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An Engineered Biomarker System to Monitor and Modulate Immune Clearance of Cell Therapies

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Abstract

Cell transplants offer a new opportunity to deliver therapies with novel and complex mechanisms of action. Understanding the pharmacology of cell transplants is important to deliver this new therapy effectively. Currently, however, there are limited techniques to easily track cells after intravenous administration due to the dispersion of the graft throughout the entire body. We herein developed an engineered cell system that secretes a luciferase enzyme to sensitively detect cell transplants independent of their locale by a simple blood test. We specifically studied a unique feature of cell transplant pharmacology, namely immune clearance, using mesenchymal stromal cells (MSCs) as a proof of concept cell therapy. MSCs are a clinically relevant cell therapy that have been explored in several disease indications due to their innate properties of altering an immune response. Using this engineered reporter, we observed specific sensitivity of cell therapy exposure to the preparation of cells, cytolysis of MSCs in an allogeneic setting, and a NK cell-mediated destruction of MSCs in an autologous setting. Our cellular tracking method has broader implications at large for assessing in vivo kinetics of various other cell therapies.

Keywords

NK cell; immune clearance; immunogenicity; MSC; engineered MSCs; luciferase

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Additional Information

The authors declare no direct competing financial interests. B.P. is a co-founder and member of the board of directors of Sentien Biotechnologies, a licensee of cell therapy patents from B.P., and holds an equity shareholding. Correspondence and requests for materials should be addressed to B.P.

Author Contributions

BP conceived and designed the study. AS, DK, SM, ML performed experiments, analyzed, and interpreted the data. AS, ML, and BP wrote the manuscript. BP provided funding of the study and revised the manuscript.

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INTRODUCTION

Cell therapies are a burgeoning class of medicines that can broaden the spectrum of drug action compared to conventional molecular therapeutics. Mesenchymal stromal cells, also referred to as mesenchymal stem cells (MSCs), are at the forefront of clinical testing with currently 493 clinical trials that administer MSCs as a therapeutic intervention¹. MSCs have many useful properties that make them a clinically attractive therapy. They are easily isolated and expanded from bone marrow or clinically discarded tissue (eg. umbilical cord blood, adipose tissue). MSCs can also be engineered *ex vivo* using both viral and non-viral methods to act as a drug delivery platform^{2,3}. A number of preclinical studies observed the potential of MSCs to enhance wound healing responses by secreting molecules that stimulate angiogenesis, endogenous stem cell populations, and a pro-resolving inflammatory response. An intravenous route of administration is the most prevalent use of MSCs for the systemic modulation⁴⁻⁷ of a dysfunctional immune system. However, despite these great strides in recent years, there lies a significant bottleneck in passing Phase III clinical trials for FDA approval⁸. One hypothesis for the ineffectiveness of MSCs in clinical studies is a short cell half-life in vivo based on estimates in mouse models³.

A critical question in the field of cell therapy is the consideration of autologous or allogeneic cells for use. Autologous cells, in principle, have the advantage of minimizing immune rejection of a cell transplant. Yet, managing supply chain logistics of autologous cell therapies is a challenge that can limit the scalability of this approach. The use of allogeneic MSCs overcomes scale up challenges of cell therapies to meet the clinical and ultimately commercial needs of >10,000 patients per year for highly prevalent indications. Unfortunately, typical allogeneic cells suffer from immunogenicity. MSCs however, have been considered minimally immunogenic through a lack of MHC Class II molecules and have been safely evaluated in mismatched transplant settings without severe immunogenic reactions^{9,10}. Whether as an autologous or allogeneic cell therapy, the immune clearance of MSCs has been underexplored with respect to their relatively short *in vivo* life span. Current literature supports that active immunologic processes may be responsible for their short in vivo persistence. Allogeneic MSCs elicited a memory T cell response and rapid clearance of subsequent intravenous doses by the immune system¹¹. Activated natural killer (NK) cells, known to eliminate foreign cells, have also been shown to facilitate MSC lysis^{12,13}. Furthermore, immunocompromised mice demonstrated higher levels to MSC-derived secreted factors after a cell transplant compared to wild type mice¹⁴. Collectively these studies suggest that recognition and immune clearance of MSCs could severely limit the bioavailability thereby greatly reducing therapeutic efficacy.

