33). Based on the success with smallpox vaccination, vaccinia and related poxviruses such as fowlpox and canarypox (genus *avipoxvirus*), which are genetically modified to express foreign/tumor

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antigens, have been used in many vaccine trials for protection against many infectious diseases and for treatment of established cancers (34,35).

Several inherent features of poxviruses make certain specific members attractive candidates for oncolytic virotherapy. Poxvirus DNA is never integrated into the host genome, as these viruses exclusively replicate in the cytoplasm of infected cells. Most poxviruses can infect a broad variety of cell types, at least *in vitro*, because no cell-specific binding/entry receptor has yet been described. But at the host organism level, infection is usually severely restricted to a small number of susceptible host species (36-38). The large genomes of poxviruses have made them amenable for genetic manipulation and use for vaccination, gene therapy, and cancer therapy (30). Poxviruses induce strong cellular and humoral immune responses that can be detected many decades later, as reported for vaccinia in smallpox eradication (39). A number of newly developed antiviral agents such as ST-246, cidofovir, and derivatives of cidofovir are available to treat orthopoxvirus infections, such as in cases of pathogenicity associated with generalized vaccinia infections (40). Over the last 40 years, both natural vaccine strains and genetically engineered vaccinia viruses (such as JX-594) have been tested in preclinical cancer models and in clinical trials (15,30).

Another poxvirus family member, called Myxoma virus (MYXV), has been recently developed as a novel oncolytic virus candidate (41). MYXV is the prototypic member of the *Leporipoxvirus* genus of *Poxviridae* family of viruses, which causes lethal disease myxomatosis in European rabbits (*Oryctolagus cuniculus*), but is completely restricted to rabbits as its host species. The South American tapeti (*Sylvilagus brasilensis*) and the North American brush rabbit (*Sylvilagus bruneii*) are the natural reservoirs for MYXV, where the virus causes only a benign infection (42). This suggests that even within related lagomorphs, host genetic variation can dictate viral pathogenicity. Other than lagomorphs, MYXV has not been reported to infect any other vertebrate species, including humans or mice (36,43). The genome of MYXV strain Lausanne has been sequenced, and many of the encoded immunomodulatory proteins that regulate tissue or cellular tropism of virus have been characterized (43-46).

At the cellular level, the rabbit-specific host restriction of MYXV replication has been studied *in vitro* and *in vivo*. For example, MYXV is unable to replicate in primary mouse fibroblasts because MYXV infection induces a robust type I IFN signaling pathway response that aborts the virus infection (47). In contrast, in primary human fibroblasts MYXV is permissive unless blocked by a synergistic anti-viral response mediated by type I IFN plus TNF (48). Since many human cell types, such as primary macrophages, robustly produce these two cytokines in response to MYXV infection, any tissues that possess cells capable of expressing IFN plus TNF can protect all the resident primary human cells in a paracrine-like fashion (48,49). In contrast, MYXV infection is fully permissive in a wide variety of different cancer cells derived from either mice or humans. *In vitro*, MYXV can selectively replicate within and kill the majority of human cancer cell lines listed in National Cancer Institute (NCI) reference (50). The mechanistic basis for this selective killing of cancer cells by MYXV is still being investigated, but two significant contributing factors are 1) the fact that most human cancer cells lack normal type I IFN and TNF synergy responses (51), and 2) many cancer cells possess excessive levels of activated Akt, which facilitates MYXV replication (52). It does seem clear that the selective cancer cell tropism of MYXV is essentially tied to the signaling environment of the cell and is independent of the origin of the tumor tissue from which the cancer cells are derived. Like cancer cells of human origin, many mouse cancer cells are also fully permissive to MYXV infection, even though mice (like humans) are not permissive hosts for the virus (53).

