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Selective Purging of Human Multiple Myeloma Cells from Autologous Stem Cell Transplantation Grafts using Oncolytic Myxoma Virus

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Autologous stem cell transplantation and novel therapies have improved overall survival of patients with multiple myeloma; however, most patients relapse and eventually succumb to their disease. Evidence indicates that residual cancer cells contaminate autologous grafts and may contribute to early relapses after autologous stem cell transplantation. Here, we demonstrate that ex vivo treatment with an oncolytic poxvirus called *myxoma virus* results in specific elimination of human myeloma cells by inducing rapid cellular apoptosis while fully sparing normal hematopoietic stem and progenitor cells. The specificity of this elimination is based on strong binding of the virus to myeloma cells coupled with an inability of the virus to bind or infect $CD34^+$ hematopoietic stem and progenitor cells. These 2 features allow myxoma to readily identify and distinguish even low levels of myeloma cells in complex mixtures. This ex vivo rabbit-specific oncolytic poxvirus called *myxoma virus treatment* also effectively inhibits systemic in vivo engraftment of human myeloma cells into immunodeficient mice and results in efficient elimination of primary $CD138^+$ myeloma cells contaminating patient hematopoietic cell products. We conclude that ex vivo myxoma treatment represents a safe and effective method to selectively eliminate myeloma cells from hematopoietic autografts before reinfusion.

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INTRODUCTION

Multiple myeloma (MM) is a clonal plasma cell malignancy that is most prevalent in adults over the age of 65 and accounts for 10% to 15% of newly diagnosed hematopoietic cancers with patients losing an average of 17 years of life expectancy per diagnosis [1]. Although recent clinical advances have improved the prognosis for patients with MM, life expectancy at diagnosis remains 2 to 5 years with a 5-year survival rate of only 34% [2,3]. Currently, the standard of care for patients with MM is treatment with high-dose chemotherapy followed by autologous stem cell transplantation (ASCT). In North America alone, ASCT is used to treat approximately 5,000 patients with MM annually. This treatment results in improved rates of disease remission as well as significantly prolonged event-free survival time compared with patients treated with conventional chemotherapy [4,5]. However, despite the improved prognosis associated with myeloablative chemotherapy followed by ASCT, the treatment is generally not curative, and a large majority of all patients with MM will suffer from relapsed disease.

The malignant cells that cause relapse are thought to originate from 2 sources: residual MM cells, which escape ablative therapy in bone marrow (BM) niches and low levels of MM cells that contaminate the autografts. To date, the relative impact of each of these 2 sources in causing MM relapse remains unclear; however, several observations suggest that residual plasma cell contamination plays a significant role. First, it has been repeatedly reported that patients with MM with lower levels of autograft contamination achieve greater benefit from transplantation than patients with higher levels of contamination [6-8]. Second, patients undergoing syngeneic transplantation, using uncontaminated BM from an identical twin donor, routinely achieve better rates of complete remission, improved long-term survival, and possibly even complete cures [9-11]. These data, particularly the

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syngeneic transplantation studies, support the conclusion that even low levels of MM cells contaminating the autograft can play a significant role in disease relapse and suggest that ex vivo manipulation of the autograft before infusion to remove all contaminating malignant cells, a process known as *purging* [12], could improve the outcomes of patients with MM.