Studying immune clearance of a cell therapy such as MSCs is particularly challenging with existing tools. Whole body imaging requires specialized equipment and is only of high utility when cells are densely concentrated; the tracking of a diffusely spread cell transplant is hindered by signal to noise limits of conventional imaging techniques. Other approaches using flow cytometry or tracer-labeled cells are invasive and require sacrificing animals over time to detect cell levels which becomes tedious and can be time and resource

intensive^{15–17}. Furthermore, these methods are equally challenged when searching for rare engrafted cells in a large tissue bed.

Since MSCs broadly distribute and encounter the immune system throughout the entire body, we engineered a reporter MSC as a cell-based biosensor that secretes a blood-based readout for minimally invasive, continuous measurements. Mouse and human MSCs were genetically engineered with constitutive expression of a secreted Gaussia luciferase (gLuc) reporter. gLuc activity can be easily quantified in a small aliquot of blood (5 µl) *ex vivo* by adding its substrate coelenterazine and measuring emitted photons using a luminometer. gLuc has a half-life of 5–20 minutes in mice circulation, and has been used as a highly sensitive reporter (detection of ~1000 cells) for quantitative assessment of stem cells *in vivo* by measuring its level in the blood^{18–20}. This reporter system was put to the test to track MSCs in clinically relevant situations of autologous and allogeneic treatments and was found to reveal new insights into formulation of MSCs, potential cytotoxicity of allogeneic MSCs and NK cell mediated clearance of autologous MSCs.

METHODS

Culture and expansion of human and mouse MSCs

Human MSCs were isolated from whole bone marrow (Lonza, SUI) following previously established protocols^{21,22}. Frozen vials of a C57BL/6 murine MSC line were purchased from GIBCO (GIBCO, MA). Cells were cultured in MSC media comprising α -minimum essential medium (GIBCO, MA) with 10% FBS, 1% penicillin-streptomycin (GIBCO, MA), 1% antibiotic-anti-mycotic (GIBCO, MA), and 2.5 µg/L human fibroblast growth factor (hFGF) (R&D Systems, MN). Cells were cultured at 37°C, 5% carbon dioxide. Cells were expanded by plating 1000 cells/cm² in a polystyrene cell culture treated flask (Corning, NY) and allowed to reach 90% confluence rate prior to sub-culturing. MSCs from passages 3–5 were used for *in vitro* experiments. For *in vivo* experiments, MSCs harvested at passage 3 were used. Brefeldin A (BA) treated MSCs were incubated for 24 hours prior to use in 5 µg/mL BA¹⁴.

Genetic Engineering of MSCs

MSCs were harvested at passage 2 for lentiviral infection. Lentivirus was obtained from the Massachusetts General Hospital Vector Core. The lentivirus was engineered to contain a G-Luciferase gene along with a GFP reporter gene. Following protocols previously established as a guide, MSC media containing 100 µg/ml protamine sulfate was used for transduction². Multiplicity of infection (MOI) of 1 to 10 was used for producing cell bank. Transduced GFP positive cells were sorted using a BD FACS Aria III (BD Biosciences, CA) cell sorter (Harvard Stem Cell Institute Flow Cytometry Core at Massachusetts General Hospital, Boston). GFP positive cells were then cultured, expanded, and used for subsequent studies.

Animals

All mice, female, C57BL/6J and BALB/cJ, were purchased from Jackson Laboratories (Jackson Laboratories, CT) at 6–8 weeks old. Animals were housed at Massachusetts General Hospital Animal Facility according to IACUC approved protocols.

Injection of Cell Suspensions and Depletion of NK cells

Tail vein injections of human and mouse MSCs were done at a concentration of 1×10^6 cells/200 μ l in serum free MSC media. Depletion of NK cells was achieved by injecting mice with 100 μ g per dose of Mouse Anti-Mouse NK-1.1 Clone PK136 (BD Biosciences, CA)^{21,23,24}. Sham mice were treated with 100 μ g per dose of Rat Anti-Mouse CD16/CD32 Clone 2.4G2 (BD Biosciences). Intraperitoneal injections were performed every three days for a week prior to injection with MSC cell suspension. Spleens were collected from mice post mortem, and cells were collected using single cell suspension methods. Depletion was characterized using flow cytometry analysis on splenocytes.

Blood & Plasma Sample Collection

Mouse tail blood samples were collected using sterile 4mm lancets. Approximately 50 μ l of blood was collected per time point into Eppendorf tubes containing 20mM EDTA in PBS. Cardiac punctures were performed using a thoracotomy to provide access to the left ventricle. Plasma was isolated by centrifugation of blood samples at 3,000 rpm for 15 minutes at 4°C. Supernatant containing the plasma was collected and stored at -80°C for further experiments.