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At the molecular level, there are several indications that MYXV distinguishes permissive versus nonpermissive cells by virtue of their variant innate signaling capacities, and not by any receptor differences at the cell surfaces. For example, compared to normal primary human somatic cells, all human cancer cells tested to date lack the ability to induce the synergistic IFN + TNF signaling responses and are not able to induce a sufficiently robust anti-viral state to block MYXV replication completely (51). As mentioned above, other cellular signaling molecules have been shown to also govern the cancer cell tropism of MYXV in human cancer cells. For example, the activation state of cellular Akt/PKB, a serine threonine kinase that plays an important role in cell survival, proliferation and cell death, also regulates MYXV tropism in cancer cells (52). Akt signaling is frequently dysregulated in a wide spectrum of human cancer cells (54), and the increased level of cellular Akt activation is directly linked to MYXV tropism in these cells (52). Screening of several MYXV host-range gene knockout viruses in human cancer cell lines has identified one particular viral host range gene, called M-T5, that regulates the ability of MYXV to productively infect different human cancer cells (50). The viral M-T5 protein interacts directly with the cellular Akt, and this protein/protein interaction regulates MYXV permissiveness in a particular class of human cancer cells called Type II (52). Cancer cells that possess naturally high levels of endogenous activated Akt (called Type I cells) are permissive for MYXV replication, whether the virus expresses M-T5 or not. A second type of human cancer cells (Type II cells) also exist, where Akt is directly activated by M-T5, and MYXV can infect those cells only when M-T5 is expressed. Thus, wild-type MYXV is permissive in type II cancer cells, but the M-T5 knockout variant of MYXV is nonpermissive in these same cells. The third type of cancer cells (called Type III) possess only low or undetectable levels of Akt activation, and MYXV cannot infect these cells *in vitro* or cause the activation of Akt even in the presence of M-T5 (52,55). The requirement of activated Akt for productive MYXV infection is independent of origin of the tumor tissue (53).

Although MYXV infection in cancer cells depends on activated Akt, at least *in vitro*, it is still not known why MYXV specifically requires this hyper-phosphorylated form of Akt. The activated Akt-mediated signaling apparently makes transformed cancer cells a better environment for MYXV replication, possibly by either blocking key anti-viral pathways or by inducing cellular factors that are required for optimal MYXV replication. Importantly, however, the cellular Akt activation level can be manipulated with certain anti-cancer drugs that can allow MYXV replication even in nonpermissive cancer cells. For example, rapamycin increases MYXV tropism for certain cancer cells *in vitro* and *in vivo* (53,56,57). However, it is also possible that other cellular signaling pathways might also regulate MYXV tropism for cancer cells. In the case of vaccinia virus, viral replication and spread depends on activation of epidermal growth factor receptor (EGFR)-Ras signaling pathway, and in most cancer cells EGFR-Ras pathway is highly activated (58-60). In order to make vaccinia virus more effective as an oncolytic therapeutic, the virus has been genetically modified to delete viral genes that might compromise infected cell killing or to reduce viral virulence. Examples include vaccinia viruses that have been engineered to lack the viral thymidine kinase, vaccinia growth factor that are deleted for self-protective genes that block cellular apoptosis (like serpins), or anti-viral response (such as inhibitors of type I IFN) (30).

Preclinical animal models of MYXV oncolysis

The potential of MYXV as an oncolytic virus has been tested in several preclinical cancer models. *In vivo* efficacy of MYXV was first demonstrated in an orthotopic model of human glioblastoma in immunocompromised mice (61). The results demonstrated that intratumoral (i.t.) injection of MYXV decreased the xenografted tumor size and prolonged the survival of mice. However, the virus was able to effectively kill only the cells within the tumor where it was directly injected and did not migrate to an implanted tumor in the contralateral hemisphere (61). This could be because of type I IFN-mediated clearance of MYXV outside of the tumor

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and/or the lack of a permissive migratory cell to carry the virus to a distant tumor site. In another study, MYXV injected by I.T. route was able to successfully reduce the burden of xenografted human teratoid/rhabdoid tumors, a highly aggressive pediatric tumor implanted by subcutaneous (S.C.) injection in CD-1 nude mice (62). In this model, MYXV reduced the tumor and increased the survival of mice only when injected intracranially but not by intravenous (I.V.) injection. MYXV was also tested in a syngeneic metastatic B16F10 tumor model in immunocompetent mice. In this model, both I.T. and systemic administration of MYXV reduced lung tumor burden (53). Additionally, a combination therapy of MYXV plus rapamycin reduced both size and number of lung metastases (53). In another study, combination therapy with rapamycin plus MYXV also increased the *in vivo* oncolysis in an orthotopic medulloblastoma tumor model, the most common malignant brain tumor in children. This combination therapy reduced spinal cord and ventricle metastases in a xenograft mouse model (57). All these studies clearly suggest that MYXV has the ability to target and kill cancer cells *in situ* even without any genetic manipulation of the virus and without exhibiting any detectable toxicity to the animals. Also, MYXV did not replicate within any noncancerous host tissues, even following systemic injection into severely immunocompromised mice.