Proposed MM purging procedures must meet 2 important criteria: (1) they must effectively remove all contaminating cancer cells from the grafts and (2) they must fully spare the normal hematopoietic stem/progenitor cells (HSPCs) in the autograft allowing for successful reconstitution of the patient's hematopoietic system. Several purging methods have been explored in ASCT [13-16], including a recent study focusing on ex vivo culture conditions that favor survival of HSPCs [17]. For MM, most of the focus has been placed on $CD34^+$ stem cell enrichment [18-20], which can reduce the level of MM contamination within the graft by 2 to 3 logs [20]. Unfortunately, clinical trials have demonstrated that this CD34-based purging does not improve clinical outcomes for patients with MM [19,21]. The results of these trials were initially interpreted as proof that myeloma relapse was primarily caused by residual disease persisting in the patient after ablative chemotherapy; however, subsequent molecular studies have shown that low levels of contaminating $CD138^+$ MM cells remain in ASCT samples even after multiple rounds of $CD34^+$ cell enrichment [22-24]. Moreover, $CD34^+$ malignant MM clones have been identified in patients which calls into questions the utility of CD34 enrichment in these patients [25,26]. Together, these data suggest that $CD34^+$ stem cell enrichment might fail to improve the prognosis of patients with MM because disease-causing MM cells remain in the autografts after positive $CD34^+$ cell selection of peripheral blood stem cells. Therefore, alternative means of ex vivo purging must be explored [12].

Previously, our laboratory demonstrated that a rabbit-specific oncolytic poxvirus called *myxoma virus* (MYXV) can eliminate primary acute myeloid leukemia cells from primary human BM samples while sparing normal HSPCs [27]. MYXV is an attractive virotherapeutic to target and eliminate human cancer cells for several reasons. First, the virus does not elicit detectable disease in any non-rabbit species, including humans or severely immunocompromised mice [28,29]. Second, the ex vivo therapeutic application of MYXV is not dependent on expression of transgenes or addition of chemotherapeutic agents and requires only a brief ex vivo incubation of the graft with MYXV before transplantation, thus making it an attractive strategy for clinical administration that minimally deviates from standard ASCT clinical practice [27,30]. Due to our previous success using MYXV to purge primary human acute myeloid leukemia cells, the virus' safety for the engraftment of normal human HSPCs, and the high rate of MM relapse after AHCT, we hypothesized that ex vivo MYXV treatment might represent an improved method for clinical elimination of MM cells contaminating patient autograft samples before reinfusion.

MATERIALS AND METHODS

Cells and Reagents

U266 (American Type Culture Collection [ATCC] #TIB-196), RPMI-8266 (ATCC# CCL-155), MM.1S (ATCC# CRL-2974), and HuNS1 (ATCC# CRL-8644) human myeloma cells as well as HL60 acute myeloid leukemia cells (ATCC# CCL-240) were obtained from ATCC and were maintained below 2×10^6 cells/mL in RPMI media supplemented with 1× pen/strep, 2 mM L-glutamine, and 20% FBS. The following Abs were used: HLA-A, HLA-B, HLA-C-APC, CD45-PE, CD45-FitC, poly ADP-ribose polymerase, HLA-A2.1-PE (BD Biosciences, San Jose, CA), caspase 3 and cleaved caspase 3 (Cell Signaling, Danvers, MA), and B actin (Ambion, Grand Island, NY). Clinical-grade heparin (1,000 USP U/mL) was a kind gift from Dr. Alexandra Lucas. Primary MM cells were obtained by patient donation under the approval of the University of Florida Institutional Review Board.

MYXV and Viral Infections

vMyx-green fluorescent protein (GFP) has been previously described [31]. Unless indicated, infections were carried out by exposing cells to vMyx-GFP at a multiplicity of infection (MOI) of 10 for 1 hour in PBS + 10% FBS in a humidified chamber at 37°C and 5% CO_2. Mock-treated cells were incubated in PBS + 10% FBS containing no virus under the same incubation conditions. Treatment with inactivated virus was performed using the same incubation conditions, but with inactivated vMyx-GFP prepared by exposing virus to UV light for 2 hours (UV inactivated) or incubating virus at 55°C for 2 hours (heat inactivated). Fluorescently tagged MYXV virions (vMyx-M093L-Venus) were created by fusing the GFP variant "Venus" protein in frame to the N-terminus of the M093 open reading frame (to be described elsewhere).

Analysis of MYXV Infection in Cultured Myeloma Cells

To measure initiation of early viral gene expression, human myeloma cells were analyzed 24 hours after infection with vMyx-GFP for expression of GFP using flow cytometry. To measure completion