Bioluminescence Assays (BLI)

BLI assays were performed by pipetting 5 μ l of sample into a white walled high binding plate (Corning, NY) and adding 100 μ l of Coelenterazine native substrate (NanoLight Technology, AZ) at 100 μ M diluted in PBS. Samples were immediately read using a BioTek Synergy 2 Multi-Mode Reader for 10 seconds at a gain of 100–200 (BioTek, VT).

Data Analysis and statistics

Graphing and statistics were performed using Adobe Illustrator 6 and Prism 7. FACS analysis was performed using Flow Jo 8.7.3. Unpaired Welch's T-tests were performed with significance level of $p < 0.05$.

RESULTS

gLuc-engineered MSCs retain expansion potential *in vitro*

The process of cell engineering (Fig. 1a) was first optimized for MSCs to ensure a durable gLuc signal while maintaining an adequate growth rate of cells post-transduction. Human MSCs were used first to optimize *in vitro* and *in vivo* protocols because of the higher propensity for lentiviral infection and expression. Human MSCs were cultured for 24 hours in media with increasing concentrations of lentiviral particles per cell (MOI) with a cationic vehicle, protamine sulfate. Protamine sulfate, approved for human use by the FDA²⁵, has comparable transduction efficiencies as polybrene, without detrimentally affecting MSC health². All conditions were non-toxic and maintained MSC viability above 95% (Fig. 1b). Conditions with a high protamine sulfate concentration (100 μ g/ml) and/or high MOI (10 particles/cell) had the highest transduction efficiency with an average of 78.6% (Fig. 1c–d). GFP⁺ cells were FACS sorted and frozen as a pure population. Thawed cells maintained a high level of viability and remained >90% pure after expansion (data not shown).

Importantly, MSC proliferation was not impaired at either highest MOI or protamine sulfate condition. There was no significant difference in doubling time between non-transduced and transduced groups (Fig. 1e). gLuc activity was detectable in purified MSCs when placed in culture and fed their substrate (Fig. 1f). Increasing the MOI resulted in a dose-dependent increase in gLuc activity (Fig. 1f). The high MOI and protamine sulfate condition was applied to mouse MSCs with a similar effectiveness (data not shown) and gLuc levels were detectable in vitro, albeit lower than human MSCs as expected.

Optimization of gLuc detection *in vivo* following MSC injection

For *in vivo* use of gLuc-MSCs, we first explored blood sample preparation to assure that a small blood sample would represent the whole systemic concentration. Human MSCs were injected intravenously and peripheral tail vein blood was compared to cardiac blood. We therefore measured gLuciferase in all four conditions (whole blood, plasma) at 24 hours after transplantation of gLuc-MSCs. Plasma detection of luminescence was higher than in whole blood for both tail vein and cardiac blood (Fig. 2a). Whole blood contains hemoglobin, which has been shown to hinder the gLuc-coelenterazine interaction *ex vivo*.^{26,27} gLuc activity was compared between plasma samples in tail vein- and cardiac-acquired blood (Fig. 2a), which gave confidence that our peripheral vein measurements were reflective of the systemic levels. Hence, for all future experiments, we analyzed gLuc activity in the plasma compartment of blood. We also tested for background signal due to auto-fluorescence or systemic factors by injecting both transduced and non-transduced hMSCs and collecting cardiac blood samples. Mice injected with non-transduced MSCs did not have any gLuc signal in blood and therefore validated that all signal was arising from the engineered gLuc biomarker (Fig. 2b).

We analyzed the preparation of gLuc-transduced MSCs on *in vivo* signal intensity. We measured luminescence in tail vein plasma post injection with either freshly cultured cells (fresh) or cryopreserved cells (frozen) thawed 24hrs before being intravenously administered to C57BL/6J mice. Tail vein blood was collected at various time points up to 24 hours post administration. Fresh cells yielded significantly higher plasma levels of gLuc than frozen cells. We observed a dramatic decline in gLuc signal between 8 and 24 hours post intravenous injection of fresh cells (Fig. 2c). The difference in gLuc signal was not attributed to a difference in the viability of freshly harvested cells compared to frozen (Fig. 2d) nor the purity of gLuc+ cells (Fig. 2e) in either preparation of the cell formulation. These results confirmed that freshly harvested cells can expose a mouse to higher levels of secreted factors and this preparation of MSCs was used for future studies in allogeneic and autologous transplant studies.