Purging of leukemic stem and progenitor cells with Myxoma virus

Apart from its natural rabbit host, MYXV has never been isolated or shown to replicate in humans, non-human primates or any other animal species outside of lagomorphs. The safety record of MYXV can be supported by the fact that even after its deliberate release in Australia in the 1950's (or later in Europe) to control the feral rabbit population, there is no report so far that the released virus caused any disease in humans or in any other animal species other than rabbits (63). Another advantage with MYXV is that, like other DNA viruses, genetic manipulation is possible by insertion of multiple therapeutic transgenes in the large genome of MYXV. Additionally, targeted deletions of MYXV virulence genes can render the virus nonpathogenic even for rabbits, while leaving its ability to infect and kill human cancer cells intact (64).

The inherent ability of MYXV to selectively target cancer cells and spare normal cells makes it a suitable oncolytic virus candidate for *ex vivo* purging of human cancer cells prior to autografts. The advantage of this specific *ex vivo* infection strategy for ABMT is that the protocol can be modified to ensure that every potential cancer cell within an autograft is exposed to virus, setting up the possibility of infecting and eliminating contaminating cancer cells in the graft. The potential exploitation of MYXV for purging of hematologic cancers has recently been tested with primary human AML cells and compared to normal CD34+ HSPCs. The purpose of this study was to investigate 1) whether MYXV infection *ex vivo* could purge AML cells and prevent their engraftment into immunodeficient mice *in vivo*, and 2) to determine if MYXV treatment had any adverse effect on normal human donor HSPC functions (65). AML is a hematologic malignancy characterized by uncontrolled proliferation and accumulation of clonal neoplastic cells due to mutations which occur in response to various cytogenetic and microenvironmental abnormalities. AML accounts for approximately 80% of all adult leukemias and its overall incidence is increasing over the last 15-20 years. Certain types of AML are high risk for relapse and death due to resistance of conventional chemotherapy (66-68).

In this study, MYXV safety with normal human donor CD34+ HSPCs was tested *in vitro* and *in vivo*. MYXV did not infect or alter the differentiation functions of virus-treated CD34+ HSPC populations, even at a high multiplicity of infection (MOI) of 10. As tested by an *in vitro* colony-forming cell (CFC) assay, MYXV-treated normal HSPCs formed all the expected types of leukocyte colonies similar to the mock-treated CD34+ control HSPCs (65). In contrast to the normal HSPCs, primary leukemic AML cells were highly infected by MYXV when

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tested *in vitro*. It has been reported that PI3K/Akt signaling is frequently activated in AML patient blasts, which contribute to the proliferation, survival and drug resistance of these cells (69). In the case of AML, the activation of PI3K/Akt signaling is probably due to mutations in FLT3, Ras or c-Kit. More importantly, MYXV productively infected AML cells *in vitro*, and the infected cells lost their ability to form colonies of leukemic blast cells (65) .

The oncolytic purging potential of MYXV was also tested *in vivo* using an immunocompromised mouse model. The safety and ability of normal human bone marrow-derived HSPCs (CD34+ selected cells) to engraft in the bone marrow was tested in severely immunocompromised NOD/scid/IL2-R□ $^{-/-}$ (NOG) mice. When these cells were treated with MYXV *ex vivo* and transplanted in sublethally irradiated NOG mice, their engraftment property remained unchanged, as confirmed by analysis of mouse bone marrow for the engraftment of human CD45+/HLA-abc+ cells (65). The level of normal human cell engraftment in the transplanted NOG mice was essentially the same in the case of mock- and MYXV-treated cells. These results confirmed that both *in vitro* and *in vivo* MYXV does not alter the engraftment properties of normal human HSPCs. The use of NOG mice and absence of any toxicity after analyzing different tissues from mice also confirmed the safety of MYXV, even in the immunocompromised animals.

In vivo MYXV purging efficacy was then tested using primary AML cells that possess a diagnostic FLT3 internal tandem duplication (ITD). The MYXV-treated AML FLT3 ITD+ mononuclear cells were transplanted in sublethally irradiated NOG mice. Analysis of BM samples for the presence of human CD45+/HLA-abc+ engraftment by FACS analysis demonstrated that MYXV was able to efficiently purge the leukemic cells, as 90% of the transplanted mice had no detectable engraftment (65). Conversely, mock-treated AML samples demonstrated robust leukemia engraftment in all transplanted mice. This suggests that MYXV is a promising oncolytic virus candidate for *ex vivo* purging, as it is safe for normal HSPCs and successfully eliminates leukemic cells from patient autografts before transplant. Future studies with this *ex vivo* purging protocol using MYXV will next focus on patient HSPC donor samples with detectable levels of cancerous cells that can be tracked and distinguished from their normal HSPC counterparts following engraftment.