Potential cell lysis detected in allogeneic MSC transplant model using gLuc cells

Mouse MSCs engineered with gLuc were then advanced for cell transplant studies in the same species to better model the clinical uses of MSCs. Since a self-inactivating lentiviral construct was used in these studies, gLuc detection *in vivo* could occur for two major reasons: (1) active cell secretion of the engineered biomarker; or 2) cell lysis and release of intracellular gLuc stores. To clarify which process was occurring, Brefeldin A (BA), a golgi apparatus inhibitor, was used to nonspecifically inhibit cell secretions as an approach to

detect cell lysis and the release of intracellular gLuc that would otherwise be inhibited by BA treatment^{28,29}. We found that pre-incubation with BA at a concentration of 5µg/mL for 24 hours inhibits gLuc secretion by MSCs to undetectable levels. BA treatment was seen to be non-toxic and reversible. Over 24 hours, a clear difference can be seen in the amount of gLuc secreted by MSCs into fresh MSC media between the two conditions (Fig. 3a).

In order to approximate an allogeneic cell transplant model, BALB/cJ mice, a mismatched mouse strain, was used as a recipient for injection with C57BL/6J MSCs. Freshly trypsinized BA treated cells or control gLuc mouse MSCs were injected into mice at a dose of 0.5×10^6 cells per mouse. Over a time course of 24 hours, tail vein blood was collected and it can be inferred that the BA treated cells elicited a gLuc signal peak at 0.5 hours, while the control cells caused a slow increase of gLuc over the time course (Fig. 3b). Since gLuc release was completely inhibited by BA treatment, these results suggest that intracellular gLuc may have been released as a bolus due to cell lysis rather than a slow release as seen untreated gLuc MSCs in this allogeneic model.

Depletion of NK cells increase bioavailability of autologous MSC transplants

We then evaluated MSC transplants in an autologous model using C57BL/6J MSCs into a recipient C57BL/6J mouse. After an initial set of cell transplants, we observed a paradoxical reduction in signal in autologous cell transplants compared to allogeneic cell transplants (Fig. 4a). The exposure, or area under the curve, to an autologous transplant was dramatically lower than allogeneic cell treatments. We further investigated the role of MSC clearance in an autologous model with a focus on NK cells as a primary mediator.

NK cells are a cytotoxic lymphocyte of the innate immune system that can recognize foreign cells in the absence of histocompatibility proteins or opsonizing antibodies enabling a faster immune clearance mechanism. *In vitro* studies have shown that NK cells can lyse MSCs, but the relevance of this phenomenon *in vivo* has not been studied. Our engineered MSC population was used for high sensitivity *in vivo* screening to assess the role of NK cell-mediated clearance. C57BL/6J mice were pre-conditioned with an NK-cell depleting antibody (anti-NK1.1) for a week and then administered C57BL/6J mouse MSCs (Fig. 4b). A control Fc receptor antibody was used as an antibody control. gLuc content was monitored through tail vein blood collection for 72 hours at which point spleens were collected for (FACS) analysis to confirm successful NK depletion. Depletion of NK cells led to a major increase in the exposure of MSC therapy seen by gLuc blood levels at all time points (Fig. 4c). At the end of the treatment, it was observed that mice with lower levels of NK cells showed higher amounts of gLuc exposure suggesting a strong correlation between the two observations (Fig. 4d). New approaches that modulate NK cell clearance of autologous MSC transplants may enable improved therapeutic efficacy by increasing the bioavailability of intravenous MSCs.

DISCUSSION

Cell therapies are an emerging class of medicine that require new pharmacological perspectives. To study the role of immune clearance of a cell therapeutic, we engineered a cell reporter system that can sensitively test for the bioavailability of a cell transplant by

indirectly monitoring a secreted biomarker. This engineered biosensor has markedly improved sensitivity over whole body imaging systems while only relying on several microliters of peripheral blood to detect a secreted biomarker. This system can detect systemic secretions from small amounts of a cell transplant, $<1 \times 10^3$ per mouse compared to whole animal imaging systems that require larger quantities of injected cells to obtain a visible signal in a highly-localized area and have difficulty detecting dispersed cells throughout the animal^{30,31}. Our *in vivo* testing verified that peripheral blood plasma was equivalent in biomarker signal to cardiac plasma which supported a simple blood test to quantify the overall level of a cell transplant in an entire subject.