Concluding remarks

Oncolytic viruses have shown great promise for the treatment of certain human tumors, but their potential as anti-cancer therapeutics is only beginning to be exploited. Although oncolytic viruses preferentially infect cancer cells compared to normal somatic cells, they might be even more effective for certain specific procedures where delivery of the virus to the target cancer cells can be rigorously controlled, such as *ex vivo* purging of cancer cells that contaminate autografts used for ABMT. Among the oncolytic poxviruses, MYXV has great potential because of its natural ability to selectively kill cancer cells, the absence of deleterious effects on normal/stem cell functions, and lack of toxicity, as demonstrated using even extremely immunocompromised animal models. *Ex vivo* purging of hematologic cancer cells using various oncolytic viruses has been tested by different groups in the past, but none of these studies have been extended to include *in vivo* engraftment studies with primary patient cancer cells, as reported here. In this *ex vivo* delivery strategy, one major concern is the safety of these viruses in humans, particularly for severely immunocompromised patients. MYXV could be an ideal candidate for both *ex vivo* and *in vivo* purging of cancer cells that contaminate autografts or reside in patient tissue niches. In the future, MYXV host range mutants generated by the targeted deletion of viral genes, like M063KO and M135KO, can also be tested for purging, as they are even safer for rabbits and to some extent may even possess superior oncolytic properties (64). Our future goal would be production of clinical grade MYXV that can be exploited for human trials for this promising new therapeutic.

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Biography

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and book chapters in the areas of stem cell engineering and cancer biology and has presented his research at several international conferences.

Christopher R. Cogle, MD Dr. Cogle is an Assistant Professor of Medicine at the University of Florida, Division of Hematology/Oncology, and is the Research Director of the Shands Hospital Stem Cell Laboratory. He is recognized for his adult hemangioblast translational research, with peer-reviewed grant support from the NIH, Florida Department of Health and Leukemia Lymphoma Society. Dr. Cogle's clinical expertise is in leukemias, myelodysplastic syndromes and blood & marrow transplantation. His laboratory focus is in human hemangioblast activity of normal and malignant adult stem and progenitor cells. His translational research focus is in translating hemangioblast therapies into the clinic. Dr. Cogle's lab uses pre-clinical animal models of human hematologic malignancies to study dependent

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pathways of hematologic malignancies. He has translated these discoveries into phase I and II clinical trials testing novel agents for treatment of leukemia and myelodysplastic syndromes.

Grant McFadden, Ph.D. Dr. McFadden is a professor in the Department of Molecular Genetics and Microbiology, University of Florida. He received his Ph.D. from McGill University in Montreal, Canada, in 1975. Dr. McFadden held several academic positions in Canada until 2006, when he relocated to the University of Florida, USA. His laboratory studies how viruses interact with the host immune system, in particular the mechanisms that determine the tropism and host range of poxviruses. The lab is also developing specific poxviruses as novel virotherapy candidates for the oncolytic treatment of human cancers. The lab has investigated the molecular basis for the host and cellular tropism specificity of poxviruses, using the myxoma virus model system as a novel platform for replication-restricted vaccine vectors and as an oncolytic virus. His lab also studies how viruses in general can occasionally leap from a long-term evolutionary host species to cause zoonotic infections in humans. The

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McFadden lab is also investigating whether myxoma virus can selectively infect and kill primary human cancer stem cells but leave normal human stem cells untouched.

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Table 1
Purging strategies to eliminate cancer cells from graft samples

Method of purging	Example of purging agents	Tumor type	Reference
Ex vivo			
Chemotherapy	4-hydroperoxycyclophosphamide (4-HC) and mafosfamide	Breast cancer	(70)
Monoclonal antibodies	Alemtuzumab, Rituximab	Chronic lymphocytic leukemia (CLL), B-cell lymphoma	(71)
Positive hematopoietic stem cell (CD34+) selection	Immunomagnetic enrichment/selection	B cell non-Hodgkin's lymphoma	(72)
Photodynamic purging process	Rhodamine	Depletion of T cells in allogenic stem cell transplant	(73)
Immunotoxins	Heregulin (HRG)-Pseudomonas exotoxin (PE) 40 toxin	Breast cancer cell lines MDA-MB-361, ZR-75-1, MCF-7	(74)
Oncolytic viruses	Herpes simplex virus type 1 (HSV-1) Adenovirus Reovirus Myxoma	Breast cancer cells Breast cancer cells Lymphocytic leukemia and lymphoma Acute myelogenous leukemia	(23,25,27,65)
In vivo			
Chemotherapy	Idarubicin, Cytarabine and etoposide	Chronic myelogenous leukemia	(75)
Monoclonal Ab	Rituximab	Chronic myelogenous leukemia	(76)