We first tested our engineered cell transplant using MSCs as a clinically relevant therapy currently being explored in both autologous and allogeneic settings. We observed that the preparation of MSCs prior to transplant is critically important. Cells freshly harvested from culture produced significantly more gLuc than cells thawed from cryopreservation and immediately transplanted. Despite both fresh and frozen MSCs having comparable viability and GFP expression, there was a clear negative impact on *in vivo* function post-thaw (Fig. 2c). The lack of gLuc expression in the engineered cells can be linked to an overall diminished MSC function following cryopreservation stress^{32,33}. Interestingly, this study demonstrated that MSC function could be restored after a 24-hour post thaw culture³². This study corroborates our findings; if given time to recover from thaw and stabilize, cryopreserved cells will regain their ability to secrete large amounts of gLuc. It is therefore beneficial to culture cells 24–48 hours using static culture or via *ex vivo* bioreactor set up prior to patient administration towards improving cell efficacy *in vivo*³⁴. This finding is of great importance as it has direct implications on current clinical practices that use cryopreserved MSCs directly after thawing for *in vivo* use³⁵. These findings further highlight the ability of a reporter system to relay the therapeutic potency of MSCs. More work should be performed to assess functional implications of this depressed MSC secretion phenotype.

We then explored our cell reporter in an allogeneic model to evaluate the use of this engineered system to detect cell lysis which limits bioavailability of cells for therapy. To detect cell lysis we adopted an intracellular release measurement of gLuc similar to a conventional chromium release assay used for *in vitro* cytotoxicity assays^{36,37}. To inhibit gLuc secretion and concentrate gLuc intracellularly, we used a well-known inhibitor to the Golgi apparatus, brefeldin A¹⁸. Moving *in vivo*, we indeed saw differences in the pharmacokinetic release of gLuc in BA treated cells. Control cells displayed increased gLuc secretions over time indicating cell survival and potential engraftment. In contrast, BA treated cells demonstrated a high initial spike followed by a rapid decline and eventual low levels of gLuc. One potential explanation indicates an initial lysis of MSCs leading to a rapid elimination of internal gLuc as seen. Though other mechanisms may be at play to explain this result warranting further investigation; since BA non-specifically blocks secretory function, it could have also disabled the mechanism by which MSCs evade the immune system by way of secreted factors themselves³⁸. Although a MSC secreted factor has not been studied in the context of immune evasion, neutralizing known immunosuppressive MSC factors, such as 2,3-IDO and PGE2, may be an initial starting point to decouple cell lysis and immune evasion.

An autologous model was then implemented to evaluate another common transplant setting. A major finding was that autologous cells were cleared quite robustly. This has major implications for studies which implement autologous models and indicate that syngeneic cells may be immunogenic. This immunogenicity may arise due to the byproducts of foreign serum components³⁹ that the autologous cells were expanded in or genetic alterations that we implemented. This result of autologous cell clearance is not unique; iPS cells that were generated from the same mice and transplanted in an autologous host were also found to be rejected⁴⁰. This indicates that it is necessary for researchers to account for immunologic cell depletion in their experiments with potential significant impacts on efficacy. We then sought to determine the mechanism by which cells were cleared. NK cells have been shown to play a significant role in immunomodulation⁴¹ and in particular in allogeneic transplants^{42,43}. Following methods as described in Harshan et al. depletion of NK cells was done through an antibody method^{24,44}. During and after depletion there was no notable difference in animal's health and behavior. Depletion of NK cells was analyzed through flow cytometry on splenocytes. We tested a pre-administration timing of a week prior to MSC injection. Through NK cell depletion, we were able to detect a higher and more prolonged gLuc signal compared to control animals. This indicates that autologous MSC clearance is, in part, due to NK cell activity. These results suggest the surface of autologous MSCs may be decorated with ligands such as fetal bovine serum albumin that is detectable by innate NK cells and prime them for clearance⁴⁵. Other timings for co-therapeutics will need to be determined. A potential reason why non-autologous cells persist longer may also be due to the slight inflammatory environment created by their immunogenicity. The activation and creation of a pro-inflammatory MSC state is known to enhance MSC function which has the potential to increase their ability for immune evasion⁴⁶.

The ability to successfully track MSCs and their secretions *in vivo* may lead to an increase in understanding of the pharmacokinetic and pharmacodynamic properties of their therapeutic effect, which could increase the success rate of many MSC based therapies. An important consideration to our engineered MSCs is that this concept and platform can be applied to numerous other cell types for other biosensing techniques. The reporter cell lines generated in this study implement a constitutive secretion method to act as a biomarker for the integrity of the cell sensor. One can further incorporate response element promoters that become activated upon a stimulus as a sensitive local biosensor with the ability to report tissue level activity by a peripheral blood test. Cell biosensors, such as this, can easily be scaled up and utilized in both larger animals⁴⁷ and humans as a useful means of cell monitoring, although caution is warranted for the use of gLuc itself as a marker system without a thorough evaluation of gLuc immunogenicity. Other natural secreted reporters, such as secreted alkaline phosphatase, may be useful in larger subjects to minimize any adverse immunogenic event risk. These studies, in collective, highlight the value of using a highly sensitive engineered MSC line in order to study the fate of transplants *in vivo* with the opportunity for further features to be added as a customizable biosensor.

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Figure 1. Characterization of gLuc engineered MSCs (MSCs)

(a) Lentiviral construct was engineered to contain a gLuciferase secretion gene and a GFP reporter. (b) FACS analysis of viability of transduction method. Transduction with lentivirus and protamine sulfate does not negatively affect viability in relation to naive MSCs. Viability above 95% for all conditions. (c) Contour plots obtained from FACS cell analysis, 100k events. FACS cell sorting method was used to sort for positive GFP expressing MSCs. Multiplicity of Infection (MOI) and protamine sulfate concentration was varied. (d) Graphical representation of efficiency of transduction from contour plots of figure 1a. Varying multiplicity of infection (MOI) and protamine sulfate concentration causes changes in efficiency of lentiviral transduction. Higher MOI and protamine sulfate concentration result in a higher transduction efficiency. (e) Comparison of doubling rates of hMSCs and gLuc transduced hMSCs in order to see if lentiviral infection affects expansion ability of hMSCs. Doubling time is comparable through passages 5 – 7. (f) Bioluminescence assays (BLI) of in vitro production of gLuc by mouse and human MSCs. Media collected from 100k cells incubated in 3ml of MSC media.

Figure 2. Optimization of *in vivo* g luciferase signal for murine model

(a) Comparison of secreted gLuc signal in mouse blood and plasma. Both tail and cardiac samples were tested. A signal can be read in all samples at 24 hours after injection of gLuc hMSCs. (b) BLI comparison of gLuc signal of non-engineered hMSCs and gLuc engineered hMSCs. (c) Comparison of gLuc signal from IV administered hMSCs that were either freshly harvested or directly thawed from cryostorage. Murine tail plasma was sampled over 24 hours. (d) Viability and (e) GFP expression of gLuc cells prepared freshly from cell culture or directly after thawing of a cryopreserved population prior to injection.

Figure 3. Brefeldin A treatment and Allogeneic Cell Transplantation

(a) GLuciferase secretion seen in control hMSCs or hMSCs treated with BA. BA cells incubated with 5 μ g/mL BA in MSC media for 24 hours. Both conditions were exposed to fresh MSC media at time 0 and media samples were taken at 8 and 24 hours later. GLuciferase concentration read in media from 5×10^6 MSC cells plated in T175 flask. BLI assay read at gain of 100. (b) GLuciferase signal throughout a time course of 24 hours. Freshly trypsinized gLuc mouse MSCs cultured over a week were injected into mice. Brefeldin A cells incubated with 5 μ g/mL BA in MSC media for 24 hours. Absolute values from BLI assay are represented. n=3 for BA condition and n=4 for Control condition.

Figure 4. Blood monitoring of gLuciferase secretion after NK cell depletion in an Autologous Cell Transplant Model

(a) Schematic of in vivo NK cell depletion murine model. Mice were injected for a week prior with NK-1.1 antibody or FC block before injection with gLuc MSCs. (b) GLuciferase signal throughout a time course of 24 hours. Freshly trypsinized gLuc mouse MSCs cultured over a week were injected into mice. Tx mice were treated with Mouse Anti-Mouse NK-1.1. Sham mice were treated Mouse BD Fc BlockTM. n=3 for Tx mice and sham mice, n=1 for Control mouse. Absolute values from BLI assay are represented. (c) Total NK cell count correlated to gLuciferase seen over 72 hours. NK count obtained through flow cytometry analysis of percentage of splenocytes and total splenocyte count. GLuciferase quantified by taking AUC of 72 hour time course.